DEVELOPMENT OF CONTROLLED RELEASE POLYMERS FOR INSECT REPELLENTS AND AGROCHEMICALS

*A thesis submitted
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy*

By

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Dedicated to
My
Beloved parents

CERTIFICATE

This is to certify that the thesis entitled **"DEVELOPMENT OF CONTROLLED RELEASE POLYMERS FOR INSECT REPELLENTS AND AGROCHEMICALS"** submitted by Md. Rabiul Hussain, Research Scholar of Department of Chemical Sciences, Tezpur University, Assam for the award of degree of Doctor of Philosophy in Science, is a record of bonafide research work done under my supervision and guidance at Department of Chemical Sciences, Tezpur University, Assam-784028. He has successfully completed the work. A few scientific papers related to the subject have been published/ accepted for publication in international and national journals of repute. A few are also under communication and under preparation.

He has fulfilled all the requirements for submitting the thesis for award of the degree of Doctor of Philosophy in Science

The results embodied in the thesis have not been submitted to any other University or Institution for award of any degree or diploma.

(Prof. T. K. Maji)

DECLARATION

I hereby declare that the thesis entitled " DEVELOPMENT OF CONTROLLED

RELEASE POLYMERS FOR INSECT REPELLENTS AND AGROCHEMICALS"

is an authentic work carried out by me under the supervision of Prof. T. K. Maji,

Department of Chemical Sciences, Tezpur University, Napaam. No part of this

work had been presented for any other degree or diploma earlier.

Place: Tezpur

Date: 01-07-09

Md. Rabiul Hussain

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ABSTRACT

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Insect repellents are substances that protect animals, plants or products from insect attack by making food or living conditions unattractive. A large number of synthetic as well as herbal repellents are available. Both synthetic as well as herbal repellents have limited persistence on skin. The ideal repellent should have long-lasting effectiveness against a wide variety of arthropods.

In general, insect repellents have been selected on the basis of persistence on the skin. However the available insect repellents have limited persistence on the skin including N, N-Diethyl-m-toluimide (DEET). The use of controlled release technology with topical repellents has provided extended protection against mosquitoes. Controlled release formulation of an insect repellent could prolong the availability of the repellent on the treated surface by reducing the loss of repellent by absorption, evaporation and abrasion.

Similar is the case with agrochemicals. They are applied to targets systemically or topically by conventional means such as broad casting, spraying etc., which may lead concentration levels locally that are too high or too low for effective action. High concentrations may produce undesirable side effects either in the target area, which could lead to crop damage, or in the surrounding environment. Subsequent periodic applications may be necessary for continued efficiency to control the pests throughout the growing season. Also, administration of the active agent at a site remote from target results in nonspecific application. Both factors, addition to the increasing cost of the treatments, produce undesired side effects on the target and the environment.

Recently, controlled release (CR) technology has emerged as an approach with potential for solving the problems associated with the applications of conventional agrochemicals. Rapid advances have been made in the use of polymers for CR in most classes of agrochemicals. The aims of controlled release formulations (CRFs) are: (a) to allow the automatic release of the active agent to the target at a controlled rate; and (b) to maintain the concentration of the active agent within the optimum limits over a specified period of time.

In the present research work controlled release polymeric systems of an agrochemical, urea and an insect repellent based on plant essential oil, *Zanthoxylum limonella oil* (ZLO) were developed and characterized. The polymeric materials used were naturally occurring polymers such as gelatin and chitosan. Microencapsulation technique was used for the synthesis of controlled release formulations of the agrochemical and insect repellent. Microencapsulation is a technique that reproducibly applies a uniformly thin polymeric coating around small solid particles, liquid droplets or solid dispersions. The polymeric wall is designed to permit controlled release of the encapsulated material under desired conditions. The release of the active agents can be controlled by crosslinking of the polymeric wall. Both synthetic and naturally occurring crosslinking agents were used in our present work. Synthetic crosslinking agent used was glutaraldehyde and naturally occurring crosslinking agent was genipin.

Chapter I. This chapter includes introduction part. This covers history of controlled release technologies, their advantages and disadvantages, types of controlled release techniques, manufacturing techniques, nature of polymers used in controlled release systems and their applications. This chapter also includes general literature relating to chitosan, gelatin and their various crosslinking agents.

Chapter II. This chapter covers the available literatures involving on controlled release agrochemicals and insect repellents (mosquito repellents) and analysis of controlled release systems in the light of stability, applicability, loading, efficiency, release characteristics, FTIR spectroscopy, thermal properties, surface properties etc. The main objectives and plan of work have also been included in this chapter.

Chapter III. This chapter covers the materials and methods, which include the raw materials used, sample preparation techniques, their characterization techniques and their application studies.

Chapter IV. This chapter includes results and discussion part. This chapter was divided into the following parts.

Part I: Microencapsulation of *Zantoxylum Limonella Oil* (ZLO), an essential oil based insect repellent in glutaraldehyde crosslinked gelatin microcapsules produced by simple coacervation technique for mosquito repellent application

In this part of work, glutaraldehyde (GA) crosslinked gelatin (G) microcapsules containing *Zanthoxylum limonella oil* (ZLO) were prepared by coacervation technique.

The minimum temperature and ratio of gelatin to sodium sulphate at which phase separation occurred were 40^0C and 1:10. This temperature and ratio were maintained during preparation of microcapsules in the subsequent experiments.

The encapsulation efficiency, oil content and release pattern from the microcapsules were determined by using UV spectrophotometer and calibration curve of oil.

The effect of variation of oil loading during microencapsulation was evaluated. It was observed that with increase in oil loading the release rate increased throughout the range of oil concentration studied. The encapsulation efficiency was found to decrease while the % oil content and release rate were found to increase.

With the increase in gelatin concentration, oil loading (%) and oil content (%) of the microcapsules decreased as expected. Encapsulation efficiency increased first and then leveled off.

With increasing in glutaraldehyde concentration during microencapsulation, an increase in oil content, oil load and encapsulation efficiency were observed. An increase in the degree of cross-linking resulted in a significant decrease in oil release rate throughout the glutaraldehyde concentration studied.

Scanning electron microscopic (SEM) study of the microcapsules showed that the microcapsules were made of spherical units linked to each other. The external surface appeared smooth at low oil loading indicating the formation of a continuous film by gelatin. At higher oil loading, a bursting look was observed.

FTIR spectra of ZLO, gelatin, physical mixture of ZLO, glutaraldehyde, gelatin and ZLO containing cross-linked gelatin microcapsules were recorded. Physical mixture was prepared using the ratio of ZLO, glutaraldehyde and gelatin similar to those of ratio used in preparing ZLO containing cross-linked gelatin microcapsules. In the spectra, the carbonyl stretching band of ZLO between 1637-1720 cm^{-1} was found to remain almost unchanged in the case of physical mixture as well as microcapsule. These results indicated the absence of any significant interaction between the ZLO and the gelatin.

The laboratory repellency test of the gelatin microcapsules mixed with petroleum jelly was conducted against female *Aedes albopictus*. Microcapsules containing mosquito repellent formulations were tested and compared with a formulation containing DEET. It was observed that ZLO containing microcapsules provided repellency in the range between 1.5 – 2.5 h under laboratory condition. On the other hand, virgin ZLO and virgin DEET containing petroleum jelly provided repellency of 1.5 and 2.5 h respectively.

Part II. Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of *Zanthoxylum limonella oil* (ZLO) using salting out method and their application as mosquito repellent

In this part of work, ZLO containing chitosan-gelatin complex microcapsules were prepared by complex coacervation process using the salting out method.

The minimum ratio of polymer mixture to salt and the temperature at which clear phase separation observed were 1:5 and 40°C respectively.

With the increase of oil loading, the release rate of the oil from the chitosan-gelatin microcapsules increased throughout the range of oil concentration studied. It was observed that the % oil content increased while encapsulation efficiency (%) decreased. At low oil loading, the dispersion of the oil into globules by the stirrer was more effective; therefore the oil vesicles were smaller. The higher oil load also resulted in the decrease of thickness of microcapsule wall, which subsequently resulted in the faster release rate of high-loaded microcapsules.

The increase in genipin concentration during microcapsule preparation resulted in an increase in oil content (%) and encapsulation efficiency (%). The results found were as per expectation.

The release rate of oil from gelatin-chitosan microcapsules was dependent on the % of chitosan present in the mixture. The higher the % of chitosan in the chitosan-gelatin mixture, the lower was the release rate. Chitosan has more average number of primary amine groups than gelatin for reaction with genipin. The more the % of chitosan in the chitosan-gelatin mixture, the higher the reaction between chitosan and genipin was. As a result, more cross-linking would take place. This would in turn produce a more compact wall resulting in the decrease of release rate.

FTIR spectra of the microcapsules when compared with that of chitosan, gelatin and ZLO, it suggested an interaction between chitosan and gelatin but no significant interaction was observed with ZLO.

On examining the scanning electron micrograph of the microcapsules with higher oil loading, a bursting look was observed and it appeared more compared to those of microcapsules prepared at low oil load.

The laboratory repellency tests of the chitosan-gelatin microcapsules mixed with petroleum jelly were conducted against hungry female *Aedes albopictus* mosquitoes. Microcapsule containing mosquito repellent formulations were tested and compared with virgin DEET and virgin ZLO each mixed with petroleum jelly. Microcapsules containing mosquito repellent formulations provided repellency in the range of 2.0-3.0 h under the same condition depending on the microcapsule behavior. Microcapsules with higher percentage of oil content and lower degree of crosslinking provided maximum protection.

Part III. Microencapsulation of *Zanthoxylum limonella oil* (ZLO) in genipin cross-linked Chitosan-Gelatin complex using coacervation technique for Mosquito Repellent Application

In this part of work essential oil containing chitosan-gelatin microcapsules cross-linked with genipin were prepared by complex coacervation process.

The optimum ratio of gelatin to chitosan and pH at which maximum coacervation observed were 1:10 and 5.9, respectively.

On examining the effect of variation of oil loading on encapsulation efficiency and release rate of the microcapsules, it was found that the more the oil load, the higher was the release rate and lower was the microencapsulation efficiency.

Again oil content (%) was found to increase with the increase in the % of oil load. As oil load (%) increased, the number of oil vesicles in the microcapsules increased which resulted in an increase in oil content.

With the increase in the concentration of chitosan in chitosan-gelatin mixture, the release rate was found to decrease. Again an increase in the viscosity of the chitosan-gelatin mixture was noticed with the increase in the concentration of chitosan.

With the increase in genipin concentration, the encapsulation efficiency was found to increase as per expectation.

On studying the SEM micrographs of genipin crosslinked microcapsules having different percentages of oil content, it was found that microcapsules with low oil content have smooth surface and those with higher oil content possess a bursting look.

Thermal stability of the ZLO containing chitosan-gelatin microcapsules was measured with the help of thermogravimetric analyzer (TGA). Both initial decomposition temperature (Ti) and residual weight %(RW) were found to increase with the increase in chitosan concentration in the chitosan-gelatin mixture. The decomposition of ZLO started at an early stage and there was no residue at 600°C.

Temperature of decomposition (T_D) values of the microcapsules, chitosan, gelatin and oil at different weight loss (%) showed that T_D values for the microcapsules increased with the increase in the % of chitosan in the microcapsules.

The FTIR spectra of the microcapsules when compared to that of virgin chitosan, virgin gelatin, virgin ZLO and their physical mixture it could be concluded that an interaction occurred between chitosan and gelatin but no significant interaction were observed with ZLO.

The differential scanning calorimetric (DSC) study of pure chitosan, pure gelatin, oil and oil loaded chitosan-gelatin microcapsules and that of physical mixture suggested

that a low compatibility in the thermal properties existed in the relation between oil and chitosan-gelatin complex.

The laboratory repellency test of the chitosan-gelatin microcapsules made with complex coacervation technique was conducted against hungry female *Aedes albopictus*. Microcapsules were mixed with petroleum jelly and compared their protection time against mosquito bites with those of DEET and ZLO. It was observed that microcapsules containing repellent formulations provided protection time in the range of 2.5-3.0 h while those of ZLO and DEET containing formulations provided protection of 1.5 h and 2.5 h respectively.

Part IV. Degree of Deacetylation of Chitosan:Determination and their influence on the behavior of genipin cross-linked chitosan-gelatin microcapsules containing ZLO prepared by Salting Out Method

In this part of work, chitosan with different degree of deacetylation (DDA) was prepared by treatment with alkali under nitrogen atmosphere for various time durations. The chitosan with various DDAs thus prepared were characterized and in the later stage utilized for the preparation of ZLO containing chitosan and chitosan-gelatin complex microcapsules cross-linked with genipin by salting out method.

The degree of deacetylation (DDA) of original and alkali treated samples were determined by potentiometric titration method and by using the ratio of absorbance of amide I (1655 cm^{-1}) to hydroxyl group (3450 cm^{-1}) in FTIR spectrum of chitosan samples. The degree of deacetylation determined by these methods were further authenticated by elemental analysis method. The degrees of deacetylation of chitosan samples determined by these methods were found to be almost same. The degree of deacetylation of the sample-1(original), sample-2(4 hours alkali treated) and sample-3(8 hours alkali treated) were found to be 55.8,61.3 and 85.34%(w/w) respectively.

The molecular weights of the chitosan samples were determined using the Mark-Houwink-Sakurada (MHS) equation: $[\eta]=K(M_W)^a$, where 'K' and 'a' are constants for given solute-solvent system and temperature. It was observed that the molecular weight of chitosan samples decreased on increasing the degree of deacetylation.

The effect of degree of deacetylation (DDA) on the behavior of the microcapsules containing ZLO was studied. The oil content and encapsulation efficiency of both chitosan and chitosan-gelatin microcapsules were found to be decreased as the degree of deacetylation (DDA) of chitosan increased.

The scanning electron micrograph (SEM) of the microcapsules clearly indicated that on increasing the degree of deacetylation, there was an increase in the surface smoothness of the microcapsules.

The FTIR spectra of the microcapsules when compared to those of different chitosans with different DDAs, virgin gelatin, virgin ZLO and their physical mixture, it could be concluded that no significant interaction occurred between chitosan-gelatin and ZLO.

Part V Controlled Release of Urea from Chitosan Microspheres prepared by Emulsification and Crosslinking Method

In this part of work, urea was microencapsulated in cross-linked chitosan microspheres. The crosslinking agents used for crosslinking of chitosan was a naturally occurring material namely, genipin.

The effect of various parameters such as effect of polymer concentration, urea loading, genipin concentration on encapsulation efficiency, and urea content in the microspheres and release profile of the urea from the microspheres were studied.

It was observed that on increasing the urea content, the release rate from the microspheres increased. The release of urea from the micro spheres was initially slow and then suddenly increased with time. Higher the content of urea in the microspheres higher was the release of urea from the microspheres.

At low loading, the release rate of urea from the microspheres was low due to higher amount of polymer. At low urea loading, higher amount of polymer increased the thickness of the wall that resulted in decrease of the release rate. Again as the initial loading of urea increased, the release was also found to increase. The release rate of urea from the microspheres was found to decrease with increasing the degree of cross-linking.

The polymer concentrations had significant effect on the micro spheres behavior. Higher polymer concentration resulted in larger sizes microspheres and lower content of urea inside the microspheres. The larger sizes resulted in lower release of urea from the microspheres.

The scanning electron microscopic study (SEM) of the urea loaded chitosan microspheres as well as unloaded microspheres confirmed that the surface of the loaded microspheres had blisters while those of unloaded microspheres had shrinkage on the surface. Furthermore loaded microspheres had cracks on the surface.

FTIR spectra of both loaded and unloaded microspheres confirmed that there was no significant interaction between urea, chitosan and genipin.

Chapter V: This chapter includes summary and conclusion part of the present work.
The salient outcomes of this thesis have been summarized.

> ➤ ZLO was microencapsulated in glutaraldehyde cross-linked gelatin successfully. The release rate of the oil was found to be dependent on various parameters such as oil content, crosslinking density and the amount of encapsulating polymer.

> ➤ ZLO was encapsulated within chitosan-gelatin complex microcapsules by using both sodium sulphate and changing pH. The release rate of ZLO was found to be dependent on polymer concentration, oil content, degree of cross-linking and the amount of chitosan present in the chitosan-gelatin complex.

> ➤ Release rate of urea was found to be dependent on polymer concentration, urea concentration and temperature of the release medium.

> ➤ The repellency study clearly demonstrated the potential of controlled release formulations based on gelatin, chitosan-gelatin and ZLO for use as topical repellent against mosquitoes.

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CHAPTER I

INTRODUCTION

Introduction

1.1. Concept of Controlled Release Technology

The concept of controlled release technology emerged actively in nineteen sixties, has attracted wide attention over the last few decades. The research and developmental effects in this area experienced in medical, agricultural and biotechnological fields [1]. The use of drugs, fertilizers, pesticides and herbicides and similar chemically active agents are inevitable to the modern society. However, the extensive and unjudged uses of these biologically active chemicals have created great alarm among scientists worldwide, because of its adverse impact on the health and environment. The uncontrolled application of these chemicals almost inevitably induces many undesired side reactions in the systems, which receive them. Controlled release technology finds its potential application in minimizing or avoiding these side reactions during the course of action of the active agents by controlled release of them to the system [1, 2].

Several low molecular weight biologically active agents are in use at present. These agents during their course of action manipulate the environment around and within us. Hence, its efficient, safe and economic use is to be strictly followed for sustaining the environment and human health. The conventional use of these chemicals in biosystems produces peak and valleys of its concentration within the system in a very short period of

time. This necessitates repeated application of these agents to the system in order to maintain its concentration level for performing the function. These periodic applications produce maximum and minimum of active agent concentration in the system. Usually these concentrations profile involve harmful and ineffective phases. Thus the efficiency of these chemicals relies on its release to their site of action at the right time and in right quantity [2].

Concentration levels of an active chemical agent in a system, when administered through different modes of conventional (uncontrolled) and controlled manner, are represented in Fig. 1.1.The upper horizontal line represents the maximum admissible level (MAL) of concentration of active agent in the system, above which damage may takes place, and the lower horizontal line represents the minimum concentration level of the active agent required in the system for the specified action to takes place. In the uncontrolled application of the application (Curve A), the period of action is very short and a part of applied active is wasted, since that is not available in the system at required level. Usually to overcome this shortfall, active agents are applied at a higher concentration (Curve B). Here the concentration level of active agent in the system goes beyond the MAL and these results in damage in the system. Though the period of effective action is for a longer time, than in case A, damage of the receiving system as well as wastage of chemicals are there.
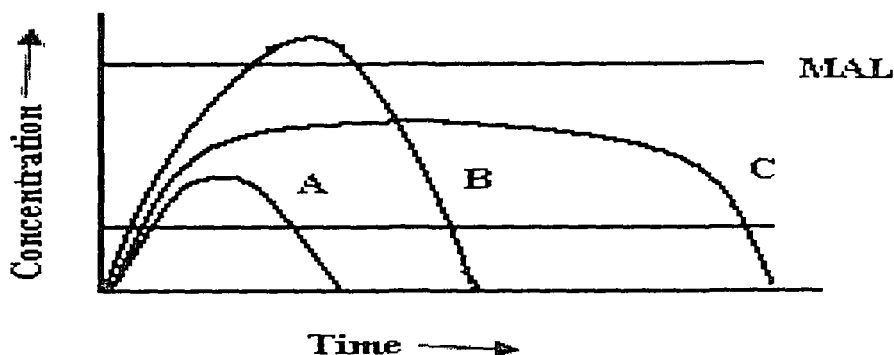
Fig 1.1. Typical active concentration v/s time profile for various modes of delivery

A: Conventional method of application B: Conventional method (Overdose)

C: Controlled release method.

The controlled release (CR) formulation delivers the active ingredient in right amount (Curve C) and period of action is much longer than in cases A or B and an ideal system can maintain the optimum level of active component in the system for a longer period. Repeated 'B' type application will result in very wide variations in concentration of the active agent in the system; sometimes too high, to set in undesired side reactions or too low such that the desired action is not performed. Thus by uncontrolled applications of active agents, loss of chemicals and environment pollution due to 'side reactions' take place. In the application of any active agent to a biosystem, a desirable regime is to release the active agent in a controlled manner and to maintain an optimum level for the required period [3].

1.2. Basic Components of Controlled Release Formulations

The components of controlled release system include (a) the active agent and (b) the polymer matrix or matrices that regulate the release of the active components.

1.2.1. Active Agents

Controlled release technology has been considered for a wide variety of applications. Broad product areas in which controlled release applications have been made are shown in Table 1.1.However, the major effort in applying controlled release principles has been in the administration of drugs/ pharmaceuticals and the application of agrochemicals [4].

In the present study two different materials namely a plant based insect repellent and urea as active agents were used.

1.2.1.1. Insect Repellents

Insect repellents are substances that protect animals, plants or products from insect attack by making food or living conditions unattractive. These substances, which may not be poisonous or only mildly toxic, are rarely, if ever, effective against all kinds insects. Repellents create a vapor shield two or three inches above the application area that confuses, thus, repels them. Insect repellents are marketed in every practicable form-aerosols, creams, lotions, suntan oils, powders, grease sticks, and cloth-impregnating laundry emulsions. Regardless of the form, the time of protection varies with the chemical, the person, the environment, the insect species, and the zeal of the insect.

Table 1.1. Broad Product Areas in Which Controlled Release has been applied

• Adhesives	• Fuels	• Perfumes
• Antifouling agents	• Growth regulators	• Photographic agents
	• Herbicides	• Pigments
• Bacteria	• Insecticides	• Plasticizers
• Blowing agents	• Repellents	• Propellants
• Catalysts	• Inks	• Solvents
• Curing agents	• Metals	• Stabilizers
• Detergents	• Monomers	• Viruses
• Drugs	• Oils	• Vitamins
• Dyes	• Paints	
• Flavours		
• Foods		

The use of insect repellent compounds dates back to antiquity, when various plant oils, smokes, tars, etc. were used to displace or kill insects. The first use of repellents goes back into the mists of time. Writings (ca 17th C) derived from the ancient Sanskrit Yoga Ratnakara also contain references to the burning of plants to repel biting insects including Vaca (*Acorus calamus*), Marica (*Piper nigrum*), asafoetidia (*Ferula asafetida*) and Nimba or Neem (*Azadiracta Indica*) . Before the Second World War, there were only four principal repellents: (1) oil of citronella, discovered in 1901, sometimes used as a hair dressing for head lice; (2) Dimethyl phthalate, discovered in 1929; (3) Indalone®, which was patented in 1937; and (4) Rutgers 612, which become available in1939. At the outbreak of World War II, the later three components were combined into a formulation

for use by the military known as 6-2-2; six parts dimethyl phthalate, two parts Indalone and two parts Rutgers 612.

Smoke is still the most widely used means of repelling mosquitoes utilized throughout the rural tropics. Waste plant materials are frequently burned in Sri Lanka as a mosquito repellent. In rural Guinea Bissau, 86% of residents used an unimpregnated bednet in conjunction with mosquito coils or plant based smoke.

Classification of Insect Repellents

a. Chemical Insect Repellents

Previously called N, N-diethyl-m-toluamide, N,N-diethyl-3- methylbenzamide (DEET) remains the gold standard of currently available insect repellents. It is a broad-spectrum repellent that is effective against mosquitoes, biting flies, chiggers, fleas, and ticks. Permethrin is both an insecticide and a repellent. It is active against a wide range of pests including lice, ticks, fleas, mites, mosquitoes, and black fries. It kills ticks on contact. Permethrin has low toxicity in mammals, is poorly absorbed by the skin, and is rapidly inactivated by ester hydrolysis. Dimethyl phthalate (dimethyl 1,2-benzenedicarboxylate) was actually the solvent in which many solid repellents were tested. They were listed among the chemicals most commonly found in insect repellents. Indalone is classified as a contact or gustatory repellent because it is only slightly volatile, and the insect must contact the treated surface before being repelled. In field study, 0.3 mg/cm^2 DEPA in alcohol provided complete protection against *Culex quinquefasciatus* mosquitoes at a mean landing rate of 9.22 mosquitoes/person/hour.

IR3535 is currently available exclusively through the Avon Corporation as Skin-So-Soft Bug Guard Plus IR3535, at concentrations of 7.5-15%. IR3535 is structurally similar to the amino acid alanine, and the EPA classified it as a biopesticide. It is labeled for use against mosquitoes, ticks, and biting flies. Data submitted by the manufacturer to the EPA revealed a protection time against mosquitoes of 2.7-4 h and protection against ticks for as long as 4 h.

b) Plant Derived Insect Repellents

Plants contain many chemicals, which are important in their defense against insects. These fall into several categories, including repellents, feeding deterrents, toxins, and growth regulators. Thousands of plants have been tested as potential sources of insect repellents. Plants whose essential oils have been reported to have repellent activity include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic, and peppermint. When tested, most of these essential oils tended to give short-lasting protection, usually less than 2 h. In terms of popularity, citronella oil from *Cymbopogan nurdus* and *C winterianus* have been reported to be the most widely used insect repellents. The lemon eucalyptus extract comes from the plant *Corymbia citriodora* (synonyms include *Eucalyptus citriodora* and *Eucalyptus maculata* var. *citriodora*) originating from China. The essential oil extract was determined to have mosquito-repelling properties. A spray type solution containing 2% α-terpinene (major component) tested for its repellent activity against *C pipiens* has been reported to show stronger repellent activity than N, N-diethyl-m- methylbenzamide (DEET). Citrosa plant *Pelargonium citrosum* ('Van Leenii'), which has been promoted as

a mosquito repellent, is claimed to be able to repel mosquitoes within a 10 ft^2 (0.93 m^2) area from oil emission. The effectiveness of the citrosa as a repellent against field populations of spring Aedes species mosquitoes has been evaluated and compared with a 75% DEET formulation. The neem tree (*Azadiracta indica*) has become a focus of attention with regard to the control of agricultural pests, and more recently against medically important insects. The repellency of neem oil to hematophagous insects has been tested, although the results have been variable. Topical application of 2% neem oil mixed in coconut oil produce varying degree of protection against different vector species and the repellent effect was more pronounced against *Anopheles spp* than against *Cx. quinquefasciatus*

Synthetic versus Natural or Herbal Repellents

o Synthetic repellent provides higher protection against mosquito bites. On the other hand, plant based or herbal repellents show lower protection time against mosquito bites.

o Synthetic repellents are of higher cost than those of plant-based repellents.

o Synthetic repellents are health hazardous whereas plant-based cause no harm to health.

o Although synthetic repellents are toxic, very low amount of it is required compared to herbal repellents which are required in higher amount to provide complete protection.

o Synthetic repellents possess unpleasant odor whereas most of the herbal repellents have pleasant odor.

o Synthetic repellents are not skin friendly as compared to herbal repellents.

o Synthetic repellents are not ecofriendly, biodegrádable whereas herbal repellents are ecofriendly and biodegradable.

Criteria of Selection of Repellents

The herbal or plant based repellents have short protection against mosquitoes compared to gold standard DEET. No repellents available to date can be termed as ideal repellent. The ideal repellent should have long-lasting effectiveness against a wide variety of arthropods; be nonirritating to the skin after topical or clothing application; be odorless or have a pleasant odor; have no effect on clothès such as staining, bleaching, or weakening of fiber but be able to withstand repeated laundering; leave no oily appearance or feel on the skin on topical application, and resist removal by wiping, washing, or sweating; be inert to commonly used plastics (e.g., eyeglass frames, pens); and be chemically stable and economically available for widespread use.

No available insect repellent meets all of these criteria.

1.2.1.2. Urea

The importance of fertilizers in agriculture is well understood erstwhile, and present agriculture production greatly depends on it. It is believed that fertilizers and pesticides are responsible for the 60% of the total agriculture production. Fertilizer consumption and production have increased several folds when compared to the nineteen fifties or sixties. Among the nitrogen (N) fertilizers, urea is the most widely used one

because of its very high N content (46%), ease of handling and comparatively low cost. It was introduced as a fertilizer in 1935, but its global acceptance and extensive use commenced only from early sixties. However, efficiency of urea in soil as per several reports is very low. Urea when applied to soil is rapidly hydrolysed to ammonium bicarbonate. This reaction is catalysed by the enzyme 'Urease' found in many species of bacteria, fungi and yeast. The ammonium bicarbonate thus produced is subsequently nitrified to nitrite and then to nitrate. The first conversion to nitrite is by the activity of 'nitrosomonas' and the subsequent conversion to nitrate is by the activity of nitrobacter species of microbes. The rapid hydrolysis of urea in soil elevates the pH around the urea granules and results in high concentration of ammonia. This situation is very conducive for ammonia volatilization. One of the major ways of urea loss is by volatilization of ammonia. The ammonia in high concentration in soil is toxic to germinating seeds. Ammonia volatilization is severe when urea is applied by surface broadcast method especially in hot countries. The loss of urea as ammonia from flooded rice fields poses very serious problems. Biological denitrification is also, a major mechanism of N-loss from rice fields that are urea fertilized. A considerable amount of applied urea-N is lost in the form of ammonia from agricultural fields. Leaching is another mechanism by which loss of urea and other ammonium forming fertilizers takes place. In such situation where an increased food production is a necessity and when that is possible only with the aid of chemical fertilizers like urea, and when serious environment problems arises out of the fertilizer application, it is the major concern of scientific community to increase the efficiency of urea like fertilizers to the environment, thus helping the farmers to produce more while sustaining the environment. Ever since the fact that low fertilizer efficiency

of urea was recognized, efforts were made to increase its use efficiency and it is still continuing.

1.2.2. Polymers in Controlled Release Technology

Polymers, both natural and synthetic, are very much useful in preparing CR formulations. Most of the drugs, fertilizers, herbicides, pesticides and pheromones are low molecular weight compounds. Tremendous progress in polymer science and technology could make it possible to combine a low molecular active agent species physically or chemically to a polymer through a pre- or post polymerization reaction. In CR technique, the active agent is allowed to release from the polymer- active agent combination over a period of time, most often to a specific target. In physical combinations, polymer acts as a rate-controlling device while in chemical combinations, it acts as a carrier for the active agent.

An important advantage of polymeric CR formulation is that the toxic natures of the chemicals are minimized. Many new pesticides are readily biodegradable and highly toxic. It poses great danger to non-target organisms also. But if is encapsulated or distributed in a polymer its toxicity will be much reduced, since the entire amount does not release at one time. Still another advantage is that the polymer combinations being solids are easy to handle [3].

The success of CR formulation relies on combining the active agent with the polymer in an economic manner, at the same time maintaining the desired release profile. These are often in opposition and one has to compromise in the ultimate cost/benefit ratio of CR formulations [2]. However there are many classes of polymers which can be

effectively employed in CR formulations. The efficiency of CR formulations depends on the following polymer properties:

- Solubility and distribution characteristics with the active agent.

- Solubility and distribution characteristics with the environmental agents.

- Compatibility with the environment should be non-toxic.

- Compatibility with the active agent should not produce undesirable products.

- Stability in the environment should not degrade during the course of action. Preferable if degraded after the desired function is over. The degraded products should not harm the environment.

- Mechanical properties should be in a suitable physical form to be easily administered to the system.

- Ease of fabrication.

- Cost.

A list of polymers that have been used in controlled release formulations is shown in Table 1.2. These polymers are used as coatings in microencapsulation, films in laminated structures, slabs in monolithic systems and flakes in many erodible devices.

Table 1.2. Polymers Used in Controlled Release Devices

Natural Polymers	Synthetic polymers	Synthetic Elastomers
• Carboxymethyl cellulose • Cellulose • Ethylcellulose • Gelatin • Gum Arabic • Starch • Bark • Methyl cellulose • Arabinogalactane • Zien • Nitrocellulose • Propylhydroxycellulose • Shellac • Proteins • Kraft lignin • Natural Rubber • Chitin and chitosan • Alginate • Guar gum • Waxes- paraffin • Carrageenan	• Polyvinyl alcohol • Polyvinyl acetate • Polyethylene • Polypropylene • Polystyrene • Polyacrylamide • Polyether • Polyamide • Polyurea • Epoxy • Ethylene vinyl acetate copolymer • Polyvinylidene chloride • Polyvinyl chloride • Polyvinyl chloride • Polyacrylate • Polyacrylonitrile • Chlorinated polyethylene • Actal copolymer • Polyurethane • Polyvinylpyrrolidone • Poly(p-xylene) • Polymethylmethacrylate	• Polybutadiene • Polyisoprene • Neoprene • Polysiloxane • Styrene-butadiene rubber • Silicone rubber • Hydrin rubber • Chloroprene • Butyl rubber • Acrylonitrile • Ethylene-propylene-diene terpolymer
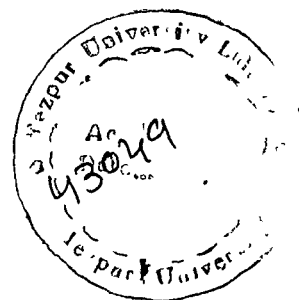
In the present study, two natural polymers namely gelatin and chitosan have been used

for preparation of controlled release systems.
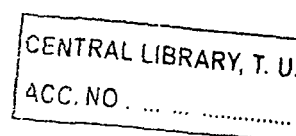
1.2.2.1. Gelatin

Gelatins are high molecular weight polypeptides derived from collagen, the primary protein component of animal connective tissues, such as bone, skin and tendon [5-7]. The name gelatin is derived from the Latin *gelatus* which means firm or frozen. Although the term gelatin is sometimes used to refer to other gel formers, it is probably applied only to the collagen derived protein materials.

Modern technological applications of gelatin depend on its high solubility in hot water, polyampholyte character, availability in a wide range of viscosities, and thermally reversible gel formation. Gelatin forms thermally reversible gels with water and the gel melting temperature (< 35°C) is below body temperature, which gives gelatin products unique organoleptic properties and flavor release. The disadvantage of gelatin is that it is derived from animal hide and bone, and hence there are problems with regard to Kosher and Halal status and vegetarians also have objections to its use. Competitive gelling agents like starch, alginate, pectin, agar, carragenan etc., are all carbohydrates from vegetable sources.

Chemistry and biochemistry of Gelatin

There are two main types of gelatin: Type A with isoionic point of 7-9 is derived from collagen with exclusively acid pretreatment. Type B with isoionic point of 4.8 to 5.2 is the result of an alkaline pretreatment of the collagen. However, gelatin is sold with a wide range of special properties like gel strength to suit particular applications. In brief,

the protein is made up of peptide triplets, glycine - X - Y, where X and Y can be any one of the amino acids but proline has a preference for the X position and hydroxyproline the Y position [8]. Approximately 1050 amino acids produce an alpha-chain with the left-handed proline helix conformation. Collagen exists in many different forms but gelatin is only derived from sources rich in Type I collagen which contains no cystine, however, hide or skin contains some Type III collagen which can be the source of traces of the traces of cystine found in some gelatins. Although Type I collagen contains no cystine, the alpha procollagen chains excreted by the cell do contain cystine at the C terminal end of the protein which is thought to be the site of assembly of 3 alpha-chains. The three chains then spontaneously [9] coil together, zipper fashion, to form a right-handed helix. After spontaneous helix formation, cross-links between chains are formed in the region of the N terminal telopeptides (globular tail portion of the chains) and then the telopeptides (containing the cystine and tyrosine of pro-collagen) are shed leaving the rod-like ca. 3150 amino acid containing triple helix. These collagen rods assemble together with a quarter-stagger to form the collagen fibre and the fibres are stabilised by further cross-links. Type A gelatin (dry and ash free) contains 18.5 % nitrogen, but due to the loss of amide groups, Type B gelatin contains only about 18 % nitrogen [10].Gelatin is abnormally stable and a special catalyst has to be used to obtain the correct Kjeldahl nitrogen content.

The amino acid composition of commercial gelatins is not routinely determined by the manufacturer and is not part of the product specifications. Gelatin has a high glycine content (33 mol%) in addition to the presence of two unusual amino acids, hydroxyproline (10 mol%) and hydroxylysine (0.5 mol%). Cysteine and tryptophan are

absent, whereas tyrosine and methionine may be present at varying levels. Tyrosine is present in the telopeptides regions, which are easily hydrolysed and lost during processing; methionine is attacked by oxidation. Typical amino acid values for Type A and Type B gelatins are given in Table 1.3. The principal nonamino acid constituents of gelatin, other than impurities, are the hexoses glucose and galactose. They are bonded covalently to hydroxylysines by glycosidic linkages and are present at 0.5 wt%.

Table 1.3. Amino acid composition of gelatins and collagen [a]

Amino acids	Gelatin Type A[*]	Gelatin Type B[**]	Collagen Type I
Alanine	112	117	114
Arginine	49	48	51
Asparagine	16	0	16
Aspartic acid	29	46	29
Cysteine	0	0	0
Glutamic acid	48	72	48
Glutamine	25	0	25
Glycine	330	335	332
Histidine	4	4.2	4.4
Hydroxyproline	91	93	104
Hydroxylysine	6.4	4.3	5.4
Isoleucine	10	11	11
Leucine	24	24.3	24
Lysine	27	28	28
Methionine	3.6	3.9	5.7
Phenylalanine	14	14	13
Proline	132	124	115
Serine	35	33	35
Threonine	18	18	17
Tryptophan	---	----	---
Tyrosine	2.6	1.2	4.4
Valine	26	22	22

[*] From acid pretreated pigskin; [**] From lime pretreated ossein

a=Residues per 1000 Residues

A typical chemical structure of gelatin is shown in Fig.1.2.

Ala   Gly   Pro   Arg   Gly   Glu   Hyp   Gly   Pro
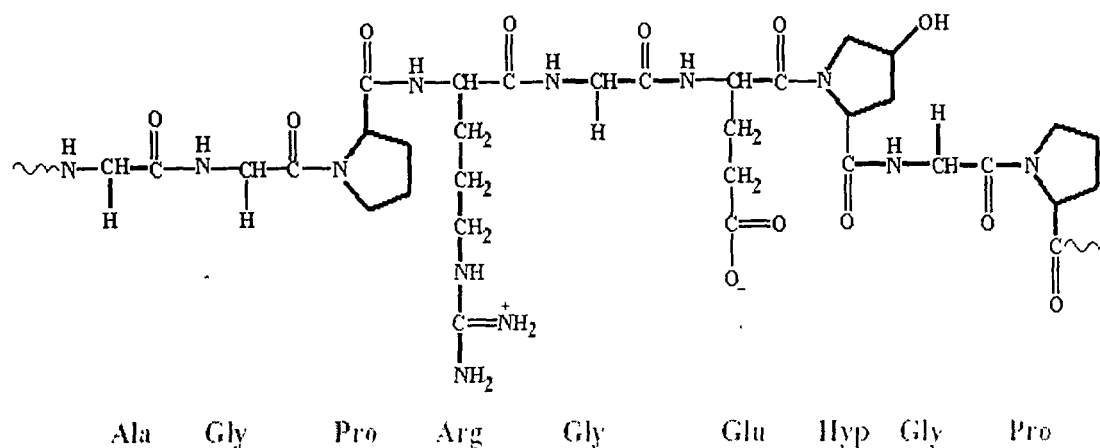
Fig.1.2. Molecular structure of gelatin

Collagen is resistant to most proteases and requires special collagenases for its enzymatic hydrolysis. Gelatin, however, is susceptible to most proteases, but they do not break gelatin down into peptides containing much less than 20 amino acids.

Cross-linking of Gelatin

The crosslinking of gelatin is used to extend the application of gelatins. In particular, treatment of gelatin films with glutaraldehyde is receiving considerable importance in order to improve their thermal resistance, decrease their solubility in water as well as their mechanical properties In Japan and Brazil, the crosslinking of gelatin using the enzyme trans-glutaminase and its use in joining gelatin to other proteins, is approved for food use.

Two fundamental methods of crosslinking have been described for gelatin: physical and chemical. Physical methods include UV irradiation and dehydrothermal

treatment, although these are inefficient and make it difficult to control the crosslinking of the gelatin matrix [11].

Chemical crosslinking agents have been categorized into two types: non-zero length and zero length. Non-zero length crosslinkers are bifunctional and polyfunctional and operate by bridging free carboxylic acid residues or amine groups between adjacent protein molecules. Examples include aldehydes (i.e., formaldehyde, glutaraldehyde, glyceraldehydes), polyepoxides, and isocyanates [11], genipin [12].

Zero length crosslinking agents activate carboxylic acid residues to react directly with amine groups on adjacent protein chains. No intervening molecules are introduced between the crosslinked residues, so this process is able to achieve gelatin matrix crosslinking without integrating foreign molecules into the network. Crosslinking agents in this category include acylazides [11], water-soluble carbodiimides [13,14].

1.2.2.2. Chitosan

Polysaccharides are distributed widely in nature with a broad range of molecular structure and properties. As understanding of their physicochemical properties and biological activities grows, interest increases. One of the most important naturally abundant and easily accessible polysaccharides is chitin (Fig.1.3). Chitin (2-acetoamido-2-dexoy-β-D-glucose) is a linear high molecular weight and crystalline polysaccharide, and like cellulose, is made of β-(1→4) linked D-glucose. Chitin is similar to cellulose in structure, but it has acetamide groups at the C-2 position in place of hydroxyl groups. Compared to cellulose, chitin has drawn less attention because of intractable bulk structure due to strong intra- and intermolecular hydrogen bonding.
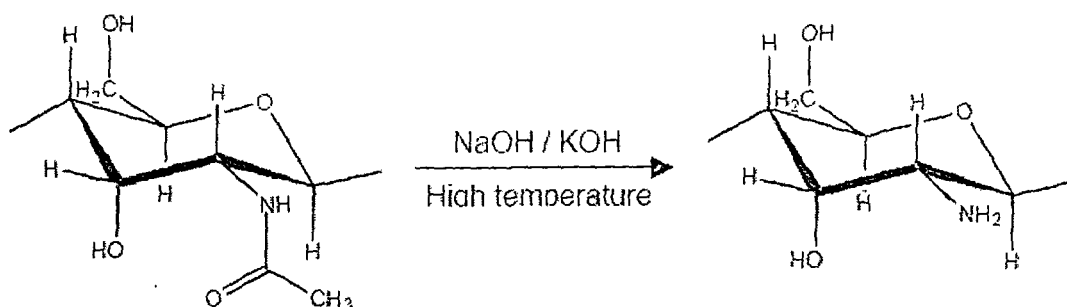
Fig. 1.3. Chemical structure of chitin (left) and chitosan (right)

Chitin is commercially produced from arthropod shells (exoskeltons) and crustacean shells, such as crabs and shrimps, which are conveniently available as waste from seafood-processing industries [15]. Two other major components of these shells are proteins and calcium carbonate. Calcium carbonate can be removed by treatment in diluted hydrochloric acid. Proteins are decomposed in sodium hydroxide solution near 100°C. Chitin remains as a residue of colorless to off-white powdery material. The N-acetyl-D-glucosamine is the basic repeating unit of chitin. Although most of the C-2 amino groups are acetylated, there are some free amine groups. Moreover, deacetylation can take place during isolation by alkaline treatment to remove proteins. Thus, chitin has different degrees of deacetylation, 0.05-0.1, depending on source and isolation mode. Three different crystalline forms (α, β, γ) of chitin have been reported. The α-chitin is the most abundant (crab and shrimp shells) and easily accessible form [16]. The α-chitin also has the most stable form of the three crystal variations. This can be explained by the molecule alignment. The X-ray studies disclosed that the α-chitin molecules are aligned in an anti-parallel fashion, which is favorable for the formation of strong intermolecular hydrogen bonding. Strong intermolecular hydrogen bonding renders α-chitin less susceptible than β- chitin to enzymatic degradation [17]. The strong intermolecular

hydrogen bonding is mainly responsible for limited solubility in common solvents. The α-chitin is soluble only in special solvents, such as N,N-dimethylacetamide containing 5-10% LiCl, hexafluoroacetone and hexafluoro-2-propanol. The solubility is generally dependent on the source of chitin. Although chitosan has been derived exclusively from α-chitin, β-chitin may be a promising alternative source of chitin. Chitosan derived from β-chitin shows the higher reactivity in modification reactions and higher affinity for solvents than that from α-chitin because β-chitin is characterized by weak intermolecular forces [16].

When chitin is treated with 40~50% aqueous alkali at a high temperature (100-160°C) for a few hours, N-acetyl groups of chitin are removed. The resulting deacetylated chitin has various degrees of deacetylation (between 70 and 95%). This considerably deacetylated chitin is called chitosan and is soluble in dilute acetic acid solution. Thus, chitosan can be considered block-type copolymers composed of N-acetyl-D-glucosamine and D-glucosamine residues linked together by β(1→4) glycosidic bonds [18,19]. Usually, chitosan has less than 40% N-acetyl-D-glucosamine. The degree of acetylation does not tend to increase by extending the reaction time. To achieve complete deacetylation, it is necessary to isolate chitosan and repeat the alkaline treatment in the same manner. However, the main chains can be depolymerized to some extent during the deacetylation, as shown by a considerable reduction in the molecular weight [20]. The degree of deacetylation and molecular weight are the most important characteristic to determine the properties of chitosan [15]. Commercially available chitosan has an average molecular weight ranging from 3,800 to 2,000,000 and degree of deacetylation from 66 to 95% [18]. Chitosan's unique characteristics make it potentially useful in a

variety of applications such as a drug carrier; for wound healing, implantation, and gene therapy because of the following advantages [21, 22].

- Chitosan is obtained from the second most abundant natural polymer chitin [23,24].

- Chitosan is nontoxic, biocompatible and biodegradable.

- Chitosan possesses more bioactivities: it has antacid and antiulcer acitivities that prevent or weaken drug irritation in the stomach [25].

- Organic solvents are not required in solubilization of chitosan.

- It is simple and easy to prepare chitosan microspheres of the desired size.

Chitosan oligomers can be prepared by cleaving the main chains with acidic or enzymatic degradation. The glycosidic linkages of chitosan are relatively stable against alkali, but cleaved with acid [20]. Hydrolysis using acids (such as hydrochloric acid and nitrous acid) is a common and fast method, but this procedure has some drawbacks (such as high cost and low yield). Enzyme degradation produces highly water-soluble low molecular weight chitosan using chitinase, chitosanase, and glucanase. This method is generally preferable over chemical treatment, because of the minimized alteration of reaction product and ease of hydrolysis control, but this process is very slow [26]. The enzymatic method is useful for preparing oligomers of a specific degree of polymerization, particularly dimer and higher ones with degrees of polymerization above 5 [20]. Other approach employs γ-ray irradiation. A rapid decrease of viscosity was observed when chitosan was irradiated in acetic acid solution. Chitosan oligomers exhibit significant physiological characteristics and functions, such as better biodegradability,

blood compatibility, antifungal activity, antitumor activity, antimicrobial activity, and immuno-enhancing activity [27].

The nitrogen content of chitosan is generally 5 to 8% depending on the degree of deacetylation. Chitosan has nitrogen mostly in the form of primary aliphatic amino groups which predominate the chemistry of chitosan. The presence of amino groups in the chitosan molecules make them suitable for further chemical modification, which in turn allow extensive adjustment of the chemical and biological properties of the chitosan.

Chitosan is insoluble in either water or organic solvents, but is soluble in most aqueous dilute acids at a pH below 6.5. Aqueous solutions such as phosphoric, sulfuric, citric, and sebacic acids are not good solvents [20]. Acetic acid has been mostly used as a standard solvent for chitosan. The extent of solubility depends on the degree of deacetylation, concentration, and type of acid and pH. Chitosans with a relatively low degree of deacetylation (40%) have been found to be soluble only up to pH 9, whereas chitosans with a degree of deacetylation of about 85% have been found to be soluble only up to a pH of 6.5 (pKa 6.5) [19,24]. Solubility diminishes with increasing concentration of acid [20]. Upon dissolution, the amine groups of the polymers are protonated and the resultant soluble polysaccharide is positively charged. One of the interesting properties of chitosan is the ability to bind to negatively charged surfaces like mucosal membranes, to act as a bioadhesive molecule. The polycationic character and ability to interact with negatively charged molecules have drawn many researchers to study chitosan particles. Chemical as well as biological properties of chitosan are listed in the table 1.4 below:

Table 1.4. Chemical and biological properties of chitosan

Chemical properties of chitosan	Biological properties of chitosan
- Cationic polyamine - High charge density at pH<6.5; - Adheres to negatively charged surfaces; - Forms gels with polyanions ; - High molecular weight linear polyelectrolyte; - Viscosity ranges from high to low ; - Form chelates with certain transition metals; - Amiable for chemical modification ; - Highly reactive amine/hydroxyl groups.	- Biocompatible; - Natural polymer; - Biodegradable to normal body constituents; - Safe and non-toxic; - Haemostatic, bacteriostatic and fungistatic; - Spermicidal ; - Anticarcinogen; - Anticholestermic; - Reasonable cost; - Versatile.

Adapted from reference [28].

Cross-linking of Chitosan

Crosslinking of chitosan is required not only to improve its mechanical properties but also to control its control release behaviour when used as shell materials. A large number of cross-linking agents are known to able to cross-link chitosan. Chemical cross-linking of chitosan achieved by treatment with aldehydes such as formaldehyde, glutaraldehyde and glyceraldehydes is well known and are reported in most of the literature [29-32]. The cross-linking with dialdehydes occurred in mild conditions without any auxiliary additive. The main drawback of dialdehyde crosslinkers is that they are suspected to be toxic in human body [33]. Other chemical crosslinking agents include glyoxal [32,34], epichlorohydrin [30], sulfuric acid [29], sodium hexametaphosphate

[32], sodium tripolyphosphate [35], diisocynate, carbodiimides etc. All these chemical crosslinking agents are relatively cytotoxic. Recently, biocompatible cross-linking agents have received much interesting in the field of biomedical application. For example, enzyme-catalyzed cross-linking methods were developed to cross-link protein-based biomaterials [36-38].

Transglutaminase and tyrosinase catalyze the glutamine and tyrosine residues in protein to link with the amino group of a lysine residue. However, it could not catalyze the cross-linking reaction of biopolymers lacking of glutamine and tyrosine residues. Genipin, a natural crosslinking agent, whose cytotoxicity, feasibility and biocompatibility have well been studied are reported [22, 39]. Genipin can be obtained from its parent compound geniposide, which may be isolated from gardenia fruits. It has been reported that genipin can spontaneously react with amino acids or proteins to form dark blue pigments [40-42]. It is 10,000 times less toxic than glutaraldehyde [39]. Generalized cross-linking reactions of gelatin and chitosan with different cross-linking agents are shown in Fig.1.4 below:

Polymer —NH$_2$ + CH$_2$—CH— (Epoxide) $\longrightarrow$ Polymer—NH—CH$_2$—CH—
                                                                            |
                                                                           OH

Epoxide

Polymer —COOH + CH$_2$—CH— (Epoxide) $\longrightarrow$ Polymer—OCO—CH$_2$—CH—
                                                                            |
                                                                           OH

Epoxide

Polymer —OH + CH$_2$—CH— (Epoxide) $\longrightarrow$ Polymer—O——CH$_2$—CH—
                                                                         |
                                                                        OH

Epoxide

Polymer —OH + O=C=N— $\longrightarrow$ Polymer—OCONH—

Isocyanate

Polymer —COOH + O=C=N— $\longrightarrow$ Polymer—CO—NH—

Isocyanate

2 Polymer —NH$_2$ + HCO (CH$_2$)$_3$ CHO $\longrightarrow$ Polymer—NH—CO (CH$_2$)$_3$ OCNH—Polymer

Glutaraldehyde

2 Polymer —OH + CH$_2$—CHCH$_2$Cl $\longrightarrow$ Polymer—O——CH$_2$ CH CH$_2$O—Polymer
                                                                            |
                                                                           OH

Epichlorohydrin

Polymer —NH$_2$ + OHC—CHO $\longrightarrow$ Polymer—N=CH—CH—
                                                                |
                                                               OH

Glyoxal

Fig.1.4. Cross-linking reactions of different functional group containing polymers (gelatin and chitosan) with various cross-linkers

1.3. Advantages and Disadvantages of Controlled Release Technology

1.3.1. Advantages of Controlled Release Technology

Controlled release technology offers advantages [43, 44] over conventional

formulations. These can be summarized as follows:

♦ Maintain of constant level of active agent: In conventional formulation, the active

agent tends to release first at an overdose then undergoes to the local

environment. Controlled releases offer a solution for this problem by maintaining

the concentration of active agent between the minimum effect and toxic level

(Figure.1.1).

♦ Use of smaller dose: This includes more efficient utilization of active agents,

resource saving, safety etc.

♦ Reduces the loss by limiting leaching, volatilization and degradation.

♦ Minimizes potential negative effect (if there any) associated with overdose.

♦ Economical because less active material is needed due to reduction of excessive

amounts for a given time interval.

♦ Facilitation of handling and masking of any odor.

♦ Toxic material becomes chemically nontoxic when attached with polymers.

♦ Extention of the duration of the activity of less-persistent or non-persistent

biocides unstable within the aquatic environment by protecting them from

leaching and degradation, hence aiding the practical applications of these

materials.

♦ Reduction of phytotoxicity by lowering the high mobility of the biocide in soil, hence reducing its residue in the food chain.

♦ Convenience: it converts liquids to solids; hence it results in easily transported materials with the reduction of flammability.

1.3.2. Disadvantages of controlled release technology

Though the advantages of controlled release are impressive, the merits of each application have to be examined individually, and the positive and negative effects weighed carefully before large expenditures for developmental work are committed. In other words, controlled release is not a panacea, and negative effects may, at times, more than offset advantages. Some of the disadvantages of controlled release or the areas that require a thorough appraisal include [4].

● The cost of controlled release preparation and processing is substantially higher than the cost of standard formulations, but this could be compensated by the fact that there is no need for repeated applications.

● The fate of using excessive amounts of polymers as matrix and its effect on the environment is very important, but this could be eliminated by using biodegradable polymers and improving weight efficiency by using polymers that may be beneficial to soil and crop growth when degraded.

● The fate of polymer additives, such as plasticizers, stabilizers, antioxidants, fillers, etc. left behind, once application is over, may cause some impact on environment.

- The environmental impact of the polymer degradation products following heat, hydrolysis, oxidation, solar radiation and biological degradation.The cost, time, and probability of success in securing government registration of the product.

1.4. Types of Controlled Release Systems

Polymer controlled release formulations are divided into two broad categories, physical and chemical combinations. In physical combination, the polymer acts as a rate-controlling device while in chemical combination, it acts as a carrier for the active agent. The choice of the best system to release the active agent in sufficient quantity to achieve the desired effect with minimum biological or ecological side effects depends on many considerations. These include the properties of the active agent, its physicochemical interactions with the polymer; the polymer nature (cross-linking degree, thermal behavior, compatibility with the active agent); stability of the combination during processing; desired release rate, shape and size of the final product; duration, seasonal conditions; cost and ease of formulation and application.

1.4.1. Physical Combinations

Two different approaches have been reported in literature in the case of the physical combination of biologically active agent with polymeric materials. Firstly, the biologically active agent can be encapsulated in a polymeric material in which the release of the active agent is controlled by Fick's law of diffusion through the micropores in the capsule walls. Fick's law of diffusion states that the rate of diffusion (R_d) depends on the

dimensional factors (A, h), which involve the geometry or dimensions of the device, and the diffusional factors (D, C_s, K, C_e), which involve active agent-polymer interaction

$$R_d = dM_t/dt = A/h \ D \ (C_s - KC_e)$$

Where M_t is the mass of the agent released, dM_t/dt is the steady state release rate at time t, A and h are the surface area and thickness through which diffusion occurs, D is the diffusion coefficient of the active agent in the polymer, Cs is the saturation solubility of the active agent in the polymeric membrane, K is the partition coefficient of the active agent and the medium which surrounds the device, Ce is the concentration of released active agent in the environment.

In the second approach, biologically active agent is heterogeneously dispersed or dissolved in a solid polymeric matrix, which can be either biodegradable or non-biodegradable. The release of the active agent is generally controlled by diffusion and erosion. Release by erosion is a surface area dependent phenomenon, and the general expression which describes the rate of release (R_r) by an erosion mechanism is:

$$R_r = dM/dt = K_e C_o A$$

Where Ke is the erosion rate constant; A is the surface area exposed to the environment; Co is the loading of active agent in the erodible matrix. The design of such physical combinations is generally not influenced by the structure of the active agent

molecule. It is also not strongly influenced by the structure of the polymer matrix. However, the polymer should fulfill the following requirements.

♦ compatibility with the active agent so that it can be readily dissolved or dispersed in the polymer, and so that there are no undesirable reactions or physical interactions

♦ low softening point is desirable in order to prevent thermal degradation of the active agent during mixing of an agent with the molten polymer

♦ low crystallinity in the polymer to avoid the alteration of the release rate by the highly ordered structure

♦ polymers must be mechanically stable , easy to fabricate and of low cost.

1.4.2. Chemical Combinations

An active agent can be chemically attached to a polymer either as a pendant side groups, or as part of the main backbone. Obviously only those biologically active agents that contain a structural moiety with at least one reactive functional group suitable for use as link to the functionalized polymer, can be used in this technique.

Polymeric chemically bonded active agents can be prepared by two synthetic methods. The first involves chemical modification of a preformed polymer with the desired active agent via a chemical bond, leading to a polymer having the active species linked to the main chain as a pendant group.

$$-\left(-R\underset{Z}{-}\right)_{n}$$
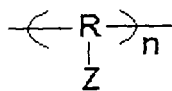
Z= active agent, R= monomer unit -

Fig 1.5. Active agent attached to polymer as side chain

The second method requires synthesis and polymerization of a biologically active monomer which leads either to polymer having the active group as repeat units in the main backbone.

$$-\left(-R-Z\right)_{n}$$
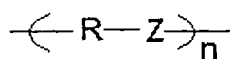
Fig.1.6. Active agent in the polymer backbone

The chemically attached active agent is released from the polymer by the hydrolytic cleavage of the active agent-polymer linkage or via a slow degradation of the polymer itself induced by the water in the surrounding environment. The kinetic expressions which describe the release rate depend on the extent of branching and crosslinking of the macromolecules, i.e., on whether the cleavage reaction occurs on the surface of an insoluble particle or in the matrix. The efficiency and duration of the action are influenced by several factors.

o   The chemical characteristics of the structure of the active agent.

o   The strength and type of the active agent-polymer bond.

o   Polymer microstructure: the structures and dimensions of the macromolecule as governed by the following:

    (a) The chemical nature of the backbone: a nondegrading polymer could maintain its activity for a long period.

(b) The chemical nature of the neighboring groups surrounding the active moieties: incorporation of hydrophobic comonomer offers protection against rapid hydrolysis and results in shortening the protection period.

(c) Stereochemistry: crystalline polymer is less susceptible to hydrolytic attack than amorphous polymers.

(d) The degree of crosslinking, porosity, particle size and swelling. a linear polymer is more susceptible to hydrolysis than a crosslinked one. Increasing the diffusion rate and swelling of the polymer accelerate the hydrolysis.

(e) Spacer groups: polymers in which the active agent is bonded directly to the polymeric backbone exhibit slow rates of hydrolysis. Increasing the distance between the biocide and the main chain by extending the pendant chain length would enhance the rate of hydrolysis since the linkage bond would be removed from the hydrophobic backbone and less sterically hindered.

(f) The degree of polymerization.

o   Release conditions:

(a) Surrounding environmental conditions that break the linkages via chemical attack (hydrolytic by moisture; thermal / photo by sunlight) or biological degradation (enzymatic by microorganisms).

(b) pH of the medium.

(c) Ionic strength of the dissolution medium.

(d) Competing ions;

(e) Electrolyte concentration

(f) Temperature.

1.5. Classification of CR systems Based on Release Mechanisms

The sought-after release profile,from a CR system is the steady state release of active agent or a zero-order release mechanism kinetically. The attraction of such a system is that the rate of release is not affected by the amount of active agent released or not released at any moment [2]. However, many of the CR formulations do not fall under this category. Depending on the rate-controlling mechanism involved; the CR systems can be classified into several classes. According to Fan and Singh [1] the major release mechanisms involved in CR formulations are:

i)   Diffusion
ii)   Erosion or Chemical reaction controlled
iii)   Swelling
iv)   Osmosis

i) Diffusion-Controlled Systems

Here the rate-determining step is the diffusion of active agent through the polymer. The polymer-environment fluid interaction is practically nil, or polymer is seldom affected by the environment factors. Mainly two categories of diffusion-controlled devices are employed in CR formulations.

a) Reservoir Systems

Here the active agent and polymer are physically combined or active agent is encapsulated in a spherical or cylindrical polymeric device. Micro-, macro- or nanoencapsulation coacervation and spray encapsulation are well-developed techniques and are employed in drug delivery systems [45]. Active agents releases out to the environment by diffusion, through the micropores of the capsule walls. Ethylene vinyl acetate co-polymers, silicone rubber, polyethylene and polyurethane are commonly used to fabricate such systems [1].

b) Monolithic Systems

Here the active agent is either dispersed heterogeneously or dissolved in the polymer. The polymer can be biodegradable or non-biodegradable. The dissolved or dispersed active agent releases out by diffusion. If interaction is possible between polymer and environment fluid, release may takes place by leaching also in addition to diffusion. If a soluble additive is incorporated in the polymer matrix, the environmental fluid can easily penetrate the matrix by dissolving the additive and interconnected channels will be formed, through which the release would be easy. These types of physical combinations need not be influenced by structure of the active agent or polymer. Hence this technique has a great applicability. A wide variety of active agents can be incorporated with a broad range of polymers to prepare CR formulations. Polymer matrix of silicone rubber, ethyl cellulose and hydroxyl propyl cellulose are widely used in this category [1, 12].

ii) Erosion or Chemical reaction controlled system

Erosion-Controlled System

The active agent is physically immobilized in the polymer matrix and release occurs only by erosion of the polymer. In an ideal system polymer matrix undergo surface erosion, releasing active agent at a rate proportional to the erosion rate. If erosion rate is constant and the matrix dimension remains unchanged a zero order release can be achieved. Poly (vinyl pyrrolidone) and coplymers of lactic and glycolic acids are used in preparing such systems [1].

Chemical Reaction Controlled System

From these chemical combinations, active agents are released only when the polymer active agent bond is cleaved, otherwise the polymer is to be degraded. When the active agent is a co-monomeric unit in polymer backbone, release may occur by polymer degradation and in such cases the release follow zero order kinetics.

iii) Swelling CR System

The active agent is dispersed or dissolved in a polymer matrix, in which it is unable to diffuse to any considerable extent. When the polymer gets into contact with an environment fluid which is compatible with it, swelling takes place and active agent present in the gel portion of the matrix diffuse out. Poly(hydroxyl methylmethacrylate), polyacrylamide and poly(ethylene glycols) are used in such systems [46].

iv) Osmosis Controlled System

In this type of devices the driving force is the osmotic force. Usually such systems consist of a solid and water-soluble active agent, which is enclosed by a water-permeable, but active agent impermeable polymer membrane with a small opening. Water is transported into the core by permeation and hydrostatic pressure will be built up in the core and subsequently, the dissolved active agent comes out [1].

Apart from these common techniques, new concepts such as magnetic or ultra sound modulation, viable cell immobilization, microspheres and nanoparticles and targeted delivery are under thorough research in the CR field [1,47-48].

1.6. Manufacturing Techniques of Controlled Release Formulations

There are several techniques for the preparation of controlled release formulations. Among them the most widely used techniques are discussed below:

1.6.1. Chemically bound

Chemically bound active agents are of two types:

(a) Those which are prepared by attaching a polymerizable site to the active ingredient, followed by polymerization of the new derivative. For example,
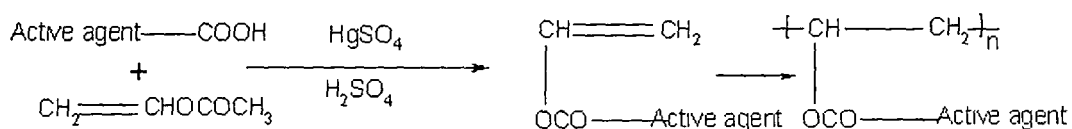
(b) Those which are prepared by chemically binding derivatives of active ingredients to a suitable polymer. For example, active agents containing carboxylic functionalities have been reacted to form acid chlorides, which in turn were attached through hydroxyl groups of natural polymers. Active agent containing primary amino functionality were reacted with phosgene to form isocyanates, which in turn were attached through the hydroxyl group of natural polymers:

Active agent——COOH
+ ————————→Active agent—COCl $\xrightarrow{\text{Cellulose}}$ Cellulose—OCO—Active agent
SOCl$_2$

Active agent——NH$_2$
+ ————————→Active agent—NCO $\xrightarrow{\text{Cellulose}}$ Cellulose—OCONH—Active agent
COCl$_2$
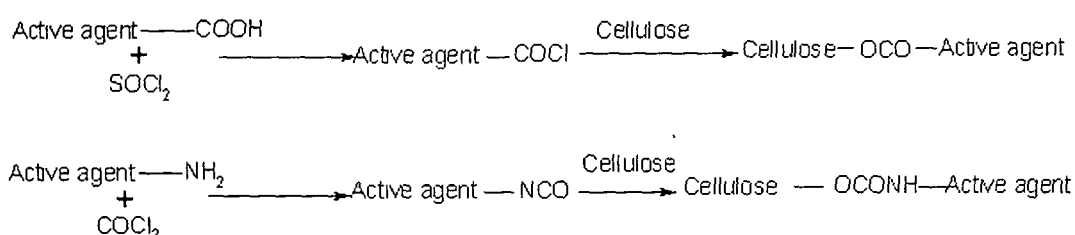
Such chemically bound combinations have found application in forestry and agronomic crops. The rate of release can be increased by lowering the molecular weight or increasing the hydrophilicity of the polymer carrier. The rate of release also depends upon the degree of substitution of the herbicide moiety within the polymer, the pH of the hydrolysis medium and the size of the particles.

1.6.2. Microencapsulation

Microencapsulation is the coating of small solid particles, liquid droplets, or gas bubbles with a thin film of coating or shell materials. The product so obtained is termed as microcapsules. Microcapsules are small particles that contain an active agent or core material surrounded by a coating or shell. At present, there is no universally accepted size range that particles must have in order to be classified as microcapsules. However, many workers classify capsules smaller than 1 μm as nanoparticles and greater than 1000μm as

macrocapsules. Commercial microcapsules typically have a diameter between 3 and 800 μm and contain 10-90 wt% core. A wide range of core materials has been encapsulated. including adhesives, agrochemicals, live cells, active enzymes, flavors, fragnances, pharmaceuticals and inks. Most capsule shell materials are organic polymers, but fats and waxes are also used.

(a)   (b)
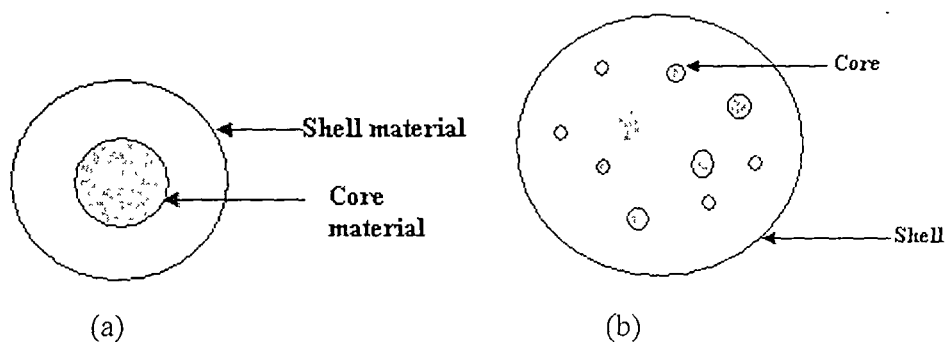
Fig.1.7.Schematic diagrams of two representative types of microcapsules: (a) Continuous core/shell microcapsule; (b) Multinuclear microcapsules [Courtesy of C.Thies]

Microcapsules can have a variety of structures. Some have a spherical geometry with a continuous shell as shown in Fig.1.7 (a). Others have an irregular geometry and contain a number of small droplets or particles of core material Fig.1.7 (b).

The morphology of microcapsules depends mainly on the core material and the deposition process of the shell. Microcapsules may have regular or irregular shapes and, on the basis of their morphology, can be classified as. mononuclear, polynuclear, and matrix types (Fig.1.8) [49].
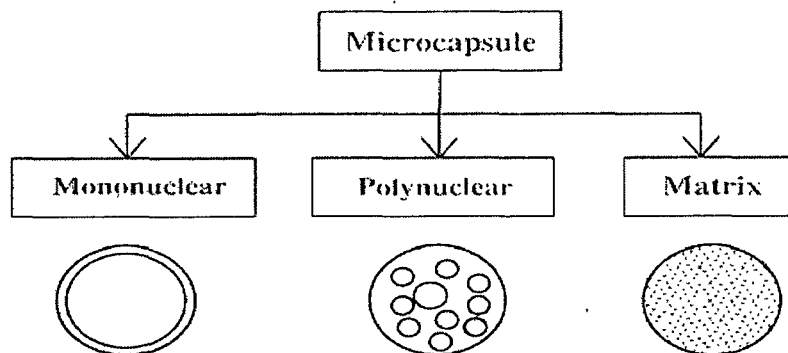
Fig.1.8. Morphology of microcapsules

Microcapsules have a number of interesting advantages, and the main reasons for microencapsulation can be summarized as follows:

- Protection of unstable, sensitive materials from their environments prior to use.

- Better processability (improving solubility, dispersibility, flowability, etc.)

- Self-life enhancement by preventing degradative reactions (oxidation, dehydration).

- Controlled, sustained, or timed release.

- Safe and convenient handling of toxic materials.

- Masking of odor or taste.

- Enzyme and microorganism immobilization.

- Controlled and targeted drug delivery.

- Handling liquids as solids.

1.6.2.1. Microencapsulation Techniques

Numerous preparation technologies available for the encapsulation of core material have been reported [50-53]. The present discussion focuses on the different microencapsulation techniques that are more relevant to the coating industries, and also provides a comprehensive review of recently developed methods. In general, microencapsulation techniques are divided into two basic groups, namely chemical and physical, with the latter being further subdivided into physico-chemical and physico-mechanical techniques. Some of the important processes used for microencapsulation are summarized in Table 1.5.

Table 1.5. Different techniques used for microencapsulation.

Chemical processes	Physical processes	
	Physico-chemical	Physico-mechanical
• Suspension, dispersion and emulsion polymerization	• Coacervation	• Spray-drying
	• Layer-by-layer (L-B-L) assembly	• Multiple nozzle spraying
• Polycondensation	• Sol-gel encapsulation	• Fluid-bed coating
	• Supercritical CO₂-assisted microencapsulation	• Centrifugal techniques
		• Vacuum encapsulation
		• Electrostatic encapsulation

Chemical Processes

Suspension, emulsion, dispersion or precipitation polymerizations, *in situ* and interfacial polymerization are the most used chemical techniques for microencapsulation [54-59]. Among them suspension, emulsion and precipitation polymerization techniques

are well known among the polymer chemist which are carried out by dispersing or emulsifying the active agents during polymerization process.

Interfacial and In Situ Polymerization

Many types of polymerization reactions can be made to occur at the interfaces, or produce polymers that concentrate at interface, thereby producing microcapsules. Accordingly, this approach to encapsulation has steadily developed into a versatile family of encapsulation processes. A unique feature of *in situ* encapsulation technology is that polymerization occurs in the aqueous phase, thereby producing a condensation product that deposits on the surface of the dispersed core material where polymerization continues. This ultimately produces a water-insoluble, highly crosslinked capsule shell.

Physico-chemical Processes
Coacervation

IUPAC defined coacervation as: "The separation into two liquid phases in colloidal systems. The phase more concentrated in colloid component is the coacervate, and the other phase is the equilibrium solution." The first systematic approach of phase separation, that is, partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium) was realized by Bungenberg and colleagues [61,62]. These authors termed such a phase separation phenomenon "coacervation". The term originated from the Latin ›acervus‹ , meaning "heap". This was the first reported process to be adapted for the industrial production of microcapsules.

Currently, two methods for coacervation are available, namely simple and complex processes. The mechanism of microcapsule formation for both processes is identical, except for the way in which the phase separation is carried out. In simple coacervation a desolvation agent is added for phase separation, where as complex coacervation involves complexation between two oppositely charged polymers.

(a) Simple Coacervation

Aqueous solutions of water-soluble polymers are phase separated in aqueous media when sufficient salt is added to such solutions. This phenomenon is called simple coacervation. As long as phase separation produces a liquid polymer-rich phase, simple coacervation can be used to produce microcapsules [62]. Microcapsules with gelatin, or poly(vinyl alcohol) or different natural polymers shell have been produced in this manner.

(b) Complex coacervation

Complex coacervation occurs in aqueous media and is used to encapsulate water-immiscible liquids or water-insoluble solids [49]. Complex coacervation is carried out by mixing two oppositely charged polymers in a solvent (usually water); the process is shown schematically in Fig.1.9.

(a)

Core material

Coacervate particle

(b)

(d)

(c)
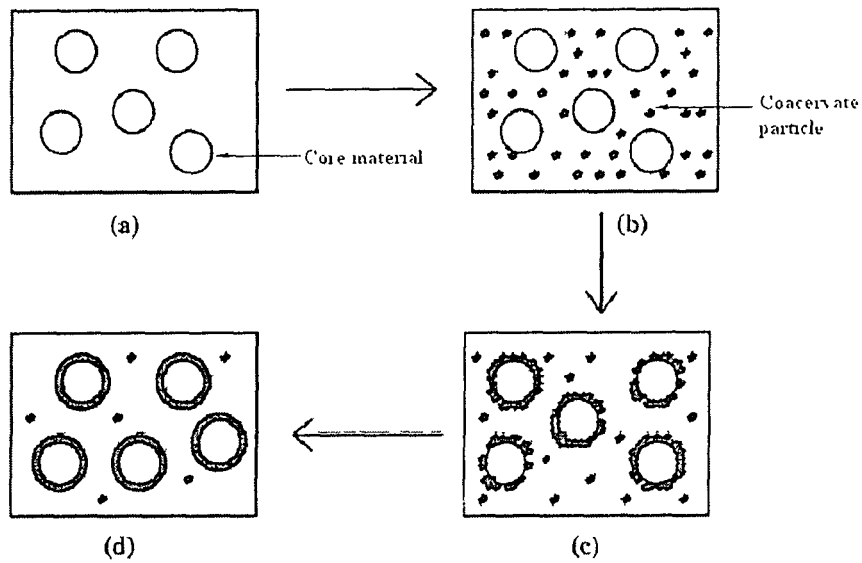
Fig.1.9. Schematic representation of complex coacervation process. (a) Core material dispersion in shell polymer solution; (b) separation of coacervate from solution, (c) coating of core material by microdroplet of coacervate; (d) coalescence of coacervate to form continuous shell around core particles.

The three basic steps in complex coacervation are: (i) preparation of the dispersion or emulsion; (ii) encapsulation of the core; and (iii) stabilization of the encapsulated particle. The core material (usually an oil) is first dispersed into a polymer solution (e.g., a cationic aqueous polymer). The second polymer (anionic, watersoluble) solution is then added to the prepared dispersion. Deposition of the shell material onto the core particles occurs when the two polymers form a complex. This process is triggered by the addition of salt or by changing the pH, temperature or by dilution of the medium. The shell thickness can be obtained as desired by controlled addition of the second polymer Finally, the prepared microcapsules are stabilized by crosslinking, desolvation or thermal

treatment. Complex coacervation is used to produce microcapsules containing fragrant oils, liquid crystals, flavors, dyes or inks as the core material. Porous microcapsules can also be prepared using this technique. When using this technique, certain conditions must be met to avoid agglomeration of the prepared capsules [63].

Figure 1.10 outlines one version of a complex coacervation encapsulation process. The first step is to disperse the core material in an aqueous gelatin solution. This is normally done at 40-60°C, a temperature range at which the gelatin forms a clear solution. After that, a polyanion or a negatively charged polymer like gum arabic added to the system, the pH and concentration of polymer are adjusted so that a liquid complex coaervate forms. The pH at which this occurs is typically between 4.0 and 4.5. Once the liquid coacervate forms, the system is cooled to room temperature. The gelatin in the coacervate gels thereby forming capsules with a very rubbery shell. In order to increase the strength of the water-swollen shell and create a gel structure that is not thermally reversible, the capsules normally are further cooled to approximately 10°C and treated with glutaraldehyde. The glutaraldehyde crosslinks the gelatin by reacting with amino groups located on the gelatin chain. Glutaraldehyde treated capsules can be dried to a free-flow powder or coated on a substrate and dried.

```
┌─────────────────────────┐                    ┌─────────────────────────┐
│  Water-immiscible liquid │                    │ Aqueous gelatin solution│
└─────────────────────────┘                    │      ( 40 - 60 C )      │
            │                                   └─────────────────────────┘
            │                                              │
            │              ┌─────────┐                     │
            └─────────────►│  Mixer  │◄────────────────────┘
                           └─────────┘
                                │
                                ▼
                    ┌─────────────────────┐
                    │ Oil-in-water emulsion│
                    └─────────────────────┘
                                │                 ┌──────────────────────────┐
                                │◄────────────────│ Aqueous gum arabic solution│
                                │                 │        ( 40-60 C )        │
                                ▼                 └──────────────────────────┘
┌─────────────────┐      ┌─────────┐
│ Water(40 -60 C) │─────►│  Mixer  │
└─────────────────┘      └─────────┘      ┌─────────────────────┐
                                │◄─────────│  Adjust pH ( 4 0 - 4 5) │
                                │          └─────────────────────┘
                                ▼
        ┌───────────────────────────────────────┐
        │ Coacervation occurs  embryo capsules form│
        └───────────────────────────────────────┘
                                │
                                ▼
                  ┌─────────────────────────┐
                  │  Cool to gel coacervate │
                  └─────────────────────────┘
                                │              ┌─────────────────────────────┐
                                │◄─────────────│ Glutaraldehyde or formaldehyde│
                                ▼              └─────────────────────────────┘
                  ┌─────────────────────────┐
                  │ Cross-linked microcapsules│
                  └─────────────────────────┘
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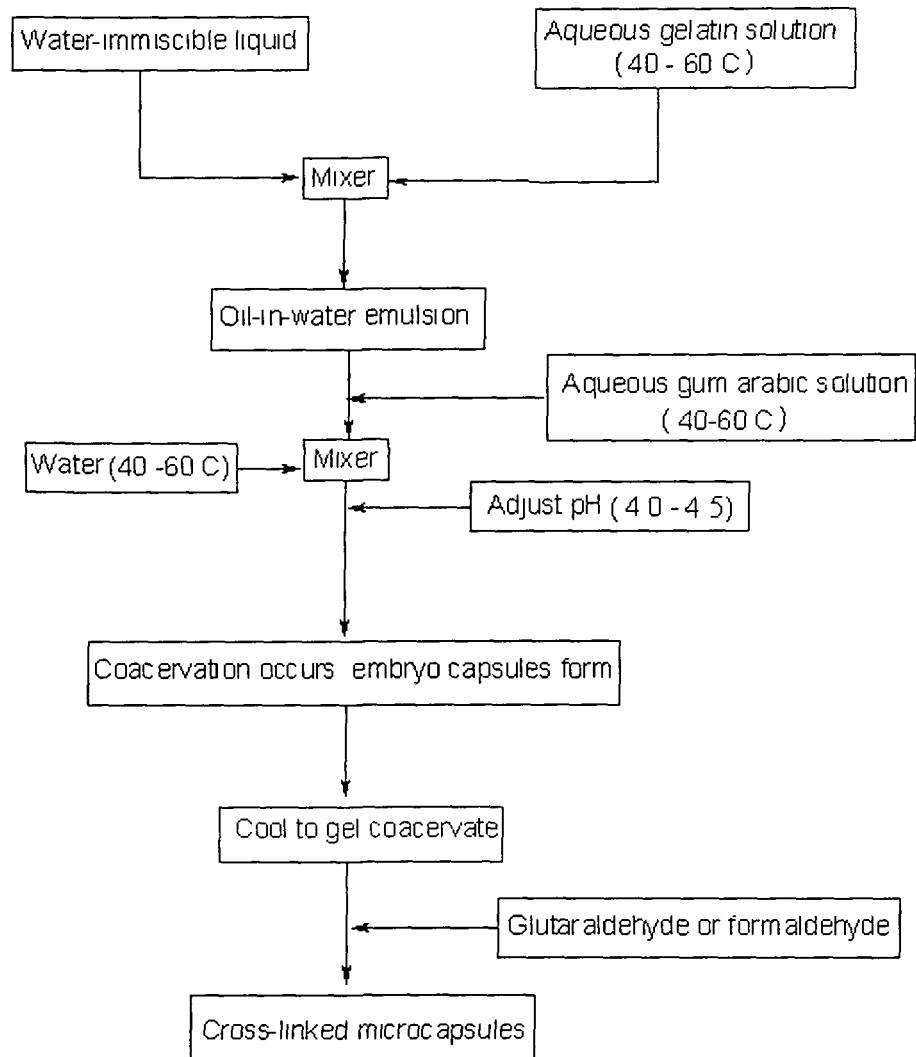
Fig.1.10.Typical encapsulation process based on the complex coacervation of gelatin with gum arabic.

Polymer-polymer Incompatibility

Polymer-polymer incompatibility occurs because two chemically different polymers dissolved in a common solvent are incompatible and do not mix in solution. They essentially repel each other and form two distinct liquid phases. One phase is rich in

polymer designed to act as the capsule shell. The other is rich in the second incompatible polymer. The incompatible polymer is present in the system to cause formation of two phases. It is not designed to be part of the final capsule shell, although small amounts may remain entrapped in the final capsule as an impurity. This type of encapsulation process generally does not involve any chemical reaction.

Solvent Evaporation

This encapsulation technology involves removing a volatile solvent from an oil-in-water, or water-in-oil, or water-in-oil-in-water emulsion [62,64]. In most cases, the shell material is dissolved in a volatile solvent, such as methylene chloride or ethyl acetate. The active agent to be encapsulated is dissolved, dispersed, or emulsified into this solution. Water-soluble core materials like hormonal polypeptides are dissolved in water that contains a thickening agent before dispersion in the volatile solvent phase that contains the shell material. The dispersed aqueous phase is gelled thermally to entrap the polypeptide in the dispersed aqueous phase before solvent evaporation occurs.

Once the active agent is dispersed in the volatile solvent phase, the dispersion produced is typically emulsified into an aqueous phase that contains a dispersing agent. The solvent used to dissolve the shell material is subsequently removed from this emulsion at atmospheric or reduced pressure. Significantly solvent evaporation processes can be used on nonaqueous media. In such cases, a volatile organic solvent is removed by evaporation from an organic solvent that has low volatility.

Encapsulation by Rapid Expansion of Supercritical Fluids

Supercritical fluids are highly compressed gasses that possess several advantageous properties of both liquids and gases. These fluids have attracted much attention in recent years; the most widely used being supercritical CO_2, alkanes (C2 to C4), and nitrous oxide (N_2O). They have low hydrocarbon-like solubility for most solutes and are miscible with common gases such as hydrogen (H_2) and nitrogen (N_2). A small change in temperature or pressure causes a large change in the density of supercritical fluids near the critical point – a property which enhances their use in several industrial applications. Supercritical CO_2 is widely used for its low critical temperature value, in addition to its nontoxic, nonflammable properties; it is also readily available, highly pure and cost-effective. It has found applications in encapsulating active ingredients by polymers. Different core materials such as pesticides, pigments, pharmaceutical ingredients, vitamins, flavors, and dyes are encapsulated using this method [65-67]. A wide variety of shell materials that either dissolve (paraffin wax, acrylates, polyethylene glycol) or do not dissolve (proteins, polysaccharides) in supercritical CO_2 are used for encapsulating core substances. The most widely used methods are as follows:

a) Rapid expansion of supercritical solution (RESS)

In this process, supercritical fluid containing the active ingredient and the shell material are maintained at high pressure and then released at atmospheric pressure through a small nozzle. The sudden drop in pressure causes desolvation of the shell material, which is then deposited around the active ingredient (core) and forms a coating

layer. The disadvantage of this process is that both the active ingredient and the shell material must be very soluble in supercritical fluids. In general, very few polymers with low cohesive energy densities (e.g., polydimethylsiloxanes, polymethacrylates) are soluble in supercritical fluids such as CO_2. The solubility of polymers can be enhanced by using co-solvents. In some cases nonsolvents are used; this increases the solubility in supercritical fluids, but the shell materials do not dissolve at atmospheric pressure. A schematic of the microencapsulation process using supercritical CO_2 is shown in Fig. 1.11.
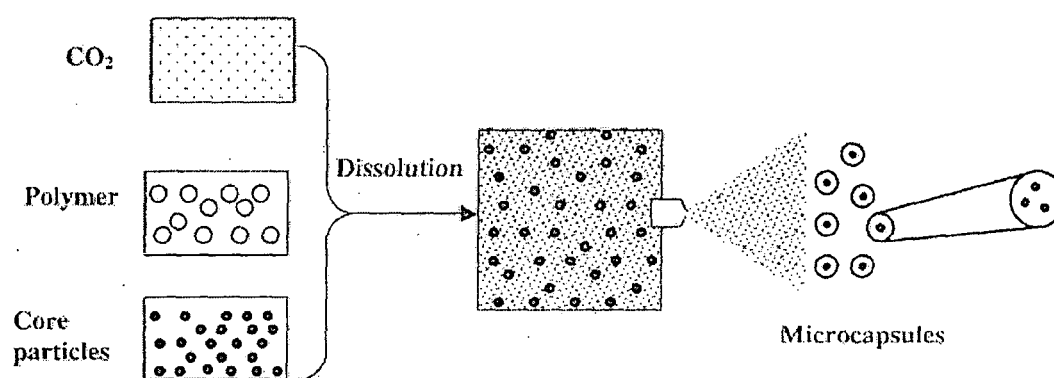
Figure.1.11.Microencapsulation by Rapid Expansion of supercritical solutions (RESS)

b) Gas anti-solvent (GAS) process

This process is also called supercritical fluid anti-solvent (SAS). Here, supercritical fluid is added to a solution of shell material and the active ingredients are maintained at high pressure. This leads to a volume expansion of the solution that causes supersaturation such that precipitation of the solute occurs. Thus, the solute must be soluble in the liquid solvent, but should not dissolve in the mixture of solvent and supercritical fluid. On the other hand, the liquid solvent must be miscible with the

supercritical fluid. This process is unsuitable for the encapsulation of water-soluble ingredients as water has low solubility in supercritical fluids. It is also possible to produce submicron particles using this method.

c) Particles from a gas-saturated solution (PGSS)

This process is carried out by mixing core and shell materials in supercritical fluid at high pressure. During this process supercritical fluid penetrates the shell material, causing swelling. When the mixture is heated above the glass transition temperature (Tg), the polymer liquefies. Upon releasing the pressure, the shell material is allowed to deposit onto the active ingredient. In this process, the core and shell materials may not be soluble in the supercritical fluid.

Within the pharmaceutical industry, preformed microparticles are often used for the entrapment of active materials using supercritical fluids under pressure. When the pressure is released, the microparticles shrink and return to their original shape and entrap the ingredients.

Physico-Mechanical Processes

Co-extrusion

The co-extrusion process was developed by Southwest Research Institute in the United States, and has found a number of commercial applications. A dual fluid stream of liquid core and shell materials is pumped through concentric tubes and forms droplets under the influence of vibration (Fig.1.12). Chemical cross-linking, cooling, or solvent

evaporation then hardens the shell. Different types of extrusion nozzles have been developed in order to optimize the process [67]
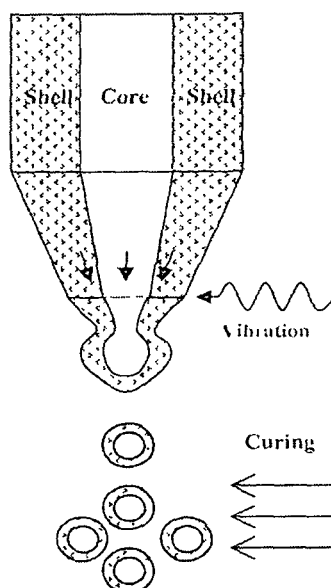
Fig.1.12. Schematic presentation of co-extrusion process

Spray Drying

Microencapsulation by spray-drying is a low-cost commercial process which is mostly used for the encapsulation of fragrances, oils and flavors. Core particles are dispersed in a polymer solution and sprayed into a hot chamber (Fig. 1.13). The shell material solidifies onto the core particles as the solvent evaporates such that the microcapsules obtained are of polynuclear or matrix type. Very often the encapsulated particles are aggregated and the use of large amounts of core material can lead to uncoated particles. However, higher loadings of core particles of up to 50–60% have been reported [68]. Water-soluble polymers are mainly used as shell materials because solvent-borne systems produce unpleasant odors and environmental problems.
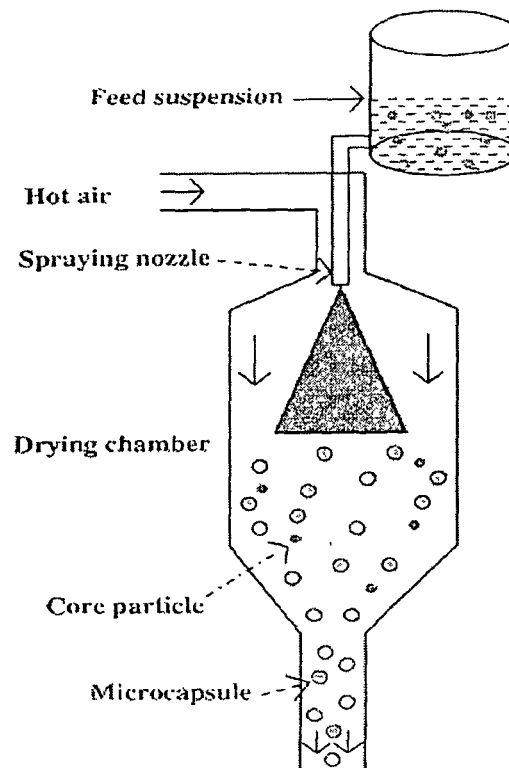
Fig 1.13. Schematic illustration of spray-drying Technique

Fluidized-Bed Technology

With the high demand for encapsulated materials in the global market, fluid-bed coaters have become more popular. They are used for encapsulating solid or porous particles with optimal heat exchange [69]. The liquid coating is sprayed onto the particles and the rapid evaporation helps in the formation of an outer layer on the particles. The thickness and formulations of the coating can be obtained as desired. Different types of fluid-bed coaters include top spray, bottom spray, and tangential spray (Fig.1.14).

- In the *top spray* system the coating material is sprayed downwards on to the fluid

bed such that as the solid or porous particles move to the coating region they become encapsulated. Increased encapsulation efficiency and the prevention of cluster formation is achieved by opposing flows of the coating materials and the particles.

- The *bottom spray* uses a coating chamber that has a cylindrical nozzle and a perforated bottom plate. The cylindrical nozzle is used for spraying the coating material. As the particles move upwards through the perforated bottom plate and pass the nozzle area, the coating material encapsulates them.

- The *tangential spray* consists of a rotating disc at the bottom of the coating chamber, with the same diameter as the chamber. During the process the disc is raised to create a gap between the edge of the chamber and the disc. The tangential nozzle is placed above the rotating disc through which the coating material is released.
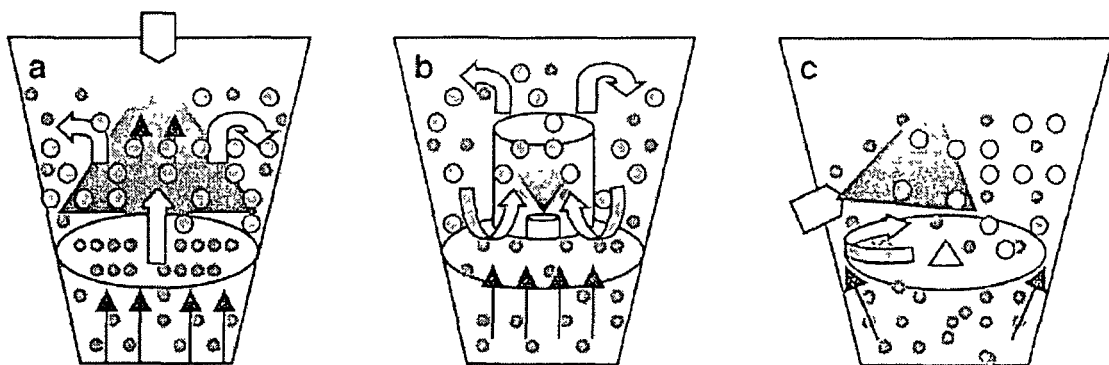
Fig.1.14. Schematic diagram of fluidized bed coating process. (a) Top spray; (b) Bottom spray; (c) Tangential spray

Spinning Disk

A schematic diagram of the process is shown in Figure 1.15. Suspensions of core particles in liquid shell material are poured into a rotating disc and, due to the spinning action of the disc, the core particles become coated with the shell material. The coated particles, along with the excess shell material, are then cast from the edge of the disc by centrifugal force, after which the shell material is solidified by external means (usually cooling). This technology is rapid, cost-effective, relatively simple and has high production efficiencies.
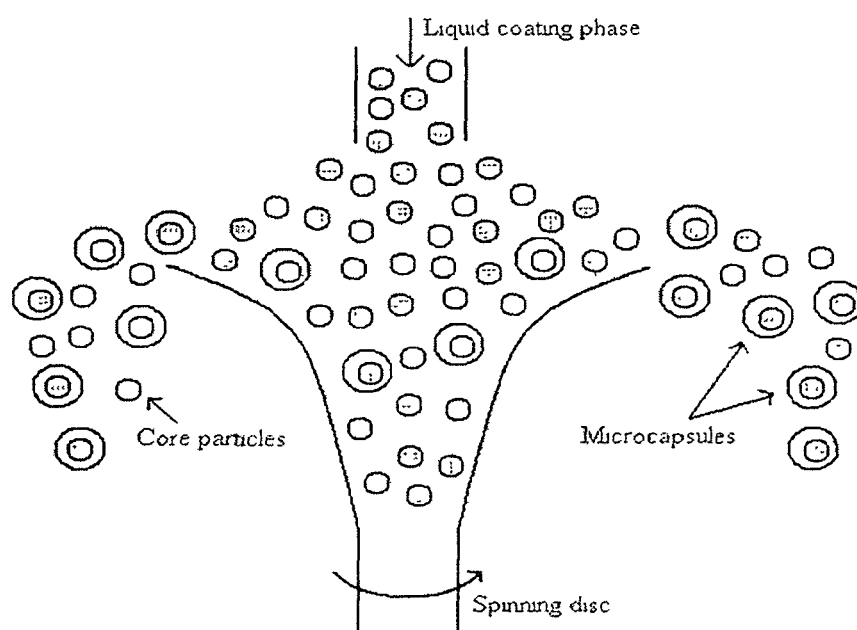
Fig.1.15. Schematic representation of microencapsulation by spinning disc

Desolvation or Extractive Drying

This encapsulation technology has been used to produce water-soluble capsules loaded with a range of flavor compounds Water-soluble shell materials used are

maltodextrins, sugars and gums. The shell material is dissolved in as little water as possible and the core material is dispersed in this solution. Heat is used to reduce the viscosity of the shell formulation so that dispersion can be achieved readily. The dispersion of core material and shell formulation is either extruded or atomized into a desolvation solvent. Because the core materials used in this process are miscible with the desolvation solvents used, capsules with no free core material are produced. Such capsules have excellent storage stability properties [70,71].

Other Process of microencapsulation

Other processes of microencapsulation are also known which includes pan coating, sol-gel method, layer-by-layer (LBL) techniques, electrostatic encapsulation, etc. A steady stream of encapsulation technologies continues to appear in the patent literature. Some are simply modifications or improvements of the established technologies, whereas others new technologies confined to laboratory scale.

1.6.3. Matrix Encapsulation Technique

Controlled release products obtained here lack a distinctive wall surrounding each particle of the active ingredient. The active agent is dispersed within a polymer and becomes entrapped within many small cells of a continuous matrix. The active ingredient may be dissolved or suspended in various polymers to yield ribbons, sheets or granules. Often an excipient is added to such formulations. Such excipients may be inorganic filler

particles or water soluble polymers that provide points at which the surrounding medium can penetrate the product in order to regulate the rate of release of active ingredients.

Beyond the above techniques some other techniques are also available for preparing controlled release product.

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CHAPTER II

LITERATURE REVIEW

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2.1 Literature Review on Insect Repellents and Their controlled Release Formulations

2.1.1. Chemically derived Insect Repellents

In general, the chemical repellents have a broader spectrum of efficacy and a greater duration of action than botanical repellents. Bueschr et al [1] reported that 50% DEET provide only about 4 hrs protection against Aedes aegypti mosquitoes, but increasing the DEET concentration to 100% provided only 1 additional hour of protection. In another study, 12.5% DEET provided over 6 hours of protection against Aedes albopictus; doubling the DEET concentration to 25% increased the protection time only to about 8 hours [2].

Literature reported the application of permithrin directly to clothing or other fabrics such as tent wall [3] or mosquito nets [4]. The combination of permithrin treated clothing and skin application of DEET based repellent produced a formidable barrier against mosquito bites had also been reported in the literature [5, 6]. Dimethyl phthalate used as a repellent against the ticks responsible for Lyme disease was reported [7].

In field studies of tick repellents, indalone was an effective repellent and appeared to be safe for use either on clothing or on direct application to the skin.

Field test of DEPA with *Culex quinquefasciatus, simulium himalayense* and the leech *Haemadipsa zeylanica* showed 1.5, 2 and 1.5 hours of complete protection, respectively. [8]. DEPA had been formulated in a commercial preparation by the Defence Research and Development Establishment (DRDE).

In one laboratory comparative study of the efficacy of insect repellents against mosquito bites, Avon Corporation's IR3535 (3-[*N*-butyl-*N*-acetyl]-aminopropionic acid)-based 7.5% repellent provided an average complete protection time of only about 23 minutes (range, 10-60 min) [9]. In another laboratory and field study, Thavara and his coworkers [10] conducted evaluations of IR 3535 in Thailand. In the laboratory, both IR3535 and deet showed equal repellency (P>0.05) for 9.8 and 9.7 h against *Aedes aegypti*, for 13.7 and 12.7h against *Culex quinquefasciatus*, and for 14.8 and 14.5 h against *Cx. tritaeniorhynchus*, respectively.

Yap et.al [11] reported the field efficacy of Bayrepel® i.e., 1-piperidine carboxylic acid, 2(2-hydroxyethyl)-1-methylpropylester against vector mosquitoes.

Research on AI3-35765 showed that it had similar efficacy to DEET against *Anopheles albimanus, An freeborni, An gambiae, An. stephensi* and *Phlebotomus papatasi* [12], *Prosimulium* and *P. fuscum* [13], *Anopheles stephensi* and *Culex quinquefasciatus* [14] as well as *Culex pipiens* both in the laboratory and the field [12].

The effectiveness of a repellent was reported to be decreased by the abrasion from the clothing, evaporation and absorption by the skin surface, wash-off from the sweat or rains, higher temperature, windy environment etc [15-17].

2.1.2. Plant Derived Insect Repellents

Citronella oil (*C. nurdus*) consists mainly of citronellal ((3,7-dimethyl-oct-6-enal)

and geraniol (3,7-dimethyl- octa-2,6(2,7)-dien-1-ol)[18].Both the components were found

to be effective alone [19,20]. Curtis et al [18] calculated the ED_{50} of citronella in

laboratory experiments to be 11.8 nl/cm2 for *Anopheles stephensi*, 20.2 nl/cm2 for

Anopheles albimanus, and 42.1 nl/cm2 for an *An. Gambiae*, which are similar to the

effective doses of freshly applied DEET.

Several articles have multiscreened a number of essential oils for mosquito

repellency with mixed outcomes. Twenty essential oils were screened by Cora et.al. [21]

against the mosquito (*A.aegypti*), fruit fly (*Drosophila domestica*), and the house fly

(*Musca domestica*), where the oils of *Juniperus communis, Valeriana officinalis, Thymus*

vulgaris, Solidago graminifolia, sylvestris, Coriandrum sativum, Larix decidua,

Pseudotsuga menziesri, Tanacetum vulgare and *Abies alba* were all found to be highly

repellent to mosquitoes. Tawatsin et al. [22] investigated the repellency effects of

essential oils of turmeric (*Curcuma longa*), keffir lime (*Citrus hystrix*), citronella grass

(*Cymbopogon winterianus*) and hairy basil (*Ocimum americanum*), against the

mosquitoes *A. aegypti, Anopheles dirus* and *C. quinquefasciatus* both in caged and open

room conditions. Tumeric, citronella and hairy basil oils especially with the addition of

5% vanillin, repelled the three species under cage conditions for up to eight hours,

comparing well against the DEET standard, which provided protection for at least eight

hours against *A aegypti* and *C. quinquefasciatus* Amer and Mehlorn et al [23] studied

the repellency of 41 essential oils and their mixture against *Aedes aegypti, Anopheles*

stephensi and *Cx. quinquefasciatus* using the skin of human volunteers to find the protection time and repellency. Trongtokit et al [24] studied the mosquito repellent activity of 38 essential oils from plants at three concentrations (10%, 50%, 100%) against the mosquito *Aedes aegypti* under laboratory conditions using human subjects. When the tested oils were applied at a 10% or 50% concentration, none of them prevented mosquito bites for as long as 2 h, but the undiluted oils of *Cymbopogon nurdus* (citronella), *Pogostemon cablin* (patchuli), *syzygium aromaticum* (clove) and *Zanthoxylum limonella* were the most effective and provided 2 h of complete repellency.

Tiwari [25] tested lemongrass oil against the housefly *Musca nebulo* and female mosquitoes of *Culex fatigans* and *Ae aegypti* and reported that lemongrass oil was less efficient than dimethyl phthalate and protection lasted only 40 min as opposed to 300 min for dimethyl phthalate. Ansari and Razdan [26] studied the effects of *Cymbopogon martini var. sofia, C citratus, C. nardus* and *Cinnamomum camphora* against local Indian mosquito species (*Anopheles cujicifacies* and *Cx. quinquefasciatus*) by exposing the feet, forearms and faces of local volunteers from dawn till dusk, dosed with 1 ml of each of the oils. Repellency of each of the *Cymbopogon* oils lasted 11 h against *A. cujicifacies* and 6–7 h against *C. quinquefasciatus*, (but less for the *Cinnamomum oil*). The oils were found to be comparable in efficacy to dimethyl phthalate and dibutyl phthalate. Oyedele et.al [27] studied the ointment and cream formulations of lemongrass oil in different classes of base and the oil in liquid paraffin solution for mosquito repellency. The 1% v/v solution and 15% v/w cream and ointment preparations of the oil exhibited 50% repellency lasting 2–3 h. This activity was comparable to that of a commercial mosquito repellent.

Li et al [28] tested extract of eucalyptus oil on *Aedes aegypti* mosquitoes and found that the protection only lasted for 1h. However, p-manthane-3, 8-diol (PMD) discovered in the waste distillate of the extract of the lemon eucalyptus plant was determined to be the active ingredient for the repellent activity of mosquitoes. Laboratory studies by Trigg and Hill showed that 30% PMD was almost as effective as DEET, the most widely available synthetic repellent [29,30].

Choi et al[31] investigated the essential oil of *Lavandula officinalis* as well as other oils such as *Eucalyptus globules, Rosemarinus officinalis* and *T.vulgaris* for their individual repellent activities against *Culex pipiens* and found that all the named oils actively repelled adult mosquitoes on hairless mice, with thyme oil showing particularly good activity in the confines of the protocol, giving a protection rate of 91% at 0.05% concentration in topical treatment, significantly extending the duration of protection.

Several species from genus *Ocimum* had been popularly used to repel or kill insects. *Ocimum americanum* (synonym: *Ocimum canum*) and *Ocimum basilicum*, for instance, have been widely employed in Africa [32]. *Ocimum* spp. leaves were reported to be rubbed on the skin as a method of repelling mosquitoes [33]. Seyoum et al.[34] reported that thermal expulsion and or direct burning of *Ocimum americanum, O. kilimandscharicum* and *Ocimum suave* were effective in repelling Anopheles gambiae in experimental huts within a screen-walled greenhouse. Seyoum et al [35] demonstrated that potted *Ocimum americanum* repelled *Anopheles gambiae* in experimental huts under semi-field conditions. Furthermore, *Ocimum americanum* volatile oil was shown to repel *Aedes aegypti, Anopheles dirus* and *Culex quiquefasciatus*, under cage conditions up to

Interestingly, when mixed with 5% vanillin, the protection times increased greatly for each mosquito species since it reduces the evaporation rates of repellents [36].

Jeyabalan et al [37] considered methanol extracts of *Pelargonium citrosa* leaf, testing for biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency and biting deterrency against *An stephensi*. At 4%, the extracts evoked strong repellent action.

Ezeonu et al. [38] used statistical studies of randomized complete block design with four replicates to show that the volatile peel extracts of *Citrus sinensis* (sweet orange) and *C. aurantifolia* (lime) possessed insecticidal activities against mosquitoes, cockroaches and houseflies.

A complete protection for 12h from the bites of all the anopheline mosquitoes species was reported by using 2% neem oil in coconut oil on the exposed part of the body [39]. However, Rajnikant and Bhatt [40] reported only 89 and 98% protection against *An. fluviatilis* and *An.culicifacies* respectively and only 68% protection against all anopheline species by using 2% neem oil. The protection from Culex and Aedes mosquitoes ranged between 76-86%. But Moore et al [41] did not find any significant protection from *An Darlingi* by using 2% neem oil, while a eucalyptus based repellent provided 96% protection for 12h. Prakash et al. [42] recorded 66.7% protection after 9 h using 2% neem oil diluted in mustard oil. Vanishing cream with 5% neem oil also provided 67 to 100% protection against malaria mosquitoes in different terrains in India [43-45]. Application of the neem cream for protection against mosquitoes was more acceptable because of its easy application, pleasant odor and more effective repellency up to 4 h after the application.

Several plant essential oils which had been demonstrate to exhibit good repellent activities against mosquitoes were *Conyza newii*, *Plectranthus marrubioides*, *Tetradenia riparia*, *Tarchonanthus camphoratus*, *Lippia javanica* and *Lippia ukambensis* [46], *Artemisia vulgaris* [47], *Lantana camara* [48,34,49], *Zanthoxylum limonella* [50], *Vitex rotundifolia* [51-52], *Zanthoxylum piperitum* [53], *Ocimum selloi* oil[54], *Eucalyptus camaldulensis*, *Mentha piperita*, *Ocimum basilicum*, *Laurus nobilis* [55], *Curcuma* spp.[56], *Cedrus atlantica* or *Juniperus* spp. (cedar, juniper) [57], *Pinus sylvestris* [57], *Syzygium aromaticum* (cloves)[57], *Tanacetum vulgare* (thujone)[58] etc.

2.1.3. Microcapsules / microspheres for Controlled Release formulations

Polymeric Materials

The use of controlled release technology with topical repellents provided extended protection against mosquitoes as reported by Gupta and Rutledge [17]. Formulations based on creams, polymer mixtures or microcapsules could prolong the effectivity of repellents. Mixing of DEET with polymers increased its water-resistance fourfold and the duration of its effect against *Aedes* aegypti by 2.5 [59]. The duration of the effect against *Aedes aegypti* and *Anopheles albimanus* with microcapsule formulations was also up to 24 h longer than that of simple lotions [60]. The efficacy of a 35% DEET cream formulation lasted longer than that of a 75% lotion against *Culex sitiens* and *Aedes vigilax* [61] against *Anopheles flavirostris*[62].A polymer cream formulation (33% Deet) and a microparticle formulation (42% DEET), tested against *Aedes aegypti*, *Aedes taeniorhynchus*, *Anopheles albimanus* and Anopheles *stephensi*,

were superior to the 75% lotion.The microparticle formulation had a longer lasting effect against *Aedes aegypti* and *Aedes taeniorhynchus* than polymer and microcapsule formulations [17].

Slow release microcapsule based formulations containing DEET considerably reduce dermal absorption [63]. In an effort to develop a new topical formulation, a liposphere lotion formulation (20%) and an alcohol solution (20%) with two insect repellents DEET and DEPA were evaluated for the extent of protection on rabbits against *aedes aegypti* [64]. The lotion formulation of DEPA and DEET were found to enhance the repellency by 1.5 and 1.25 times respectively compared to the alcohol solution of the repellents. Gupta et.al [65] reported that the protection time of DEPA against mosquitoes may be increased by developing its slow release formulations using polysiloxanes as matrix systems.

The 3M Company (St. Paul, Minnesota) developed a slow-release, polymer-based product containing 35% DEET. When tested under laboratory and several different environmental and climatic field conditions, the 35% DEET polymer formulation by the 3M Corporation was as effective as 75% DEET in repelling mosquitoes [66-68] One study showed that Minnetonka Brands' 6.5% liposphere microdispersion of DEET was effective for up to 2.5 h and that their 10% product was effective for about 1 h longer [68].

Urea-formaldehyde microcapsules containing lemon oil were prepared by in situ interfacial polymerization. The particle size and their distribution under different experimental conditions were measured and reported [69]. Thimma and Tammishetti [70] studied the complex coacervation of gelatin with carboxymethyl guargum and applied it

for microencapsulation of clove oil and sulphamethoxazole. Microcapsules containing fragnant oil were synthesized by in situ polymerization method and the microencapsulation efficiency and other physical properties were analyzed by Lee and his coworkers [71]. Heng et al [72] studied the encapsulation of wheatgerm oil and evening primrose oil using sodium alginate by emulsification method. They also investigated the physical appearance of microspheres, amount of oil to be encapsulated, flow property, size distribution and mean size of microsphere produced. Bachtsi et al [73] synthesized and studied the release behaviour of oil from oil containing poly(vinyl alcohol) microcapsules prepared by simple coacervation technique. Rosenblat et al [74] studied the effect of electrolytes, stirring rate and surfactant concentration on coacervation and microencapsulation process of gelatin. Effect of wall thickness of microcapsule on the release characteristics was studied and reported by Madan [75]. Sun et al [76] prepared a series of gelatin microspheres by emulsification –coacervation method and studied the influence of preparation parameters like concentration of gelatin, emulsifier, emulsifying time, stirring speed etc. on particle size, surface morphology and dispersion of gelatin microspheres. Shu and Zhu [77] studied the interaction of chitosan with three kinds of anion (tripolyphosphate, citrate and sulphate) by turbidimetric titration and reported that the electrostatic interaction took place in a certain region of solutions. Yan et al [78] prepared crosslinked chitosan / poly (vinyl alcohol) blend with high mechanical strength. Chitosn microspheres were prepared using sodium sulphate as precipitant. The microspheres were loaded with drugs and the loading property was investigated by spectrophotometry. The loaded microsphere were characterized by SEM and DSC [79].

Mani and Jun [80] developed a method, employing salt and wetting agents, to improve the loading efficiency of a water-soluble drug. Microencapsulation of hexadecane in a vegetable protein by salting out method and the effect of different process parameters on microcapsules characteristics were investigated by Mauguet et al [81]. Kulkarni et al [82] reported the use of urea formaldehyde resin for the controlled release of diclofenac sodium. Iwanga [83] and coworkers studied the release rate of insulin from gelatin microspheres with crosslinking densities. The release rate of insulin showed initially a burst effect followed by a slow release phase regardless of the crosslinking density.

Crosslinking Agents

Aminabhavi et al [84] studied the effect of various crosslinking agents on the release behaviour of diclofenac sodium encapsulated chitosan microspheres. The effect of crosslinking agent on the release of lactic acid from gelatin microsphere was studied by Dinarvand et al [85]. Varieties of crosslinking agents like glutaraldehyde [86], formaldehyde [87], epoxy compounds [88] were reported to be employed for improving controlled release behaviour of controlled release polymer. Genipin, a natural crosslinker whose cytotoxicity, feasiblility and biocompatibility were studied and reported [89,90]. Sung et al [5] reported that genipin was 10,000 times less toxic than glutaraldehyde. Genipin crosslinked alginate-chitosan microcapsules for live cell encapsulation was reported by Chen et al [91]. In another study, Chen et al [92] investigated the fluorogenic characteristics of chitosan-genipin reaction for microencapsulation purposes.

Deacetylated Chitosan

Li et al [93] reviewed the various aspects of chitosan and reported that the degree of deacetylation (DDA) could be employed to differentiate between chitin and chitosan. Baxter et al [94] reported that an increase in either temperature or strength of sodium hydroxide solution could enhance the removal of acetyl groups from chitin, resulting in a range of chitosan molecules with different properties. Different methods were available in the literature for characterization of degree of deacetylation of chitosan. These were ninhydrin test [95], infrared spectroscopy [96], linear potentiometric titration [97,98], circular dichroism spectroscopy [99], NMR [100,101], UV spectroscopy [102], elemental analysis [103] etc. Spherical beads of chitosan, crosslinked with different concentration of glutaraldehyde were prepared and used for controlled release of drugs [104]. The effect of molecular weight and degree of deacetylation of chitosan on inherent viscosity, crystallinity and swelling were evaluated by Taghizadeh and Davari [105].

2.2. Literature Review on Controlled Release Agrochemicals

The different classes of polymers viz., elastomers, plastics and fibres were extensively used in agriculture for varied purposes. The major application fields included CR pesticides, herbicides and fertilizers, soil conditioning, plant protection, seed coating and gel planting [106].

Several pesticides like sevin, dimethoate, ethyl trithion, methyl trithion, diazinon, malathion, chloropyriphos and temephos could be incorporated in plasticized poly(vinyl chloride) to obtain CR products [107].

El-Refaie and coworkers [108] prepared controlled release formulations based on crosslinked polyacrylamide derivatives. The release data of the herbicide 2,4-D in vitro from formulations were described.

Micro-or macro encapsulation of active agents using polymers is one of the methods widely used for the preparation of CR products. Condensation polymerization reactions yielding polyamide, polyester, polyurea, polyurethane, polycarbonate and polysulphonamide could be well utilized to prepare CR formulations. Crosslinking of the polymer wall provided durable and storage stable capsules [109,110]. Several controlled release pheromone formulations were also synthesized by microencapsulation. The utilization of starch as a polymer matrix for CR agrochemical was reported [111].

Pfister, Bahadir and Korte [112] claimed another system based on calcium alginate with a series of herbicides. Starch was used as an encapsulating material for S-ethyldipropylthiocarbamate (EPTC), atrazine and trifluralin [113-116]. Teft and Friend [117] synthesized controlled-release polymeric microspheres of herbicides Dicamba(DA) based on ethylcellulose , polyarylsulfone or a combination of the two.

Kulkarni, Kumbar, Dave and Aminabhavi [118] reported the release kinetics and encapsulation efficiency of urea-formaldehyde (UF) crosslinked matrices of starch, guar gum (GG) and starch+guar gum (St+GG) for controlled release of solid (chloropyrifos) and liquid (neem seed oil) pesticides. In another report, Kulkarni and his group [119] claimed the synthesis of novel polymeric sodium alginate interpenetrating network (IPN) beads for the controlled release of chloropyrifos. They also synthesized IPN beads of poly(vinylalcohol)-g-poly(acrylamide) with sodium alginate for the controlled release of cypermethrin pesticide[120].

Marei et al. [121] compared carbofuran encapsulated controlled release formulation with the granular formulation in term of mobility of carbofuran and reported that leaching potential of alginate formulation decreased more than nine times compared with granular formulation. Chitosan gel beads and film were assessed for their ability to control the release of herbicide atrazine and fertilizer urea [122]. Elabahni et al. [123] developed a technique for encapsulation of herbicide inside ethyl cellulose microsphere and evaluated the shape and size of microspheres by scanning electron microscopy. Polysaccharides like cellulose, chitin, amylose and amylopectin were found to be useful natural polymers for the CR formulations of 2,4-dichlorophenoxyacetic acid and metribuzin [106]. CR formulation of kraft lignin and propachlor had been successfully prepared by Wilkins and Blackmore [124]. It was reported that rice husk lignin could be combined with 2,4- dichlorophenoxyacetic acid [125]. The application of lignin in CR formulations was reviewed by Wilkins [126].

Zhu and Zhu [127] synthesized a new starch-g-poly(butylacrylate) for encapsulating carboxylic group containing herbicides. Polymerizable derivatives of pesticides containing acid groups could be prepared by a reaction with alcohols having a vinyl group [128,129]. Copolymers of vinyl 2,4- dichlorophenoxyacetate and trimethl amine methacrylamide were reported to be used for CR application [125]. Increased release of herbicide was obtained as the hydrophilic co-monomer content increased.

Kenawy and his group [130] prepared controlled release systems based on polyureas and poly(Schiff's bases). The effects of structure and temperature of the aqueous environment on the hydrolysis rate of the obtained polymer had been reported. Cheillini and Akelah [131] synthesized polymeric herbicides containing 2,4-D and

MCPA by modification of oligoethyleneoxylated styrene/divinylbenzene(DVB) resins. The release features for these systems were greatly affected by the pH.

Akelah et al. [132] reported chemical modifications of a series of polyamides containing hydroxyl groups with 2,4-D in the presence of dicyclohexylcarbodiimide(DCC) as a condensating agent to yield a series of polymer. They reported that the rates of release of 2,4-D from the formulations were mainly dependent on hydrophilicity, the pH and the temperature of the release medium.

Pesticides containing acid groups were converted to more reactive acid chlorides, which could react with polymers containing pendant hydroxyl or amino groups. Acylation of synthetic and natural polymers were possible in this manner [133-136]. Pentachlorophenol intercalated on mineral clay was reported by Akelah and Rehab [137]. The release of pentachlorophenol from the formulations was studied in different media at 30^0C and it was concluded that the release of pentachlorophenol from the formulations was dependent on the structure, swelling degree and the medium of release.

A series of preformed polymers modified with pesticides were reported [125]. Chitin [138-140] as a naturally occurring polymer was used as carrier for herbicide metribuzin and the system showed slow release when polymer was directly attached to metribuzin.

Fertilizers

Development of synthetic CR fertilizers had been conducted, mainly with nitrogen sources for many years [137]

Based on the synthetic method and nature of the products CR or slow release fertilizers are divided in to following categories:

a) Slowly Releasing Organic-N Compounds

Urea formaldehyde (UF) is the most popular organic-N compound used for the slow release of nitrogen, and the most widely used of all SRF/CRFs [140-141]. The release of N from these compounds thus depends strongly on soil properties such as biological activity, clay content, pH, and external conditions such as moisture content, wetting and drying, and temperature [140,142-144]. Additional nitrogen compounds based on the reaction of urea with other aldehydes were cited in the literature [140,143,145].

b) Coated Fertilizers

Resin, plastic, lac, silica, sulphur, natural rubber, polyolefin, starch and gypsum were reported to be used for preparing CR urea fertilizers [111,146-148]. The preparation of sulphur and gypsum coated urea (SCU) were reported by several workers [149-153]. The use of polymers to the sulfur-coated urea for improving attrition resistance of coated granules was reported [154].

The alkyd-type coated fertilizer was reported by Lambie[155] and Goertz [143]. Moore [156,157] developed a polyurethane-like coating on fertilizer.The release of nutrients from these products was mainly temperature dependent, while moisture content

in the soil, pH, wetting and drying, and soil microbial activity have little effect on the release [157-159].

Coating of granular fertilizers with thermoplastic materials such as polyethylene by dissolving the coating material in a chlorinated hydrocarbon and spraying it on the granules in a fluidized bed reactor was reported in the literature [160-163].

Polyolefin-coated urea fertilizer (POCU) was developed in Japan [148]. Several types of POCU with varying release rates were reported to be commercially available in Japan. Release was reported to be less sensitive to soil pH and soil moisture [148].

Korean Advanced Institute of Science and Technology (KAIST) had developed several batches of Silicate and Polymer Coated Urea (SPCU) and they had observed satisfactory results for their product on rice [164]. The dissolution rate was adjusted by varying the thickness of coating. The polymer latex used for coating was prepared with styrene, 2-ethylhexyl acrylate and acrylic acid.

c) Matrix-Based Slow Release Fertilizers

Efforts have been made to prepare SRFs or CRFs by mixing nutrients with materials that reduce their dissolution rate. Different materials were used for this purpose: rubber [165], gel-based materials [166], and thermoplastic polymers. Natural rubber based slow release fertilizer had been formulated by Hepburn et al, [172]. It was reported that the crosslinking influenced the rate of release of fertilizer from the matrix.

CR fertilizers were made by using water-absorbing polymers as a carrier for fertilizer solutions. Smith and Harrison [168] had carried out experiments on acrylate, vinyl alcohol and starch based polymers that can be expanded in fertilizer solution. Wu et

al. [169] prepared a double coated slow release NPK with a superabsorbent and water retaining crosslinked poly(acrylic acid)/diatomite- containing urea granules (the outer coating), chitosan (inner coating). The product had a water absorbency of 75 times its own weight if allowed to swell in tap water for 2 h.

2.3. Objectives and Plan of Work

Insect repellents are substances that protect animals, plants or products from insect attack by making food or living conditions unattractive. A large number of synthetic as well as herbal repellents are available. Both synthetic as well as herbal repellents have limited persistence on skin. The ideal repellent should have long-lasting effectiveness against a wide variety of arthropods. The use of controlled release technology with topical repellents has known to provide extended protection against mosquitoes. Controlled release formulation of an insect repellent can prolong the availability of the repellent on the treated surface by reducing the loss of repellent by absorption, evaporation and abrasion. Few reports are available regarding the development of controlled release formulation based on plant-based repellents. Plant-based repellents are ecofriendly, safe and lesser toxic compared to synthetic repellents.

The need for agrochemicals is absolute; thus, there exists an urgent necessity to improve the production and the use of active agrochemicals. This can be achieved by using and applying controlled release technology. Many of the controlled release formulations are highly efficient in sustaining the release of the biologically active components. It should be recognized that the polymeric material to be used in controlled release must degrade to some fashion before there can be any environmental impact in

the chemical, biochemical or biological sense. If polymers for use in controlled release were completely inert or their degradation rate was measured in geologic time, the cumulative aspect would be a matter of concern. However it is possible to use naturally occurring polymers or degradable synthetic polymers.

Keeping in view all the above backgrounds, the aim of the present work was set and the present work had been undertaken. In the present research work controlled release polymeric systems of an agrochemical, urea and an insect repellent based on plant essential oil, *Zanthoxylum Limonella oil* (ZLO) were developed and characterized. The polymeric materials used were naturally occurring polymers such as gelatin and chitosan. Microencapsulation technique was used for the synthesis of controlled release formulations of the agrochemical and insect repellent. Microencapsulation is a technique that reproducibly applies a uniformly thin polymeric coating around small solid particles, liquid droplets or solid dispersions. The polymeric wall is designed to permit controlled release of the encapsulated material under desired conditions. The release of the active agents can be controlled by crosslinking of the polymeric wall. Both synthetic and naturally occurring crosslinking agents were used in our present work. Synthetic and natural crosslinking agents used were glutaraldehyde and genipin, respectively.

The plan of work was divided into the followings:

- To develop and optimize the microencapsulation of active agents (mosquito repellent, urea) in crosslinked natural polymers for controlled release (CR) application.

- To study the effect of various parameters like concentration of active agents, polymer and crosslinking agent on percent encapsulation, active agent content and release rate.

- To characterize the encapsulated product by spectroscopy, differential scanning calorimetry (DSC), thermogravimetry and scanning electron microscopy.

- To study and compare the repellency of the developed CR formulations of mosquito repellent against standard repellent such as DEET.

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CHAPTER III
EXPERIMENTAL

CHAPTER III

EXPERIMENTAL

This chapter covers the materials and methods, which include the raw materials used, sample preparation techniques, their characterization techniques and their application studies.

3.1. Materials Used

The chemicals used in the present study and the manufacturers are listed below:

Materials	Suppliers
(i) Gelatin	E-Merck, India
(ii) Chitosan (Medium molecular weight)	Sigma-Aldrich Inc., USA
(iii) Gelatin Type-B (from bovine skin, 225 bloom)	Sigma-Aldrich Inc., USA
(iv) Tween 80	E-Merck, India
(v) Glacial Acetic acid	E-Merck , India
(vi) Urea GR grade	E-Merck, India
(v) Sulphuric Acid (98%)	E-Merck, India
(vi) Sodium sulphate (anhydrous)	Sd Fine chemicals, Mumbai
(vii) Sodium Hydroxide (pellets)	E-Merck, India
(viii) Genipin (Mw=226.23)	Challenge Bioproducts Co., Taiwan

(ix) Glutaraldehyde 25%(w/v)   E-Merck, Germany

(x) Sodium chloride   E-Merck, India

(xi) Potassium chloride   E-MERCK, India

(xii) N, N-diethyl-m-toluimide (DEET)   Sigma-Aldrich Inc., USA

Other reagents used for the study were of analytical grade.

3.2. Methods

3.2.1. Extraction of Essential Oil

Seeds of *Zanthoxylum Limonella* (locally called: *Zabrang*) a big tree available in the Solmara area of Tezpur, Assam, India were collected and dried in shed for one week. After air drying, steam distillation of *Z.Limonella* seeds was carried out to isolate the essential oil. Five hundred grams of dried and finely ground material of *Z. limonella* seeds were placed in an extraction column connected to a round-bottomed distillation flask containing distilled water. The flask was heated to about 100°C and allowed to reflux until distillation was completed. The liquid formed, together with distillate oil, was collected in a separating funnel. The mixture was allowed to settle and the water (lower) layer was slowly drawn off until only the oil layer remained. The volatile oil obtained was dried by treatment with anhydrous sodium sulphate and then collected. The dried oil was transferred into an ambered colored glass bottle and kept at 4°C for subsequent use.

3.2.2. Coacervation Behaviour Study

3.2.2.1. Simple Coacervation behavior of gelatin / or gelatin-chitosan with sodium sulphate

To optimize the coacervation process, the study of phase separation behaviour of aqueous gelatin or gelatin-chitosan solution in presence of sodium sulphate solution is essential. The coacervation process depends on several factors like polymer to salt ratio, temperature etc. The minimum temperature and polymer-salt ratio at which clear phase separation occurred indicate the optimum condition for coacervation .The temperature at which a homogenous solution separates into two phases namely, a polymer phase and a diluted one is known as the cloud point temperature.

a) Coacervation of gelatin by salting out method

A series of experiments were carried out to determine the cloud point temperature of a gelatin solution as a function of sodium sulphate concentration.

Flask containing a certain amount of gelatin was immersed in a thermostatic water bath maintained at 5°C. A predetermined amount of aqueous sodium sulphate solution (10 % w/v) was added to the flask under stirring condition and the temperature of the water bath was gradually raised. The temperature at which the onset of phase separation started was recorded.

b) Coacervation of chitosan-gelatin by salting out method

0.25 %(v/v) aqueous solution of chitosan in 1% (v/v) acetic acid and 0.25% aqueous solution of gelatin in deionised water were prepared. The solution of chitosan and gelatin were mixed at different ratio (1: 0.5 – 2) at room temperature ($\sim$30^{0}C) under stirring condition. Now a predetermined amount of aqueous sodium sulphate solution (20%w/v) was added to each polymer mixture containing chitosan and gelatin at different ratio at room temperature. The ratio of total polymer to sodium sulphate was varied from 1:2 to 1: 30. The temperature was varied from 30^{0}C to 50^{0}C. The minimum temperature and polymer-salt ratio at which clear phase occurred was recorded.

3.2.2.2. Complex coacervation of chitosan and gelatin

Complex coacervation is a process where the phase separation is induced by the interaction of two oppositely charged macromolecules. It can be experimentally determined by measuring the coacervate yield and turbidity. Complex coacervation also depends on several factors like ratio of one polymer to other, pH, temperature, etc.

Coacervate Yield

0.25% (w/v) aqueous solution of chitosan in 1%(v/v) acetic acid and 0.25-1.0%(w/v) aqueous solution of gelatin in double distilled deionised (DDI) water were prepared. The prepared solution of chitosan and gelatin were mixed at different ratios at room temperature ($\sim$30^{0}C) under stirring condition. The ratio of chitosan to gelatin was

varied from 1: 1 to 1:40. The pH of the polymer solution was adjusted with either dil. HCl or NaOH solution and it was measured using digital pH meter. The investigated pH range was 5.0 to 6.0. This range was choosen as it was above the isoelectronic point of gelatin and below the pH at which precipitation for chitosan occurred. The charge and charge density of the polymer would vary with change of pH. Accordingly interaction between chitosan and gelatin would vary leading to formation of different coacervate yield. The dry coacervate yield was obtained by decanting the supernatant and drying the coacervate phase till attainment of constant weight.

Turbidity measurement

Mixture of chitosan and gelatin was taken in a beaker. The pH of the solution was kept initially low (below 5 0) using dil. HCl. The solution appeared clear. Now dil. NaOH was added drop wise from a burette. Turbidity would appear due to formation of coacervate particles in the continuous phase. The change in transmittance due to turbidity was monitored continuously using UV spectrophotometer at 450nm. The pH at which maximum absorbance noticed was recorded. The absorbance was expressed as 100 - %T (transmittance). This pH would produce maximum turbidity as well as maximum coacervate yield.

3.2.3. Preparation of glutaraldehyde cross-linked gelatin microcapsules containing *Zanthoxylum Limonella oil* **(ZLO) by simple coacervation technique**

To a reaction vessel, certain percentage (4-10 % w/v) of an aqueous solution (50ml) of gelatin was taken at room temperature (30° C). To this, a certain amount of essential oil (3-15 ml) was added under high agitation to form an emulsion. The

temperature of the vessel was then raised to 40°C. Coacervation was brought by gradual addition of aqueous sodium sulphate solution (20 % w/v) for about 90 mins. The vessel was kept at this temperature for another 30 mins. The temperature of the vessel was then brought down slowly to about 5°C using ice crystals. The crosslinking of the polymer capsule was achieved by slow addition of certain amount of glutaraldehyde (1-10 mmol/g of gelatin) solution which consists of methanol 16.67%, acetic acid 5%, sulphuric acid 0.17% and glutaraldehyde 25%. The temperature of the vessel was then raised to 40°C and stirring was continued for about 3- 4 h in order to complete the crosslinking reaction. The vessel was cooled to room temperature. The microcapsules were filtered, washed with 0.3% Tween 80 surfactant solution, dried and stored inside a glass bottle.

3.2.4. Preparation of genipin cross-linked chitosan-gelatin microcapsules containing ZLO using complex coacervation technique

To a beaker, certain amount of 2%(w/v) chitosan solution previously made in 1%(v/v) aqueous acetic acid and 2%(w/v) aqueous gelatin solution were taken. Total amount of polymer is kept constant at 1 gm. The mixture of polymer solution was stirred by mechanical stirrer under high agitation after adding one drop of silicon antifoaming agent at 40°C. The temperature was maintained at 40°C .To this, essential oil (1-10ml) was added under high agitation to form an emulsion. Using 0.1N NaOH, the pH of the emulsion was brought to the range of 5.4-5.8 to attain the maximum coacervation. Once the coacervation took place with the formation of microcapsules the system was brought to room temperature (30°C) to harden the microcapsules. The cross linking of the polymer capsule was achieved by slow addition of certain amount of genipin (0.05-0.5

mmol/g of polymer) solution (0.5%w/v aq.solution). The temperature of the vessel was then raised to 40°C and stirring was continued for about 3- 4 h in order to complete the crosslinking reaction. The vessel was then cooled to room temperature. The microcapsules were filtered, washed with 0.3% Tween 80 surfactant solution, dried and stored inside a refrigerator in a glass ampule.

3.2.5. Preparation of chitosan-gelatin microcapsules containing ZLO using salting out technique

2.50 g of chitosan flakes were dissolved in 100ml of 1%(w/v) acetic acid solution by stirring overnight in a conical flask until a clear solution was obtained. 4.0 g of gelatin (type B) was dissolved in 100ml double distilled water by heating for 1-2 hour to get a clear solution. Variable amounts of chitosan and gelatin solution were taken in a beaker at room temperature (~30°C) so that the weight ratios of chitosan to gelatin were 1/0,1/1, ½, 2/1 and 0/1.The temperature of the beaker was raised to 40°C .To this mixture, a drop of silicon antifoaming agent and essential oil (1-10ml) were added under high agitation by mechanical stirring to form an emulsion. Initially the coacervation of chitosan was brought about by gradual addition of aqueous sodium sulphate solution (20%w/v) for about 2-2.5h.In this stage ZLO encapsulated chitosan particles/microcapsules were formed. The pH of the entire mass of the beaker was then brought between 7.0-8.0 to induce interaction between chitosan, gelatin (type B) and genipin. The crosslinking of the chitosan-gelatin microcapsule was achieved by addition of certain amount of aqueous genipin solution (0.1-0.3mmol/g of polymer). The temperature of the vessel was

maintained between 40-50°C and stirring was continued for another 3 hr. The vessel was then cooled to room temperature .The microcapsules were filtered, washed with 0.3% Tween 80 solution to remove excess oil adhered to the surface, dried and kept in a storage vial.

3.2.6. Preparation of chitosan microspheres containing urea using emulsification and cross-linking method

Emulsification and crosslinking method was adapted for the preparation of urea containing chitosan microspheres. 75ml paraffin oil and 25 ml petroleum ether were taken in a beaker. To this solution, 2.0g Tween 80 surfactant was added. They were mixed together and to the above solution, urea and chitosan mixture (mixed in another beaker) were added gradually with continuous stirring with the help of high speed homogenizer. A water in oil (W/O) emulsion resulted and then the temperature of the whole mass were brought to about 40°C after the addition of predetermined amount of crosslinking agent. Crosslinking reaction was allowed to occur for 3 h. The microspheres formed were separated from the paraffin oil and petroleum ether mixture by filtration. The filtered microspheres were washed thrice with petroleum ether, once with acetone and finally with water to remove surface urea (if any). The filtered and washed microspheres were dried under vacuum at about 60°C. The dried microspheres were kept in tightly stoppered bottle in vacuum desicator.

3.2.7. Preparation of deacetylated chitosan

Chitosans with different degree of deacetylation (DDA)s were prepared from the procured chitosan sample 10 g chitosan sample was refluxed with 100ml of 40 %(w/v) NaOH solution at 80^0C for 4 h under nitrogen atmosphere. Similarly, another sample of chitosan was refluxed with NaOH solution at 80°C for 8h to obtain chitosan with higher DDA. The refluxed samples were separated, washed with hot and cold distilled water till it was freed from alkali and finally dried in vacuum oven. Since reaction temperature affects the rate of deacetylation, deacetylation in chitosan samples was carried out at fixed temperature [1].

3.2.8. Determination of calibration curve for ZLO using UV-Visible spectrophotometer

Calibration curve for the system under study was not available in the literature and therefore a calibration curve was required to estimate the release rate of the encapsulated oil in the eluting solvent medium. It was observed that 1 gm of ZLO could be dissolved easily in 100 ml water containing 0.3 gm Tween 80.

A known concentration of essential oil in distilled water containing 0.3% Tween 80 was scanned in the range of 200- 400 nm by using UV visible spectrophotometer. For ZLO having concentration in the range 0.005 to 0.1 gm/100ml, a sharp peak at 256 nm was noticed. The absorbance values at 256nm obtained with the respective concentrations were recorded and plotted From the calibration curve, the unknown concentration of ZLO was obtained by knowing the absorbance value.

3.2.9. Determination of % Oil loading, % Oil content and % Encapsulation efficiency

A known amount of accurately weighed crushed microcapsules was taken inside a volumetric flask containing a known amount of 0.3% aqueous Tween 80 solution and kept overnight with continuous stirring to ensure complete extraction of oil in Tween 80 solution The encapsulation efficiency (%), oil content (%) and oil loading (%) were calculated by using the calibration curve and the following formulae

$$\text{Encapsulation efficiency (\%)} = w_1 / w_2 \times 100$$

$$\text{Oil content (\%)} \qquad = w_1 / w \times 100$$

$$\text{Oil load (\%)} \qquad = w_2 / w_3 \times 100$$

Where w = weight of microcapsules

w_1 = actual amount of oil encapsulated in a known amount of microcapsules

w_2 = amount of oil introduced in the same amount of microcapsules

w_3 = total amount of polymer used including cross-linker

3.2.10. Determination of degree of deacetylation of chitosan

The degree of deacetylation of the original and alkali-treated chitosan samples were determined by using the following techniques.

Linear Potentiometric Titration (LPT) method

Approximately 0.1g of chitosan was dissolved in 25.0ml of standard 0.1M HCl solution. The solution was then topped up to 100ml with distilled water and calculated amount of KCl was added to adjust the ionic strength to 0.1M. The titrant was the solution of standard NaOH containing 0.1M KCl. The standard titrant solution was added to chitosan solution gradually. Both the volume of NaOH added and pH values of the solution were recorded using digital pH-meter. The differential and integral titration curves were drawn between pH and the volume of titrant added, which produced an integral curve with two inflexions. The differential volume ($\triangle$V) of alkali between first and second neutralization point corresponds to the acid consumed by amino groups present in the chitosan. The degree of deacetylation was calculated using the following equation [2]:

$$DDA = [203 \; Q/(1 + 42 \; Q)] \times 100\% \text{ and } Q = N\triangle V/m$$

Where 'm' is the weight of chitosan sample and 'N' is the strength of NaOH used in titration.

Infrared Spectroscopic method

For determination of degree of deacetylation (DDA) of chitosan samples, both KBr disk and film samples were used. The KBr disk was prepared according to the method of Sabnis and Block [3] with slight modification. Approximately 20mg of chitosan powder and 120mg of KBr was blended and triturated with mortar and pestle for

approximately 10 min. The mixture was compacted to form a disk. The disk was conditioned in a desiccator placed in an oven at 80^0C for 16h before analysis.

Chitosan solution in acetic acid (1% w/v) was prepared and casted on a glass plate. This was dried in a oven at 60°C for 12 h [4]. The chitosan films were deprotonated by washing 3-4 times with methanolic ammonia solution followed by distilled water and methanol. The chitosan films were kept in a desiccator placed in an oven at 80°C for 16h before scanning. The spectra of chitosan samples in the form of KBr disk and film were obtained using an IR instrument (Nicolet ,model Impact-410) with a frequency range of 4000-400 cm ^{1}.The degree of deacetylation (DDA) was evaluated by recording absorbance at 1655 cm^{-1} for amide-I and at 3450 cm^{-1} for OH group in chitosan. The absorbance of chitosan was used to calculate the degree of deacetylation (DDA) using the following equation [5]:

$$DDA = [1- (A_{1655}/ A_{3450})/1.33 \times 100] \%$$

Where the factor 1.33 represents the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan.

Elemental Analysis method

The degree of deacetylation of chitosan samples was verified by elemental analysis. The following derived relationships between weight percent of elemental carbon and nitrogen and degree of deacetylation (DDA) were used [6].

$$DDA = (\frac{9600}{364\,W_C + 2400}) \times 100\%$$

$$DDA = (\frac{1400}{364\,W_N}) \times 100\%$$

Where 'W$_C$' and 'W$_N$' are the weight percent of carbon and nitrogen in the chitosan samples.

3.2.11. Molecular weight of Chitosan

The molecular weight of chitosan samples was determined using the Mark-Houwink-Sakurada (MHS) equation [7]

$$[\eta]=K(M_W)^\alpha$$

$[\eta]$ and Mw are the intrinsic viscosity and molecular weight while K and α are constants for given solute-solvent system and temperature. Six different concentrations (0.0156-0.5%, w/v) solutions of chitosan in 0.1M acetic acid-0.2M NaCl (1:1, v/v) were prepared. The solution was filtered to remove insoluble materials. The ubbelohde-type capillary viscometer was used to measure the flow time of the solutions through the capillary in a constant temperature bath at 25^0C.Three measurements were made on each sample. The running times of the solution and solvent were recorded as seconds (sec) and used to calculate intrinsic viscosity $[\eta]$.

$$\eta_{rel} \text{ (Relative viscosity)} = \frac{t_{solution} \text{ (efflux time of solution)}}{t_{solvent} \text{ (efflux time of solvent)}}$$

$$\eta_{sp} \text{ (Specific viscosity)} = \eta_{rel} - 1$$

$$\eta_{sp} \text{ (Specific viscosity)} = \eta_{rel} - 1$$

$$\eta_{red} \text{ (Reduced viscosity)} = \eta_{sp} / C$$

$$\eta_{inh} \text{ (Inherent viscosity)} = (\ln \eta_{rel})/C$$

$$[\eta] = \lim \eta_{sp} / C = \lim (\ln \eta_{rel})/C$$
$$C \to 0 \qquad C \to 0$$

Where C is concentration of chitosan solution (g/dl)

Both η_{red} and η_{inh} were plotted on a same graph. The common intercept of the two plots on the ordinate at C=0 gives intrinsic viscosity, [η] (dl/g). The intrinsic viscosity was obtained by extrapolating the reduced viscosity vs. concentration data to zero concentration.

The viscosity average molecular weight of chitosan samples was calculated using the MHS equation.. The literature values of K and α are 1.81×10^{-5} and 0.93, respectively [8].

3.3. Release characteristics

3.3.1. Release characteristics of ZLO containing microcapsules

Oil release studies of encapsulated oil were evaluated using UV – visible spectrophotometer (UV -2001 Hitachi). A known quantity of microcapsules was placed into a known volume of 0.3% Tween 80 surfactant solution. The microcapsule- Tween 80 mixture was magnetically stirred at a constant rate and the temperature throughout was maintained at 30°C (room temperature). An aliquot sample of known volume (5 ml) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 256 nm for the determination of cumulative amount of oil release up to a time t. Each

determination was carried out in triplicate. To maintain a constant volume, 5 ml of 0.3% Tween 80 solution was returned to the container.

3.3.2. Release characteristics of urea from chitosan/urea crosslinked microspheres

The conventional method for determining the dissolution for encapsulated urea is a static test in which a certain amount of urea containing microspheres were placed in a conical flask containing 100 ml of distilled water [9]. The temperature was controlled by using a water bath. The refractive index of the solution was measured at 25°C as a function of time (daily for 7 days). The amount of urea released in water could be known from the standard calibration curve (a correlation between refractive index and concentration).

3.4. Water Uptake or Swelling Studies

A known amount (0.4-0.5 g) of microcapsules / microspheres was immersed in distilled water for a stipulated time period to reach equilibrium. Before swelling in water, all the active agents from the microcapsules / microspheres were extracted in a suitable solvent and then dried. Water uptake at equilibrium was determined according to the following equation [10]:

$$\text{Water uptake (\%)} = (W - W_0) / W_0 \times 100$$

Where W_0 and W are the weights before and after immersion in water for a certain period, respectively.

3.5 Elemental Analysis Study

Elemental analysis for determination of degree of deacetylation was performed in a CHN elemental analyzer of Perkin Elmer (model CHN 2400).

3.6 Fourier transform infrared (FTIR) study

FTIR spectra were recorded using KBr pellet in a Nicholet (model Impact-410) spectrophotometer Microcapsules/microspheres, physical mixtures of polymer with active component, active component alone, were finely grounded with KBr and FTIR spectra were recorded in the range of $4000\text{-}400 cm^{-1}$.

3.7. Scanning electron microscopic study

The samples were deposited on a brass/copper holder and sputtered with gold. Surface characteristics of the microcapsules / microspheres were studied using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 10Kv/15kv and at room temperature.

3.8. Thermal properties study

Thermal properties of ZLO containing microcapsules, ZLO, gelatin, chitosan each individually and chitosan/gelatin mixture were evaluated by employing thermogravimetric analyzer (TGA) and differential scanning calorimeter (DSC). TGA study was carried out using thermogravimetric analyzer (model TA 50, shimadzu) at a heating rate of $10^{0}C/min$ up to 600°C. DSC study was done in a differential scanning

calorimeter (model DSC-60, shimadzu) at a heating rate of 10^0C/min up to 400°C. Both the study was done under nitrogen atmosphere.

3.9. Laboratory Evaluation of Mosquito repellency of ZLO containing microcapsules

A predetermined amount of microcapsules prepared under different conditions were sieved. Microcapsules of approximately similar sizes were considered for the formulations. The formulation comprises of petroleum jelly and 20% active repellent. Both were mixed thoroughly in an appropriate proportion to prepare the petroleum jelly based cream. The amount of microcapsules containing 20 % active repellent was calculated on the basis of oil content (%) of the corresponding microcapsules. Base cream (petroleum jelly) without repellent was used as control. In most of the commercial formulations, N, N, Diethyl-3-methyl-benzamide (DEET) were used as one of the ingredients. Therefore, another formulation consisting of petroleum gelly and DEET was prepared for comparison.

The repellent trials were conducted in a repellent test chamber (30 x 30 x 62.5 cm) under laboratory conditions. Before application of the repellents, hands were washed and cleaned thoroughly with rectified spirit. Male and female *Aedes* (S.) *albopictus* mosquito progenies obtained from laboratory colony was maintained in honey solution in a cloth cage (50 x 50 x 50 cm) under controlled temperature (28±2°C) and relative humidity range (75–80%). About 50 to 60 hungry (3 days old) female *Ae* (S.) *albopictus* were introduced into the repellent chamber through the hole on top. The test sample (1-2 g) was smeared on dorsal side of one hand (wrist to finger tips) of each of the subject.

After 30 min of application, the hand was placed inside the repellent chamber for 10 min through a hole up to wrist and plugged with cotton to prevent escape of mosquitoes in order to facilitate the female mosquitoes to bite on. The test was repeated at every 30 min interval. The interval between the application of repellent and the first two consecutive bites occurring within 30 min was considered as protection time against the bites afforded by each of the concentrations of the test repellents [11] The test was replicated 3 times for each concentration of the repellents. Control readings were obtained by placing hand inside the repellent chamber without applying any repellent before the experiment

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CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Microencapsulation of *Zanthoxylum Limonella Oil* (ZLO), an essential oil based insect repellent in glutaraldehyde crosslinked gelatin microcapsules by simple coacervation technique for mosquito repellent application

In this part of work, the author has chosen simple coacervation technique for the preparation of glutaraldehyde crosslinked gelatin microcapsules. The effect of various parameters like oil loading, gelatin concentration, crosslinker concentration, pH of the reaction medium, stirrer speed etc. on the microcapsule properties have been explained here. The mosquito repellent behaviour of the microcapsules has been evaluated.

Results and Discussion

4.1.1. Coacervation of gelatin with sodium sulphate solution:

The results showing the phase separation experiment are shown in Table 4.1.1.

The temperature and minimum weight ratio of gelatin to sodium sulphate at which phase separation occurred were 40^0C and 1:10. This ratio was maintained during preparation of microcapsules in the subsequent experiments. All experiments were carried out in triplicate and results presented were the average values.

Table 4.1.1 *Cloud point observation for study of phase separation of gelatin and*

sodium sulphate solution

Gelatin : Na$_2$SO$_4$	Temperature (°C)										
Solution mass ratio	5	10	15	20	25	30	35	40	50	55	60
1:5	g	g	g	t	t	t	t	t	t	t	t
1:7.5	g	g	g	t	t	t	t	t	t	t	t
1:10	o	o	o	o	o	o	o	p	s	s	s
1:12.5	o	o	o	o	o	o	o	p	p	s	s
1:15	o	o	o	o	o	o	o	p	p	s	s
1:20	o	o	o	o	o	o	o	p	p	p	s

g : gelled ; t : transparent (one phase); o : separation in gummy form ; p : clear

separation in fine particle / colloidal form ; s : separation requires more time, very fine

particle.

4.1.2. Effect of variation of Oil-loading on gelatin microcapsule behavior

The effect of variation of oil loading on the encapsulation efficiency and release

rate are shown in Table 4.2 and Fig.4.1.1. With the increase in oil loading, the release

rate was found to increase throughout the range of oil concentration studied The

encapsulation efficiency was found to decrease while the % oil content and release rate

were found to increase. A possible explanation for the lower encapsulation efficiency at

higher oil load might be due to the higher percentage of oil loss during isolation. At low

oil load, the disperse force by the stirrer was more effective, causing the formation of

smaller oil vesicles. The amount of gelatin present in the system was sufficient to encapsulate the oil vesicles properly. At higher oil load, the dispersive force of the stirrer became less efficient and larger oil vesicles were produced as a result. At this stage, gelatin would try to encapsulate the larger oil vesicles at the expense of decrease of thickness of microcapsule. Also the amount of gelatin might not be sufficient to encapsulate all the oil vesicles. Some of the oil vesicles might exist without encapsulation. These oil vesicles might get lost during isolation. The faster release rate might be due to the decrease of the thickness of the capsule wall. With decrease in wall thickness, diffusional path for the oil release became short which resulted in an increase of release rate [1, 2]

Again oil content (%) was found to increase with the increase in the percentage of oil load. At low oil load, many of the microcapsules probably contained few oil vesicles indicating that there was an abundance encapsulating polymer for the oil present. As oil load (%) increased, the number of oil vesicles in the microcapsules increased which resulted in increasing oil content.

Table 4.1.2. Effect of variation of oil loading, gelatin and glutaraldehyde
concentration on the behavior of microcapsules

Gelatin : (2-5 gm) ; glutaraldehyde : (1-10 mmol/gm of gelatin) ; oil : (5-15 ml) ;
water: 50 ml ; temperature : 30°C

| Sample particulars			oil load (%)	oil content (%)	encapsulation
gelatin	glutaraldehyde	oil			efficiency(%)
3	10	5	74.10	42.90±0.51	98.20±1.13
3	10	7	106.42	49.69±1.29	96.42±2.50
3	10	10	148.30	50.26±0.57	88.42±1.07
3	10	13	192.60	51.60±2.0	87.89±3.04
3	10	15	222.30	59.82±1.2	78 00±1 73
2	5	7	207.48	55.90±0.64	78.35±0.89
3	5	7	142.00	51.16±0.54	97.46±1.02
5	5	7	79.95	29.90±0.54	96.34±1.74
3	1	7	193.82	38.98±0.73	59.10±1.11
3	2	7	177.00	41.80±0.87	65.32±1.36
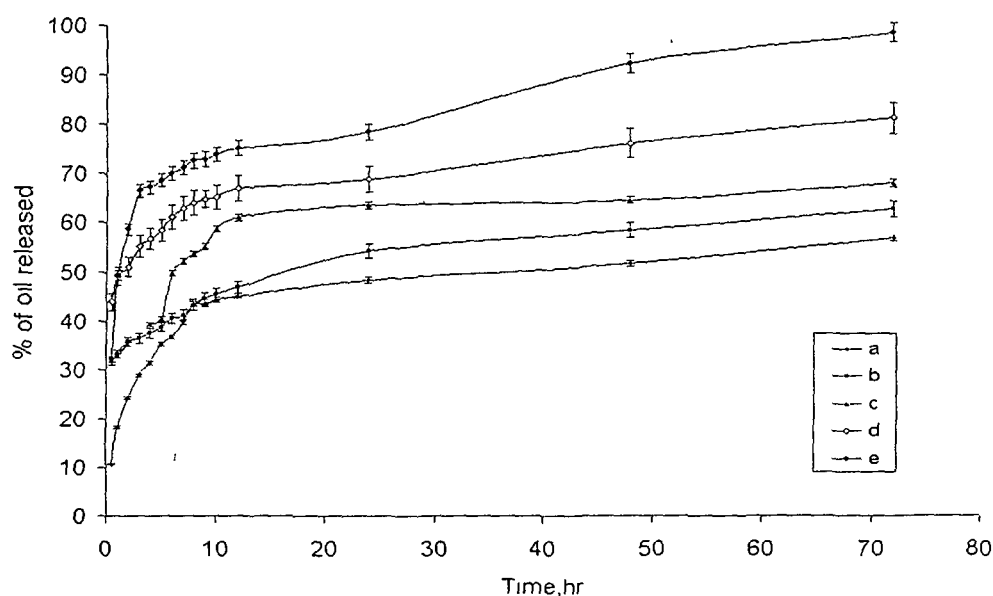
Fig.4.1.1. Effect of variation of oil loading on the release rate

a: G 3 g, GA 10mmol, ZLO 5 ml; b: G 3 g, GA 10mmol, ZLO 7ml;

c: G 3 g, GA 10mmol, ZLO 10ml; d: G 3 g, GA 10mmol, ZLO 13ml;

e: G 3 g,GA 10mmol, ZLO 15ml

4.1.3. Effect of variation of gelatin concentration

Table 4.1.2 shows the effect of variation of gelatin concentration. As expected, oil loading (%) and oil content (%) were found to decrease as polymer content increased. Encapsulation efficiency (%) increased first and then leveled off. With the increase in polymer content, more and more gelatin would be available to encapsulate the oil vesicles and thereby efficiency would be increased. At certain polymer content, all the oil vesicles present would be encapsulated by the polymer. After that, excess polymer would be used to thicken the microcapsule wall which resulted in leveling off the efficiency. Fig 4.1.2

shows the release profile with the variation of gelatin concentration. The concentration of gelatin was varied from 2 to5 g .The release rate was found to decrease with increase in gelatin concentration. The increase in wall thickness of the microcapsule might be responsible for this type of behavior.
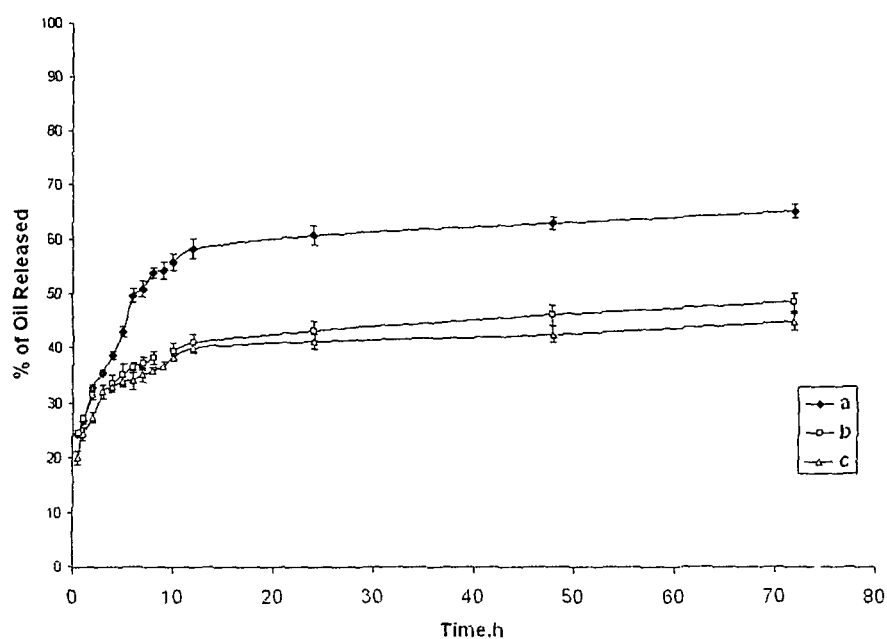
Fig.4.1.2.Effect of variation of gelatin concentration on the release rate

a: G 2g, GA 5mmol, ZLO 7ml; b: G 3g, GA 5mmol, ZLO 7ml

c: G 5g, GA 5mmol, ZLO 7ml

4.1.4. Effect of variation of crosslinker concentration

The related results are shown in table-4.2 and fig.4.1.3.The trend of oil loading (%) and oil content (%) shown in the table was as per expectation. The increased encapsulation efficiency (%) could be due to the improvement in oil retention capacity of the microcapsules caused by the reaction between gelatin and glutaraldehyde. An

increase in the degree of cross-linking, as expressed by molar concentration of glutaraldehyde used, resulted in a significant decrease in oil release rate throughout the glutaraldehyde concentration studied (1 mmol/g of gelatin - 5 m mol/g of gelatin). As degree of crosslinking of gelatin increased, the microcapsule wall became denser resulting in the decrease of diffusion rate of oil through the microcapsule wall. Similar types of observations were reported in the literature [3, 4]. Dinarvand and co-workers also investigated and reported the effect of crosslinker on the release rate of lactic acid from gelatin microspheres [5].
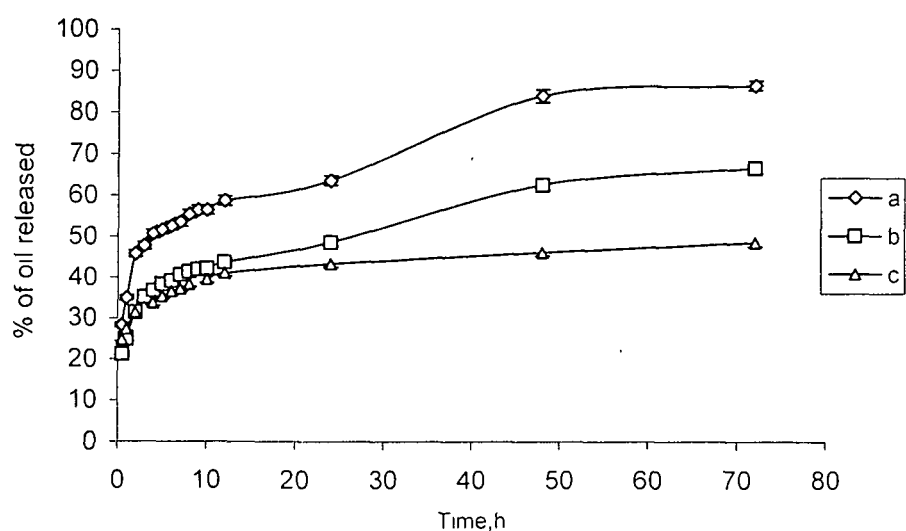
Fig.4.1.3. Effect of variation of crosslinker on the release rate

a: G 3g, GA 1mmol, ZLO 7ml; b: G 3g, GA 2mmol, ZLO 7ml

c: G 3g, GA 5mmol, ZLO 7 ml

4.1.5. Effect of temperature on Release rate

Fig.4.1.4 shows the effect of variation of temperature of release medium, on the oil release characteristic of microcapsules. The higher the temperature of the release medium, the higher was the rate of release of oil as expected. The dependence of the dissolution rate of the encapsulated material on temperature was probably due to the increased solubility of the core material and a higher diffusion rate as the temperature increased. Similar type of result was reported in literature whereby encapsulated fertilizer dissolution rate increased with the increase in temperature of the release medium from polysulfone-coated fertilizers [6].
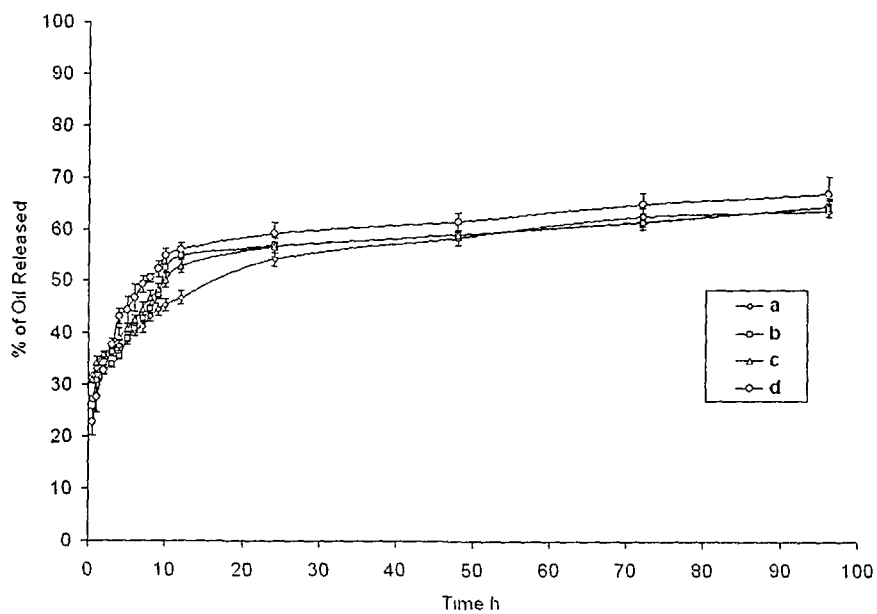
Fig.4.1.4. Effect of variation of temperature on release profile [sample: G 3 g, GA 10 mmol, ZLO 7 ml and pH=7.5]: (a) 25°C; (b) 30° C; (c) 40°C; (d) 50°C

4.1.6. Effect of variation of pH on Microcapsule properties

The effect of variation of pH during cross-linking stage of microcapsule formation on microcapsule properties and release rate is shown in Table 4.1.3 and Fig.4.1.5. From table, it was found that the higher the pH of the reaction medium, higher was the oil content (%), encapsulation efficiency (%) and lower was the release rate of the microcapsules. Lower pH did not favour well the cross-linking reaction between gelatin and glutaraldehye. Gelatin is polyelectolyte in nature and at very low pH (1.5), because of an excess of hydrogen ions (H $^+$), the amino groups of gelatin molecule could get protonated (NH$_3^+$) and cause repulsion among the gelatin chains. This repulsion caused the crosslinking between gelatin and glutaraldehyde difficult. However, this repulsion decreased with the increase in pH of the reaction medium. This would favour the crosslinking reaction between gelatin and glutaraldehyde. The increased oil encapsulation efficiency and oil content at higher pH could be due to the improvement in oil retention capacity of the crosslinked microcapsules. At higher pH, the improved crosslinking might be responsible for the decrease in the release rate of oil.

Table 4.1.3. Effect of variation of stirrer speed and pH on the behavior of gelatin microcapsules

Sample particulars					Oil load (%)	Oil content (%)	Encapsulation efficiency (%)
Gelatin (g)	Oil (ml)	Glutaraldehyde (mmol)	pH	Stirring speed (rpm)			
3	7	5	7.0	750	138.29	44 32±1.06	76.42±1.83
3	7	5	7.0	1000	138.29	50.52±0.92	86.94±1.58
3	7	5	7.0	1500	138.29	55.79±0.76	96.08±1.32
3	7	10	7.0	1000	103.72	49.76±1.08	97.78±0.55
3	7	5	1.5	1000	138.29	40.84±0.14	71.87±0.25
3	7	5	4.0	1000	138.29	47.63±0.75	81.34±1.29
3	7	5	6.3	1000	138.29	51.17±0.05	86.88±0.096
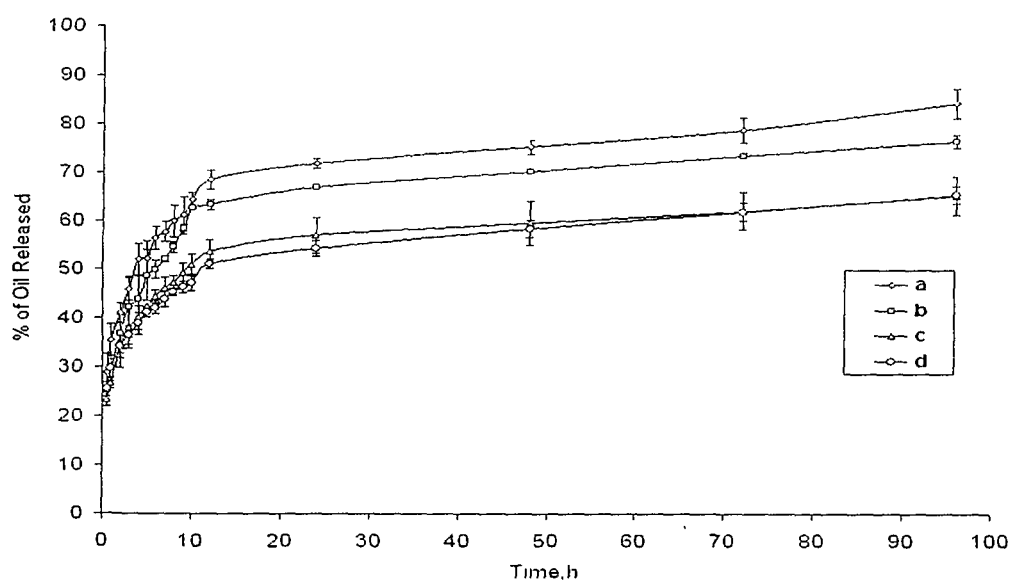
Fig.4.1.5. Effect of variation of pH on the release profile [sample: G 3 g, GA 5 mmol, ZLO 7 ml]: (a) pH = 1.5; (b) pH = 4.0; (c) pH = 6.33; (d) pH = 7.5

4.1.7. Effect of variation of stirring rate keeping other parameters constant

Stirring rate is a kind of physical factor, which plays an important role in the process of emulsification-coacervation. In the process of emulsification/ microencapsulation, three ranges of stirring rate were chosen. It was observed that, the microcapsules obtained at lower stirring rate (750 rpm) had relatively higher proportions of larger microcapsules along with lower percentage of smaller microcapsules adhering to the large ones. On the contrary, microcapsules obtained at higher stirring rate (1000 rpm and 1500 rpm) had higher percentage of smaller microcapsules. Moreover, it was observed (Table 4.1.3) that the microcapsules, produced at higher stirring rate, had higher oil content (%) and encapsulation efficiency (%). At this stage, the droplets might experience difficulties in flocculating to each other because of their stability caused by the stirred centrifugal force. At high rpm, the average size of the oil droplets would be less due to high dispersive force of stirrer. The high dispersive force of the stirrer resulted in lower size microcapsules was reported [7]. The amount of polymer used might be sufficient to encapsulate all the oil droplets and thus prevent them from coalescence. As a result, higher percentage of smaller microcapsules would be produced and oil content including encapsulation efficiency would enhance. Higher stirrer speed produced lower size particles were reported by Sun and coworkers [7].

The release rate of oil from microcapsules prepared under different stirring rate is shown in Fig.4.1.6. Microcapsules produced from higher stirrer speed showed highest release of oil. This could be attributed to the particle size of microcapsules obtained at

higher stirring. Higher the stirring rate, higher was the possibility of formation of lower

particle size and as a result total surface area increased and thereby release rate.
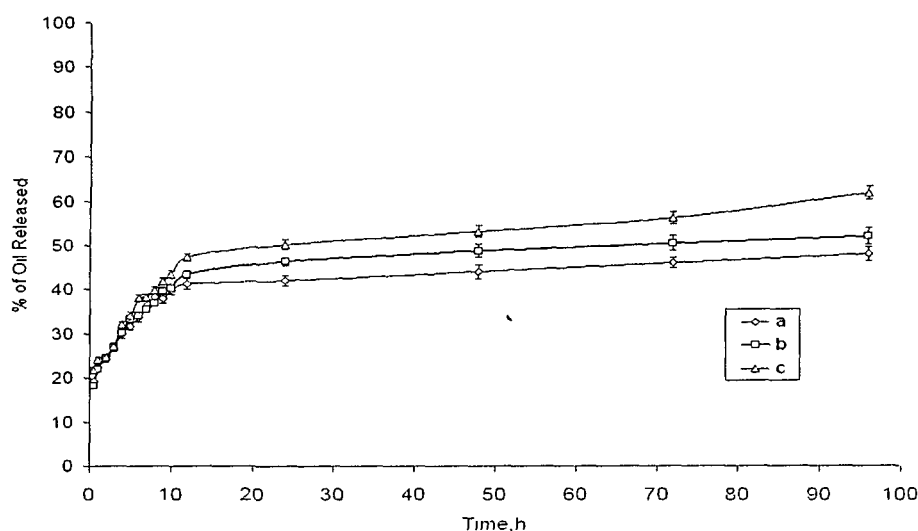

Fig. 4.1.6. Effect of variation of stirring speed on release rate [sample: G 3 g, GA 5

mmol, ZLO 7 ml and pH=7.0]; (a) 750 rpm (b) 1000rpm; (c) 1500 rpm

4.1.8. Differential Scanning Calorimetric Study

Fig.4.1.7 shows the DSC results of pure and oil encapsulated gelatin

microcapsules. Pure gelatin (curve a) showed peaks at 65°C, 225°C and 277°C. Pure oil

(curve b) showed peaks at 94°C and 200°C. Oil encapsulated microcapsules (before

extraction) (curve c) showed endothermic peaks at 120°C, 190°C and 252°C respectively.

Others peaks appeared in the range of 290-330°C. Curve-d showed the thermograms of a

physical mixture of oil and gelatin. The % of oil was kept similar to that of oil present in

oil encapsulated microcapsules. The peaks appeared in the thermogram of the physical

mixture were at 100°C, 223°C and 280°C. Others peak appeared were in the range 310-

340°C. There was no remarkable difference between the thermograms of physical

mixture as well as encapsulated microcapsules. These results suggested a low compatibility in thermal properties in relation between oil and gelatin.
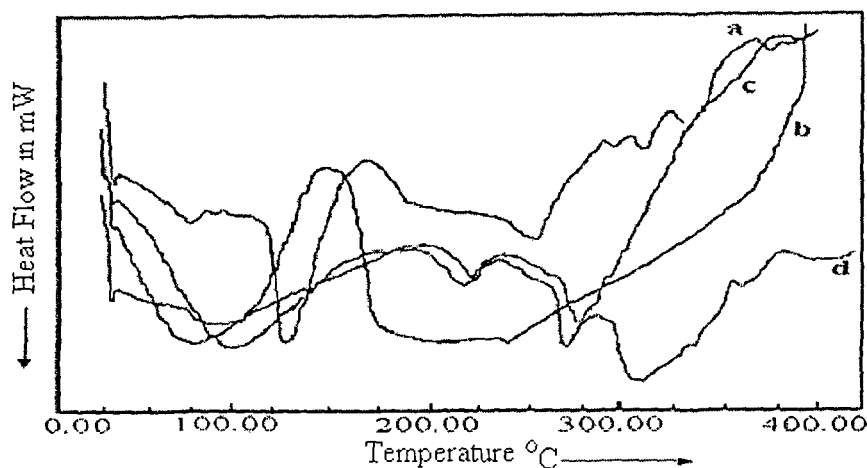
Figure 4.1.7. DSC curve for (a) pure gelatin, (b) pure oil, (c) oil containing microcapsules and (d) physical mixture of oil and gelatin

4.1.9. Fourier Transform Infrared Spectroscopic Study

FTIR spectra of ZLO (curve a), gelatin (curve b), physical mixture of ZLO, glutaraldehyde, gelatin (curve c) and ZLO containing cross-linked gelatin microcapsules (curve d) are shown in Fig.4.1.8. Physical mixture was prepared using the ratio of ZLO, glutaraldehyde and gelatin similar to those of ratio used in preparing ZLO containing crosslinked gelatin microcapsules. The carbonyl-stretching band of ZLO between 1637-1720 cm^{-1} remained almost unchanged in the case of physical mixture as well as microcapsule. The other notable peaks appeared at 1457.70 cm^{-1}, 1377.78 cm^{-1}, 1232.73 cm^{-1}, 1167.53 cm^{-1} and 1019.89 cm^{-1} which were due to CH$_2$ asymmetric deformation,CH$_2$ symmetric deformation, C-N,C-C and C-O stretching vibration also remained almost unchanged in the physical mixture and microcapsules .These results indicated the absence of any significant interaction between the ZLO and the gelatin polymer.

Fig.4.1.8. FTIR spectra of (a) ZLO, (b) gelatin, (c) physical mixture of gelatin, ZLO and

glutaraldehyde and (d) gelatin microcapsules

4.1.10. Scanning Electron Microscopic Study

Fig.4.1.9 shows the SEM photographs of glutaraldehyde crosslinked gelatin

microcapsules of varying oil content. Microcapsules appeared to be made of spherical

units linked to each other. The external surface appeared smooth at low oil loading

indicating the formation of a continuous film by gelatin. At higher oil loading, a bursting

look was observed. The microcapsules prepared at low oil loading, appeared dry and

powdery on physical verification. Where as those prepared at higher oil loading appeared

oily and agglomerated.
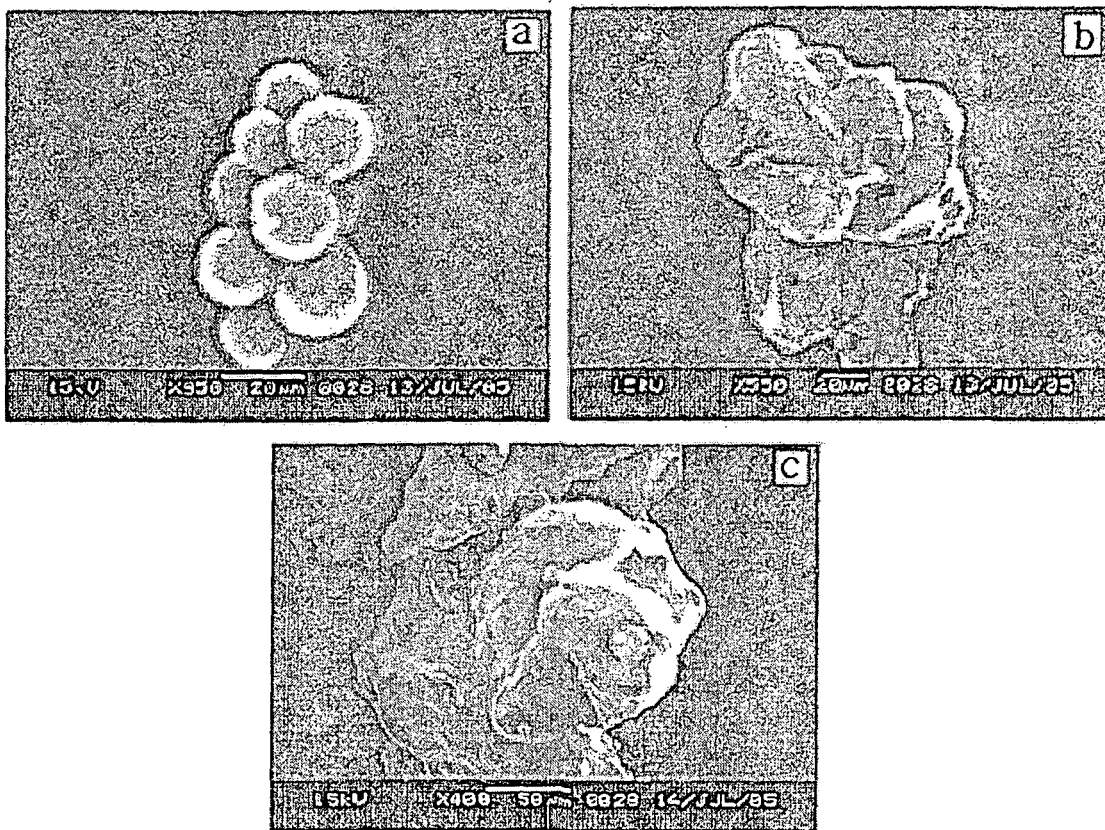
Fig.4.1.9. Scanning electron microphotograph of microcapsules prepared with oil

load (%)   (a) 144.7   (b) 200   (c) 438.8·

4.1.11. Laboratory Evaluation of mosquito repellency of microcapsules

Table 4.1.4 shows the repellent properties of different prepared formulations. The amount of oil or microcapsules were taken in such a way so that the percentage of oil remained same in all the formulations. It was observed that the protection time varied between 0-2.5h. The formulation (A) did not provide any protection as there was no active repellent agent. The formulation (shown in G) based on DEET provided protection

lasting for 2.5 h. The mean protection time for formulation based on gelatin microcapsules (C-F) was in the range 1.5-2.5 h.

The lower, and higher protection time observed in gelatin microcapsule based formulations could be explained as follows. Microcapsules used in the formulations were prepared by varying amount of oil and crosslinker concentration. Formulations (E and F) had shown similar protection time although the amount of crosslinker was different. This indicated that the present level of crosslinker used (1.67-3.33 mmol/g of gelatin) for preparation of microcapsules had little role in the present study compared to that of oil loading. The crosslinking efficiency did not improve further beyond a minimum level of crosslinker (1.67 mmol/g of gelatin) At lower amount of oil loading, the polymer was sufficient to encapsulate the oil droplets. At higher amount of oil loading, the polymer could able to encapsulate the large oil droplets only at the expense of decrease of thickness of microcapsule wall. The higher the oil loading, the lower was the thickness of the microcapsule wall. This would lead to increase the release rate of oil and thereby protection time. ·

It was reported that protection time increased with increasing oil concentration.15% concentration of p-menthane diol (PMD) obtained from Lemon Eucalyptus distillation showed 4.4 h protection against *Aedes aegypti*. The same oil at 50% concentration gave 13h protection against the same mosquito vector [8]. High concentration of *C nardus*, *P cablin*, *S. aromaticum* and *Z limonella* (fruit) provided 2 h complete repellency against *Aedes aegypti* was reported in the literature [9]. The protection time of these oils were less when they were diluted. At 50% concentration *C nardus*, *P cablin*, *S. aromaticum* and *Z. limonella* showed 50, 60, 70 and 80 min

protection respectively, and the repellent activity decreased to 30 min or less when diluted to 10%.

Table 4.1.4. Mosquito repellency of different formulations

Formulae Name	Formulations	Mean protection time (h)
A	Petroleum Jelly	0
B	Petroleum jelly + 20% ZLO	1.5
C	Petroleum jelly + 20% ZLO containing gelatin microcapsules (a)	1.5
D	Petroleum jelly + 20% ZLO containing gelatin microcapsules (b)	2.0
E	Petroleum jelly + 20% ZLO containing gelatin microcapsules (c)	2.5
F	Petroleum jelly + 20% ZLO containing gelatin microcapsules (d)	2.5
G	Petroleum jelly + 20% DEET	2.5

(a): G 3 g, GA 10mmol, ZLO 5 ml; (b): G 3 g, GA 10mmol, ZLO 7ml
(c): G 3 g, GA 10mmol, ZLO 10ml; (d): G 3 g, GA 5mmol, ZLO 10ml

4.2. Preparation of Genipin cross-linked Chitosan-Gelatin Microcapsules for *Encapsulation of Zanthoxylum limonella Oil* (ZLO) using Salting Out method and their application as mosquito repellent

Introduction

In this part the author has chosen salting technique for the encapsulation of *Zanthoxylum limonella oil* (ZLO) in genipin crosslinked chitosan-gelatin microcapsules. The effects of various parameters such as oil loading, degree of crosslinking, ratio of chitosan to gelatin etc. on oil content, encapsulation efficiency and the release rate of ZLO have been reported here. FT-IR spectroscopy has been interpreted to describe the interaction between the polymers and oil. Scanning electron microscopy (SEM) has been shown to study the morphology of the prepared microcapsules. Lastly the microcapsules were mixed with petroleum jelly and their effectiveness as mosquito repellent was studied in the laboratory against standard mosquito repellent and reported.

Results and Discussion

4.2.1. Optimization of salting out of polymer

The phase separation behaviour of chitosan and gelatin (type B) were studied at first in order to get an idea regarding minimum temperature and polymer –salt ratio to be required individually. Gelatin (type B) solution did not produce any coacervate at any sodium sulphate concentration and temperature where as chitosan produced coacervate throughout the temperature range and salt ratio studied. In the case of the mixture of

chitosan –gelatin, the minimum ratio of polymer mixture to salt and temperature at which clear phase separation observed were 1:5 and 40^0C respectively. Therefore, all the experiments for microencapsulation were done by maintaining the ratio of polymer to salt at 1:5 and 40^0C respectively. Microcapsules prepared from gelatin (type A) were used whenever comparison to those of chitosan-gelatin microcapsules required.

4.2.2. Effect of Variation of Oil-loading

The effect of variation of oil loading on the encapsulation efficiency, oil content and release rate is shown in Table 4.2.1 and Fig.4.2.1. With the increase of oil loading, the release rate of the oil from the chitosan gelatin microcapsules increased throughout the range of oil concentration studied. It was observed that the % oil content increased while encapsulation efficiency (%) decreased. At low oil loading, the dispersion of the oil into globules by the stirrer was more effective, therefore the oil vesicles were smaller .At this stage, the amount of polymer present in the system was enough to encapsulate properly the oil vesicles. However, as more oil was introduced, the dispersive force of the stirrer became less efficient and larger oil vesicles were produced as a result. Also there was an increased tendency for the oil vesicles to coalesce at higher oil loads, so that the larger oil vesicles were formed and more oil could be encapsulated with the same amount of encapsulating material at the expense of decrease of thickness of microcapsule wall. At this time, the amount of polymer might not be sufficient for encapsulation of all oil vesicles. The chances of existing of some oil vesicles without encapsulation became more. The loss of these oil vesicles during isolation might cause a reduction in encapsulation efficiency. The faster release rate of the microcapsule at higher loading

might be due to decreased wall thickness of the microcapsule. With the decrease in wall thickness of the microcapsule, diffusional path for the oil release became short which resulted in an increase of release rate [1,2]. Again with the increase in % oil load, the oil content (%) increased. At low oil load, many of the microcapsules have few or no oil vesicles in them indicating that there was an abundance of the encapsulating polymer for the oil present. When the amount of the used oil increased, there was an increase in the number of oil vesicles in the microcapsules which resulted in an increase of oil content.
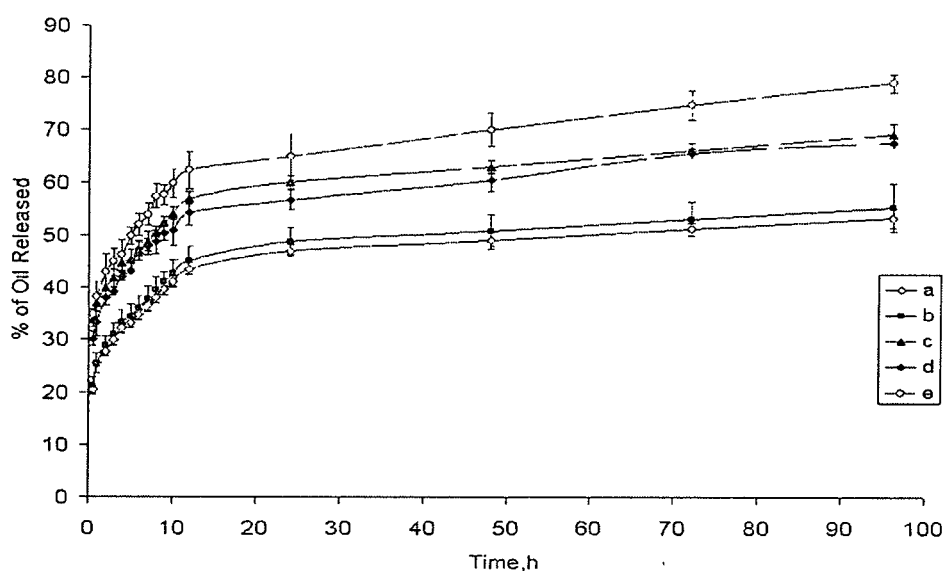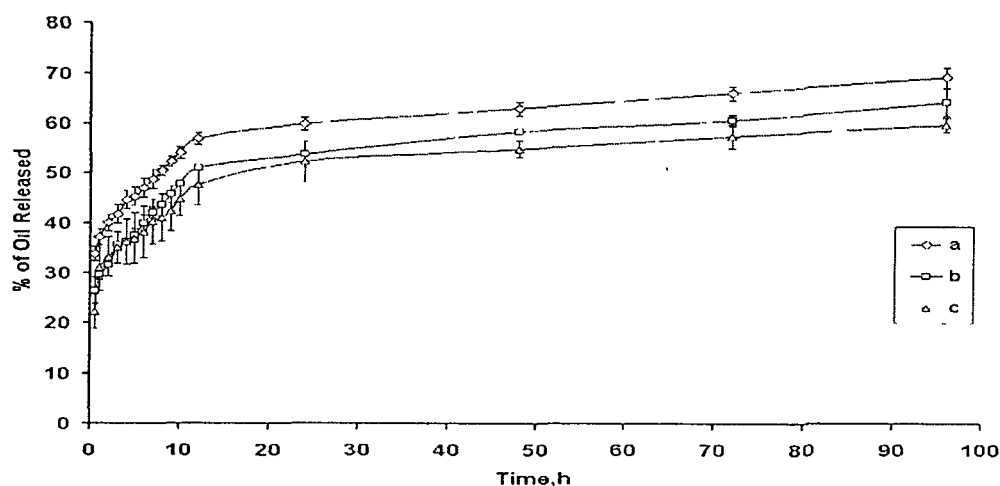
Fig.4.2.1. Effect of variation of oil loading on the release rate

a: CG(0.5/0.5),ZLO 1.0ml,Gp 0.1mmol; b: CG(0.5/0.5),ZLO 2.0ml,Gp 0.1mmol ; c: CG(0.5/0.5),ZLO 4.0ml,Gp 0.1mmol; d: CG(0.5/0.5),ZLO 6.0ml Gp 0.1mmol; e: CG(0.5/0.5),ZLO 8.0ml,Gp 0.1mmol.

Table 4.2.1. Effect of variation of oil loading, chitosan to gelatin ratio and genipin concentration on the behavior of microcapsules [Chitosan/gelatin mixture: 1 gm; Genipin: (0.1-0.3) mmol/gm of mixture; oil: (1-8) ml; water: 100ml; temperature 40 ± 1^0C]

Samples			Oil load (%)	Oil content(%) (mean$\pm$ SD)	Encapsulation efficiency(%) (mean$\pm$ SD)
Chitosan/gelatin (C/G)	Genipin	Oil			
0/1 *	0.1	4.0	343.67	37.66$\pm$3.39	48.6$\pm$4.38
0.33/0.67	0.1	4.0	343.67	42.2$\pm$1.67	48.36$\pm$1.52
0.5/0.5	0.1	4.0	343.67	37.59$\pm$0.075	48.53$\pm$0.097
0.67/0.33	0.1	4.0	343.67	46.25$\pm$2.36	52 65$\pm$2 38
1/0	0 1	4 0	343 67	62 60$\pm$2 1	80 8$\pm$1 2
0.5/0.5	0.2	4.0	343.67	39.78$\pm$1.52	51.36$\pm$1.96
0.5/0.5	0.3	4.0	343.67	51.3$\pm$1.02	66.22$\pm$1.31
0.5/0.5	0.1	1.0	85.92	25.17$\pm$0.345	54.46$\pm$0.747
0.5/0.5	0.1	2.0	171.83	25.8$\pm$1.01	40.82$\pm$1.6
0.5/0.5	0.1	6.0	515.5	39.92$\pm$0.202	47.67$\pm$0 243
0.5/0.5	0.1	8.0	687.34	45.06$\pm$3.03	51.6$\pm$3.47

* For comparison, gelatin type A was used for coacervation study as no coacervatiom formed with gelatin type B

4.2.3. Effect of Variation of Cross-linker concentration

The effect of variation of crosslinker concentration on encapsulation efficiency (%), oil content (%) and release rate are shown in Table 4.5 and Fig.4.2.2, respectively.

The results found were as per expectation. The increased encapsulation efficiency (%) might be due to the improvement of oil retention capacity of the microcapsule caused by the reaction between crosslinking agent genipin and microcapsule wall material, chitosan and gelatin. An increase in the degree of crosslinking as expressed by molar concentration of genipin used, resulted in a decrease in oil release rate throughout the genipin concentration studied (0.1mmole per gram of polymer-0.5 mmole per gram of polymer). As the degree of crosslinking of microcapsule wall material increased, the microcapsule wall became denser resulting in the decrease of diffusion rate of the oil through the microcapsule wall. Similar type of observation was reported in literature [3]. The probable reactions between gelatin, chitosan and genipin is presented in Fig.4.2.3.

Fig.4.2.2. Effect of variation of cross-linker on the release rate

a: CG(0.5/0.5),ZLO 4.0ml,Gp 0.1mmol ; b : CG(0.5/0.5),ZLO 4 0ml,Gp 0 2mmol

c: CG(0.5/0.5),ZLO 4.0ml,Gp 0.3mmol
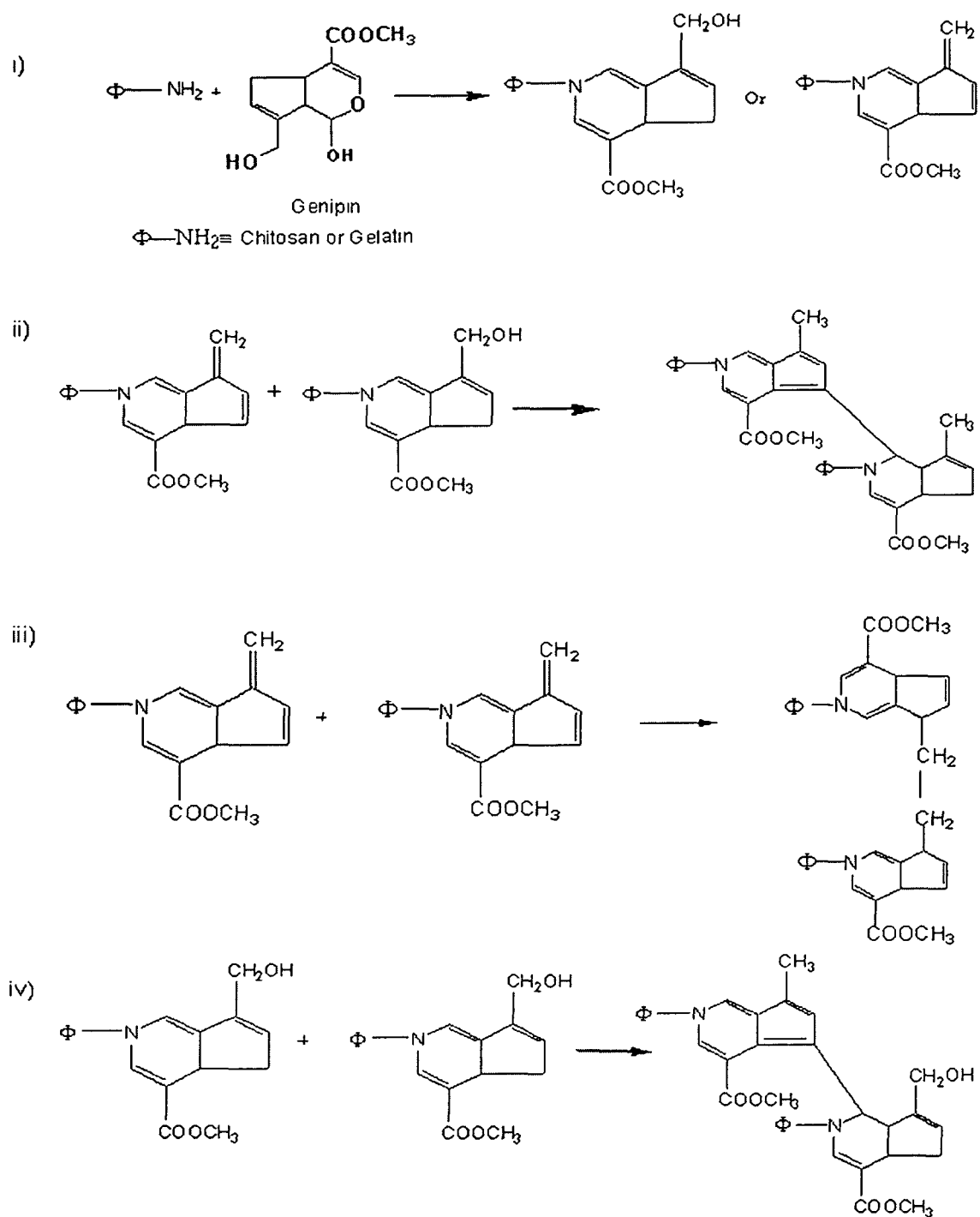
Fig.4.2.3. Reaction scheme between chitosan , gelatin and genipin.

4.2.4. Effect of Variation of Chitosan to Gelatin Ratio

The related results are shown in Table 4.2.1 and Fig.4.2.4. The release rate of oil from gelatin-chitosan microcapsules was dependent on the % of chitosan present in the mixture. The higher the % of chitosan in the chitosan-gelatin mixture, the lower was the release rate. The lower release rate might be due to the formation of microcapsules having more compact wall. It was known that every glucosamine unit of chitosan could react with genipin where as only primary amine groups of lysine and arginine residues on gelatin could react with genipin. The lysine and arginine residues in gelatin are very less. On the other hand, chitosan has more average number of primary amine groups than gelatin for reaction with genipin [10]. The more the % of chitosan in the chitosan-gelatin mixture, the higher the reaction between chitosan and genipin. As a result, more cross linking would take place. This would in turn formed a more compact wall resulting in the decrease of release rate. A decrease in the release rate was reported in the literature during studying of release behaviour of triclosan encapsulated in chitosan – gelatin microcapsules [11].
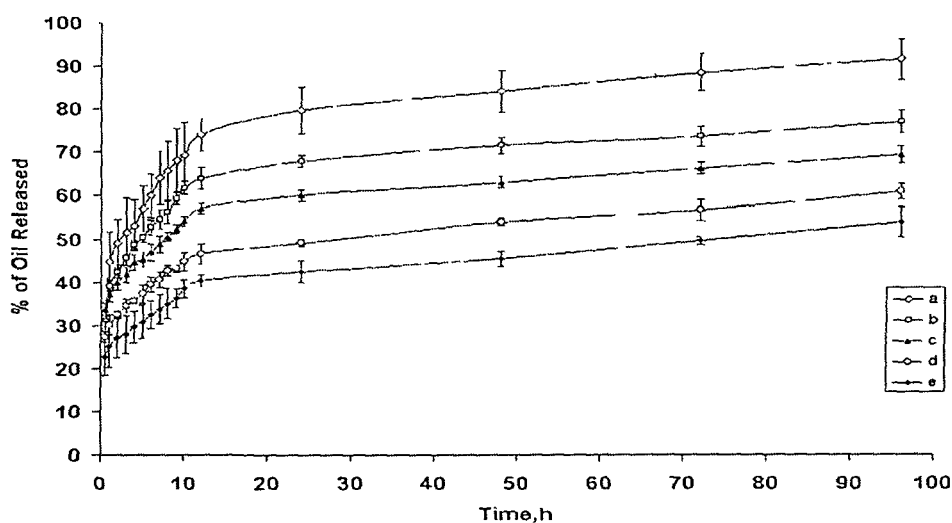
Fig.4.2.4. Effect of variation of chitosan to gelatin ratio on release rate

a: CG(0/1),ZLO 4.0ml, Gp 0.1mmol ; b: CG(0.33/0.67,)ZLO 4.0ml,Gp 0.1mmol

c: CG(0.5/0.5),ZLO 4.0ml,Gp 0.1mmol; d: CG(0.67/0.33),ZLO 4.0ml,Gp 0.1mmol

e: CG(1/0),ZLO 4.0ml,Gp 0.1mmol

4.2.5. FTIR spectroscopic study

Fig.4.2.5 shows the FTIR spectra of gelatin, chitosan, ZLO and ZLO containing chitosan-gelatin microcapsules. The spectrum of ZLO displayed peaks around 1673 and 1720 cm^{-1} which were due to carbonyl stretching band. The other peaks appeared at 1463 and 1383 cm^{-1} were due to CH_2 assymetric and CH_2 symmetric deformation. The spectrum of chitosan showed an amide characteristic peak at 1633 cm^{-1}. Gelatin was characterized by its carbonyl peak and amino band appeared at 1624 and 1547 cm^{-1} respectively. The shifting of carbonyl band to 1641cm^{-1} indicated an interaction between gelatin and chitosan. The position of this peak did not alter when compared to that of spectrum of ZLO. The position of other peaks also were found to remain unchanged. This

suggested that there was no significant interaction between chitosan-gelatin complex and ZLO.

Fig.4.2.5.FTIR spectra of a) gelatin B, b) chitosan, c) oil, d) oil containing microcapsules

4.2.6. Scanning Electron Microscopic Study

Fig.4.2.6 shows the SEM photographs of microcapsules having different % of oil loading. At higher oil loading (Fig.4.2.6 b), a bursting look was observed and it appeared more compared to those of microcapsules prepared at low oil load (Fig. 4.2.6 b). Moreover, on physical examination, the surface of the microcapsules containing higher % of oil appeared more oily and agglomerated compared to those of microcapsules containing lower % of oil.
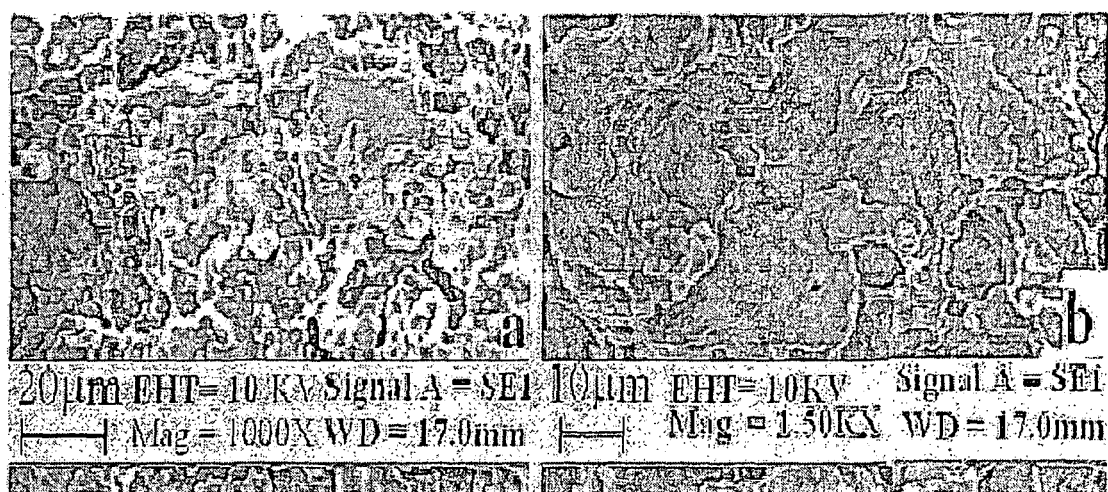
Fig.4.2.6. Scanning electron micrographs of microcapsules prepared with oil

load (%) a) 343.67 b) 687.34

4.2.7. Laboratory Evaluation of Mosquito Repellency of Microcapsules

Table 4.2.2 shows the repellent properties of different prepared formulations. It was observed that the protection time varied between 0-3.5h. The formulation (A) did not provide any protection as there was no active repellent agent. The formulation (shown in G) based on DEET provided protection lasting for 2.5 h. The mean protection time for formulation based on chitosan-gelatin microcapsules (C-F) was in the range 2.0-3.5 h

Table.4.6. Repellency of different Mosquito repellent formulations

Formulae name	Formulations	Mean protection time (h)
A	Petroleum Jelly	0
B	Petroleum jelly + 20% ZLO	1.5
C	Petroleum jelly + 20% ZLO containing microcapsules (a)	2.0
D	Petroleum jelly + 20% ZLO containing microcapsules (b)	2.5
E	Petroleum jelly + 20% ZLO containing microcapsules (c)	3.5
F	Petroleum jelly + 20% ZLO containing microcapsules (d)	3.0
G	Petroleum jelly + 20% DEET	2.5

(a): CG(0.5/0.5),ZLO 4.0ml,Gp 0.1mmol; (b): CG(0.5/0.5),ZLO 6.0ml,Gp 0.1mmol

(c): CG(0.5/0.5),ZLO 8.0ml,Gp 0.1mmol; (d): CG(0.5/0.5),ZLO 8.0ml,Gp 0.2mmol

In all the formulations, the amount of oil / or microcapsules were taken in such a way that the percentage of oil remained same in every formulations. Formulation E showed highest protection time. The order of protection time were as follows: E > F > D or G > C > B> A. Microcapsules used in the formulation were prepared by varying the amount of oil and crosslinker concentration. It was observed that formulations based on higher oil loading based microcapsules showed better protection compared to those of

lower oil loading microcapsules. The present level of crosslinker used (0.1-0.2 mmol) had little role compared to oil loading. The crosslinking efficiency remained same within the range of crosslinker concentration studied. Microcapsules prepared by variation of oil loading had much role in manifesting the protection time. The thickness of microcapsules prepared from higher oil loading was less as discussed earlier. This led to an increase in the sufficient release of oil to produce a vapour shield and thereby protection.

4.3. Microencapsulation of *Zanthoxylum limonella oil* (ZLO) in genipin crossliked Chitosan-gelatin complex using coacervation technique for Mosquito Repellent Application

Introduction

In this part the author has chosen complex coacervation technique for the encapsulation of *Zanthoxylum limonella oil* (ZLO) in genipin crosslinked chitosan-gelatin microcapsules. The effects of various parameters such as oil loading, degree of crosslinking, ratio of chitosan to gelatin etc. on oil content, encapsulation efficiency and the release rate of ZLO have been reported here. FT-IR spectroscopy has been interpreted to describe the interaction between the polymers and oil. Thermal properties have also been studied and interpreted. Scanning electron microscopy (SEM) has been shown to study the morphology of the prepared microcapsules. Lastly the microcapsules have been mixed with petroleum jelly and their effectiveness as mosquito repellent was studied in the laboratory against standard mosquito repellent and reported in this part.

Results and Discussion

4.3.1. Complex Coacervation between chitosan and gelatin

Pure gelatin B solution was scanned between 450-600nm at different pH using UV spectrophotometer. The % transmittance studied in the above wavelength was found to follow more or less similar trend at different pH. For chitosan, the % transmittance at the above scanned wavelength remained unchanged up to a certain pH (~6.00), beyond that the % transmittance decreased due to precipitation.

Chitosan-gelatin mixture of different ratios showed the trend similar to those of chitosan. However, in the case of both chitosan and chitosan/gelatin mixture, the maximum absorption occurred at lower wavelength. Therefore all the successive measurements were done at 450nm and reported.

To optimize coacervation behaviour, the study of phase separation behavior is essential. This was determined by measuring turbidity as well as coacervate yield.

Turbidity measurement

Turbidity measurements were done to optimize the coacervate system. Fig.4.3.1 shows the plot of absorbance vs. pH at two different chitosan-gelatin ratios. At both the chitosan-gelatin ratio, the absorbance remained unchanged up to a certain pH. Thereafter it increased attaining to a maximum and then decreased with the increase in the pH of the medium. Maximum absorbance was noticed in the pH range 5.7-5.9. The higher the absorbance, the higher was the turbidity. The appearance of turbidity, which is related to

coacervate formation, was due to the presence of scattered particles in the medium. The concentration as well as size of the dispersed particles was very much important for scattering of light.
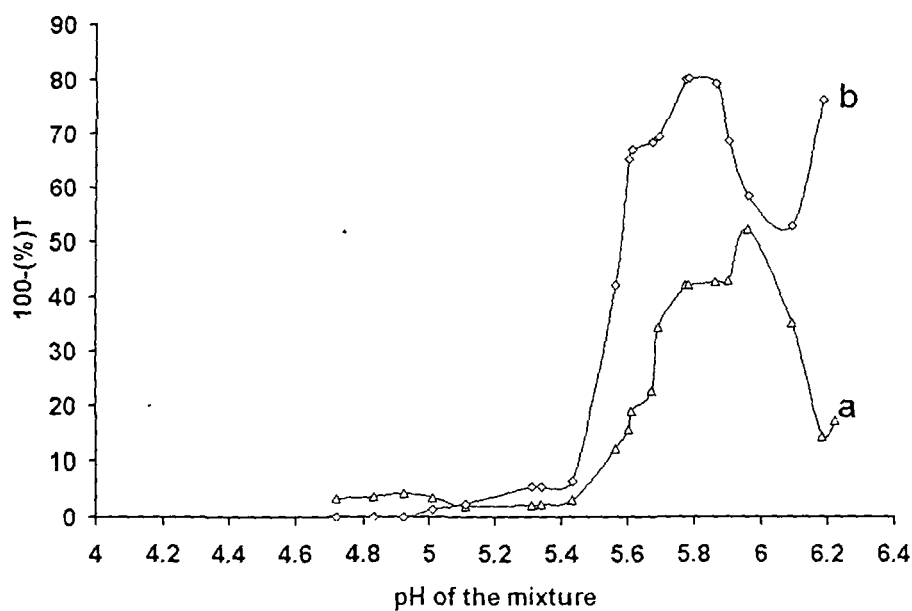

Fig.4.3.1 Absorbance of chitosan-gelatin mixture at different pH

[a: total polymer conc. = 0.91%(w/v), gelatin : chitosan =1:10 ; b: total polymer conc. =0.975%(w/v), gelatin : chitosan =1:40]

Coacervate Yield

Preliminary investigations showed that polymer ratio, total polymer concentration, pH etc. played a significant role on coacervate yield. Therefore the effect of the above parameters on coacervate yield was studied and reported.

a) Effect of variation of gelatin-chitosan ratio on coacervate yield

The effect of different chitosan gelatin ratio on coacervate yield (%) at 0.95 %

(w/v) total polymer concentration is shown in Fig.4.3.2.The pH of the system was kept

fixed at 5.50. Coacervate yield (%) was found maximum at 1:10 gelatin-chitosan ratio,

thereafter it decreased with the increase of the gelatin-chitosan ratio. At this ratio,

maximum interaction between oppositely charged groups of chitosan and gelatin

probably took place, which resulted in the formation of maximum yield. Above or below

this ratio uncomplexed positively or negatively charged groups of either chitosan or

gelatin were excess, thus keeping the complex in solution. Similar results were reported

by Lopez et al [12].
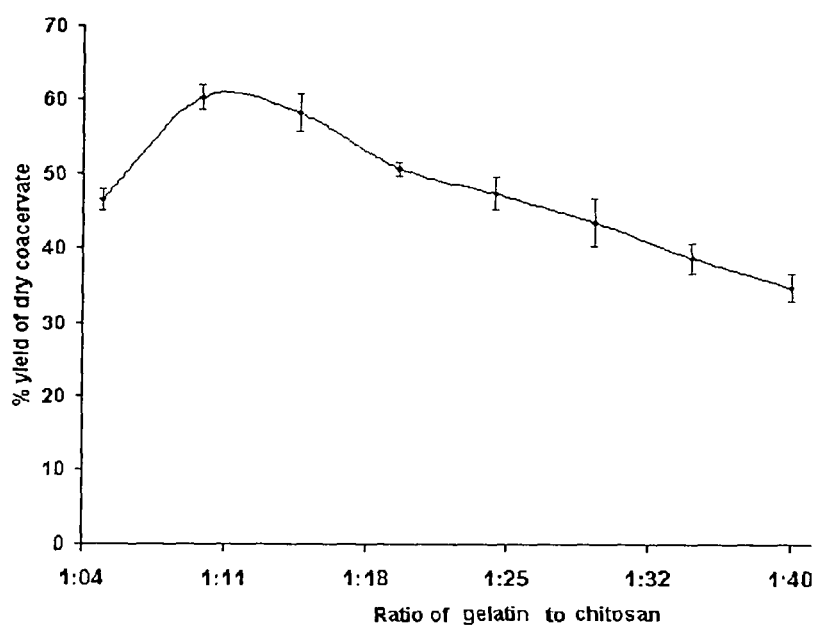
Fig.4.3.2 Effect of variation of chitosan to gelatin ratio on coacervate yield

[Total polymer concentration=0.95% (w/v); pH=5.50;

Temperature=30 ± 1^{0}C]

b) Effect of total polymer concentration on coacervate yield

Table 4.3.1 shows the effect of total polymer concentration on coacervate yield (%). Total polymer concentration (%) and pH were varied from 0.25 to 0.95 and 5.24-5.85 respectively. As expected, the amount of coacervate formed increased with the increase in the total polymer concentration. But the coacervate yield (%) remained almost constant within the pH range studied. Lopez and Bodmeier [12] observed and reported similar results.

Table 4.3.1. Effect of variation of total polymer concentration on coacervate yield

[Gelatin: Chitosan = 1:20; pH = 5.24-5.85; Temperature=30±1⁰C]

pH	Coacervate yield (%) at total polymer concentration (%)		
	0.25	0.47	0.95
5.29±0.05	48.34±0.99	47.34±2.77	50.61±3.18
5.5±0.05	49.7±0.10	49.33±0.95	50.8±0.66
5.8±0.05	48.47±0.28	49.38±0.24	49.12±0.22

c) Effect of variation of pH on coacervate yield

Fig. 4.3.3 shows the effect of variation of pH on coacervate yield. The coacervate yield was studied in the pH range 5.0 to 6.0 using different polymer concentration and polymer ratio. In all the cases, maximum yield was formed between 5.4 -5.9. Chitosan remained positively charged in solution whereas the charge of gelatin in solution was dependent on the pH. The yield was found low either below or above this

pH range. In the above-mentioned pH range, oppositely charged particles of chitosan and gelatin neutralized each other completely forming strong binding and maximum coacervate yield. Above or below this pH range, interaction between chitosan and gelatin decreased causing a reduction in coacervate yield.
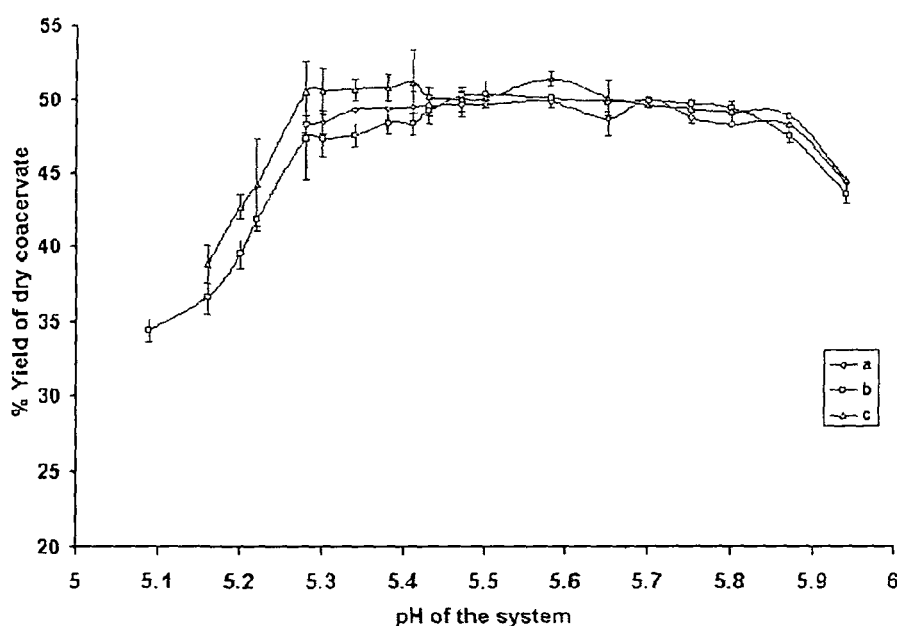

Fig 4.3.3 Effect of variation of coacervate yield with pH at different polymer
concentration (Gelatin : Chitosan =1:20, Temperature=30±1°C) [a:
total polymer conc.=0.25%(w/v); b: total polymer conc.=0.47%(w/v);
c: total polymer conc.=0.95%(w/v)]

Oil Release Studies

4.3.2. Effect of variation of oil loading on release rate

The effect of variation oil loading on encapsulation efficiency and release rate for 1:1 chitosan-gelatin microcapsules are presented in Table 4.3.2 and Fig.4.3.4.The more

the oil-load, the higher was the release rate. The lower in encapsulation efficiency might be due to the higher % of oil loss during isolation of microcapsules.

At low oil load, small oil vesicles were formed as the dispersive force of the stirrer was more effective. The % of chitosan-gelatin mixture was enough to encapsulate properly the oil vesicles. With the increase in oil-load, the dispersive force of the stirrer became less efficient which resulted in the formation of large oil vesicles. At this stage, chitosan-gelatin mixture could be able to encapsulate the large oil vesicles only at the expense of decrease in thickness of microcapsule wall. Besides this, the amount of chitosan-gelatin mixture might not be sufficient to encapsulate all the oil vesicles. Some oil vesicles might present without encapsulation. These oil vesicles got lost during recovery of microcapsules. As wall thickness decreased, the diffusional path for the oil became short which resulted in an increase of release rate [1,2].

Again oil content (%) was found to increase with the increase in the % of oil load. At low oil load, many of the microcapsules probably contained few oil vesicles indicating that there was an abundance of encapsulating polymer for the oil present. As oil load (%) increased, the number of oil vesicles in the microcapsules increased which resulted in an increase in oil content.

Table 4.3.2. Effect of variation of oil loading, chitosan to gelatin ratio and genipin concentration on the behaviour of microcapsules [Total polymer =1 g; genipin =(0.05-0.5mmol/gm of polymer; oil = (1-4 ml); water = 100ml; Temp.= 40 ± 1^0C]

Sample particulars			Oil load (%)	Oil Content (%)	Encapsulation efficiency (%)
Chitosan : gelatin	Genipin (mmol)	ZLO (ml)			
0.25: 1	0.05	4	308.75	26.45±1.20	34.14±1.74
0.66: 1	0.05	4	308.75	25.15±0.57	32.47±1.07
2: 1	0.05	4	308.75	30.65±0.54	39.56 ±1.74
4: 1	0.05	4	308.75	37.65±0.75	48.60±1.14
1: 1	0.05	1	77.20	22.32±0.15	48.31±0.84
1: 1	0.05	2	154.37	23.68±0.51	44.3 ± 0.35
1: 1	0.05	3	231.56	28.0 ± 0.12	32.87± 0.96
1: 1	0.10	4	280.27	42.0 ± 0.45	54.22 ±1.12
1: 1	0.20	4	236.60	45.34±0.87	58.53 ±1.36
1: 1	0.50	4	161.26	46.50±0.64	60.05 ± 0.89

Fig.4.3.4   Effect of variation of oil loading on release profile

[a: polymer 1.0 gm; crosslinker 0.05mmol; ZLO 1.0 ml , b: polymer

1.0 gm; crosslinker 0.05mmol ; ZLO 2.0ml   c: polymer 1.0 gm;

crosslinker 0.05mmol ; ZLO 3.0 ml]

4.3.3. Effect of variation of chitosan/gelatin ratio on release rate

The effect of variation of chitosan-gelatin ratio on oil loading, encapsulation

efficiency and release rate are shown in Table 4.3.2 and Fig.4.3.5. The release rate of oil

was governed by the % of chitosan present in the chitosan-gelatin mixture. With the

increase in the concentration of chitosan in chitosan-gelatin mixture, the release rate was

found to decrease. Again an increase in the viscosity of the chitosan-gelatin mixture was

noticed with the increase in the concentration of chitosan.
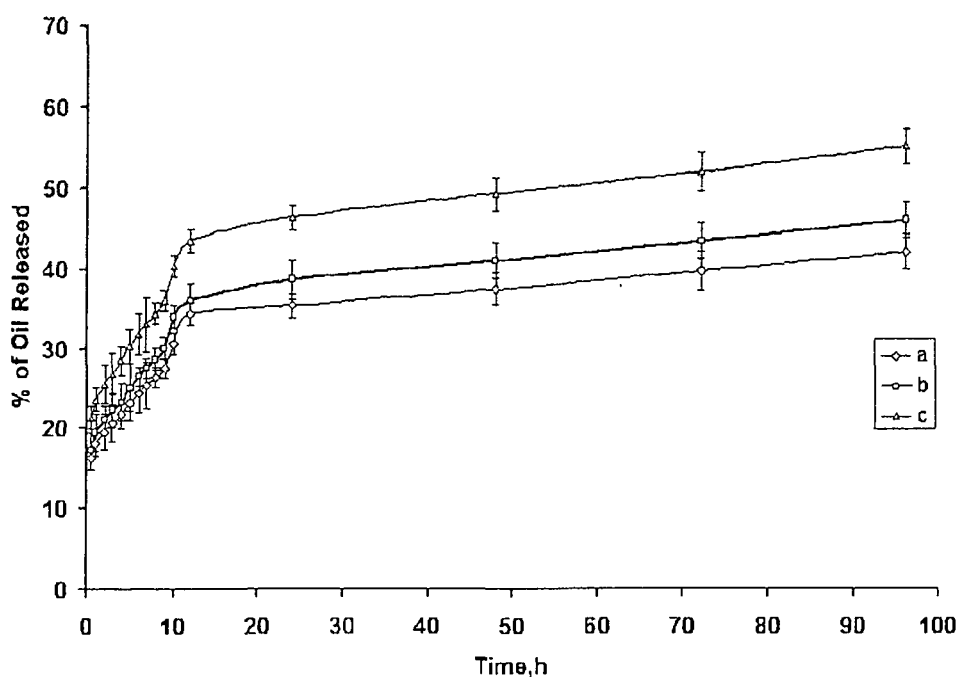
Fig.4.3.5.Effect of variation of chitosan to gelatin ratio on release profile

[Total polymer 1.0 gm; Ratio of chitosan to gelatin in a: 0.25:1,

b=0.66:1,c=2:1,d=4:1; ZLO 4.0ml; crosslinker 0.05mmol]

The higher viscosity might decrease the dispersive force of the stirrer. As a result

large oil vesicles were formed. The decrease in surface area could be responsible for the

decrease in release rate. Moreover, chitosan has more average moieties of primary amine

groups than gelatin. Chitosan could react with genipin to form sufficient crosslink bridges

compared to gelatin. This might also play a role in reduction of release rate. Similar

observations were reported by Kim et al [11] during the study of the release behaviour of

triclosan encapsulated within chitosan-gelatin microcapsules.

Both oil content (%) and encapsulation efficiency were also found to increase

with the increase in the chitosan concentration. As explained earlier, the increase in

viscosity of the medium resulted in the formation of large oil vesicles. These large oil

vesicles had a tendency to coalesce at higher oil load to form further large oil vesicles and therefore more oil could be encapsulated with the same amount of encapsulating material.

4.3.4. Effect of variation of concentration of genipin on release rate

Results showing the oil load (%), oil content (%) and encapsulation efficiency are shown in Table 4.3.2. The release profile of the oil is shown in Fig.4.3.6. The trend shown by both oil loading and oil content was as per expectation. Encapsulation efficiency increased with the increase in genipin concentration. The concentration of genipin was varied from 0.1 –0.50 m mol/g of polymer mixture. The increased efficiency was due to the higher oil retention capacity of the microcapsules caused by the formation of crosslinking. The crosslinking reaction took place between genipin, gelatin and chitosan. The release rate of oil was found to decrease as the % of genipin increased. The microcapsule wall became compact as degree of crosslinking increased. This resulted in the decrease of diffusion rate through the microcapsule wall. Similar findings were cited in the literature [2].
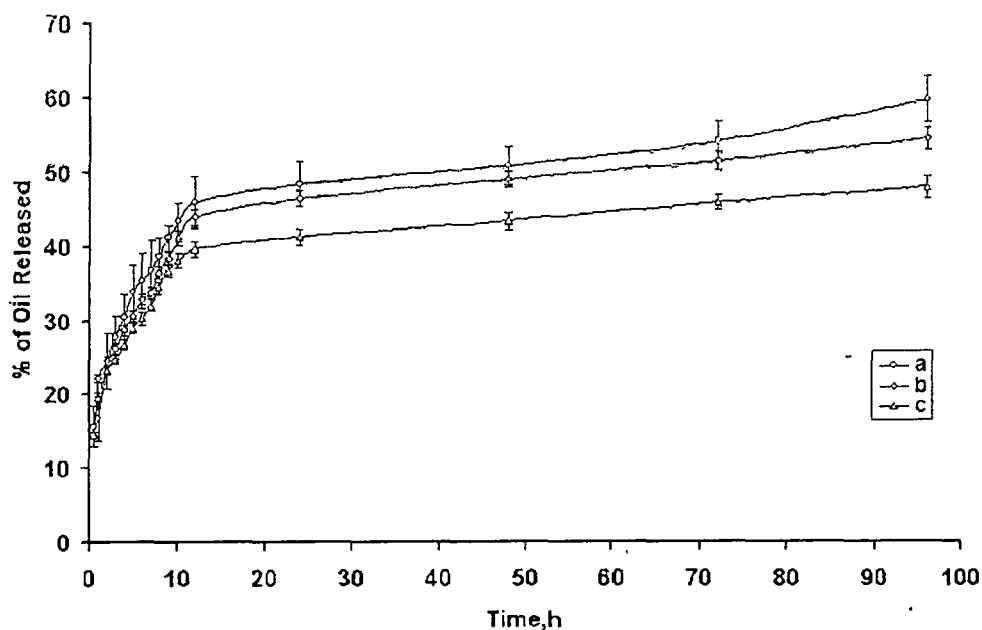
Fig.4.3.6 Effect of variation of crosslinker concentration on release profile

[a: polymer 1.0 gm; crosslinker 0.1mmol ; ZLO 4.0 ml , b: polymer

1.0 gm; crosslinker 0.2mmol ;ZLO 4.0ml c: polymer 1.0 gm;

crosslinker 0.5mmol ; ZLO 4.0 ml]

4.3.5. FTIR Study

FTIR spectra of chitosan, gelatin, ZLO and chitosan/gelatin microcapsules containing ZLO were recorded and presented in Fig.4.3.7.The spectrum of chitosan displayed a strong amide characteristic peak at $1632cm^{-1}$. Similarly gelatin spectrum also showed an amino band at $1547cm^{-1}$ and carbonyl peak at $1624cm^{-1}$. In ZLO, the peaks appeared between $1638-1720cm^{-1}$ were due to carbonyl stretching band. Besides this, the other notable peaks appeared at $1457 cm^{-1}$ and $1378cm^{-1}$ were due to CH_2 asymmetric deformation and CH_2 symmetric deformation. In the microcapsules, the carbonyl band shifted to $1641cm^{-1}$ indicating an interaction between chitosan and gelatin complex. The

position of these peaks remained almost unchanged when compared to that of spectrum

of ZLO. The position of other peaks which were due to CH_2 asymmetric deformation was

also remained unchanged. This suggested that there was no significant interaction
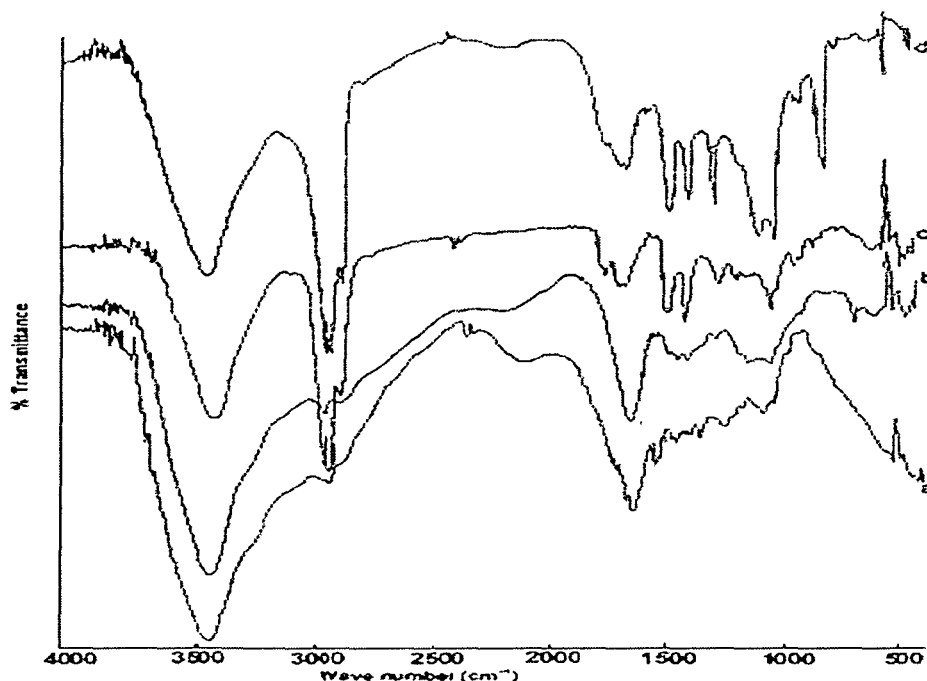
between ZLO and chitosan gelatin complex.

Fig.4.3.7.FTIR spectra of a) gelatin B b) chitosan c) oil d) oil containing
Microcapsules

4.3.6. Thermogravimetric Analysis

Table 4.3.3 shows the initial decomposition temperature (Ti) and residual weight

(RW, %) of virgin polymers (chitosan and gelatin), ZLO and ZLO containing

microcapsules. Both the Ti (^{0}C) and RW (%) were found to increase with the increase in

chitosan concentration in the chitosan-gelatin mixture. ZLO started to decompose at an

early stage and there was no residue at 600^0C.

Table 4.3.3.Thermal Analytical data for virgin polymer and oil containing

microcapsules

Sample		Ti (^{0}C)	RW(%)
Gelatin : Chitosan	Oil (ml)		at 600^{0}C
1: 0	-----	236	8.75
1:0.5	4.0	175	9.33
1:1	4.0	190	23.06
0:1	....	273	37.74
.....	*Oil	----	------

Ti : Initial decomposition temperature

RW: Residual weight

*   : Started decomposing from the very beginning

Temperature of decomposition (T_D) values of ZLO/chitosan/gelatin microcapsules, chitosan, gelatin and oil at different weight loss (%) are shown in Table 4.3.4. T_D values for the microcapsules increased with the increase in the % of chitosan in the microcapsules. This observed high values might be due to the decreasing chance of elimination of small molecules like NH_3, CO_2 etc. with the formation of crosslinking by genipin. Gelatin contains lower % of lysine and arginine residues as primary amine groups. Chitosan contains glucosamine unit in larger percentage. Genipin could react with the primary amine group of gelatin and glucosamine unit of chitosan. The reaction rate of chitosan and genipin was reported more compared to that of gelatin [10]. So

chitosan could form more crosslink bridges compared to that of gelatin and thereby would lead to more thermally stable microcapsules.

Table 4.3.4. Temperature of decomposition (T_D) at different weight loss (%) of virgin polymer and oil containing microcapsules

Sample particulars		Temperature of decomposition (T_D)(^{0}C) at different weight loss (%)				
Gelatin : Chitosan	oil	20	40	60	70	80
1: 0	----	286	331	394	438	529
1: 0.5	4	236	300	354	370	446
1: 1	4	200	302	366	448	---
0: 1	---	294	320	543	---	---
---	oil	90	115	155	---	228

The difference in Ti values for various samples could be explained on the basis of their difference in rate of decomposition. The crosslinking reaction of genipin with chitosan was higher compared to that of gelatin as per explanation given above. Moreover oil decomposed at fast rate. Both of these influenced the rate of decomposition and were responsible for different Ti values.

4.3.7. Differential Scanning Calorimetric Study

The DSC thermogram of pure chitosan (a), pure gelatin-B (b), oil (c) and oil loaded chitosan/gelatin microcapsules (d) are presented in Fig.6. Pure chitosan showed

peaks at 98 °C, 271 °C and 340 °C respectively. Pure gelatin B showed peaks at 95 °C and some multiple peaks in the temperature range 226-323°C. Pure oil showed a peak at 90 °C and another broad peak having average peak temperature at 200 °C. Oil encapsulated chitosan/gelatin microcapsules showed a sharp peak at 120 °C and another two peaks (in shoulder form) having average peak temperature at 240 °C and 320°C.The peaks appeared in the temperature range 95-98 °C were due to the removal of moisture. The position of one peak appeared in the thermogram (not shown) of physical mixture of chitosan/gelatin/oil at 95 °C was found to disappeared and a new peak appeared (in shoulder form) having average peak temperature at 240 °C and 320 °C.The peaks appeared in the temperature range 95-98 °C were due to the removal of moisture. The position of one peak appeared in the thermogram (not shown) of physical mixture of chitosan/gelatin/oil at 95 °C was found to disappear and a new peak appeared (shown at 120 °C) when genipin was used (crosslinked samples). The position of other two peaks in the thermogram of physical mixture remained almost unchanged irrespective of addition of genipin. The peaks found at 240 °C and 320 °C in crosslinked oil loaded microcapsules were mainly due to the decomposition of oil and chitosan-gelatin complex respectively. The position of these peaks exhibited in both the thermograms of physical mixture and crosslinked microcapsules suggested that a low compatibility in thermal properties existed in the relation between oil and gelatin –chitosan complex.
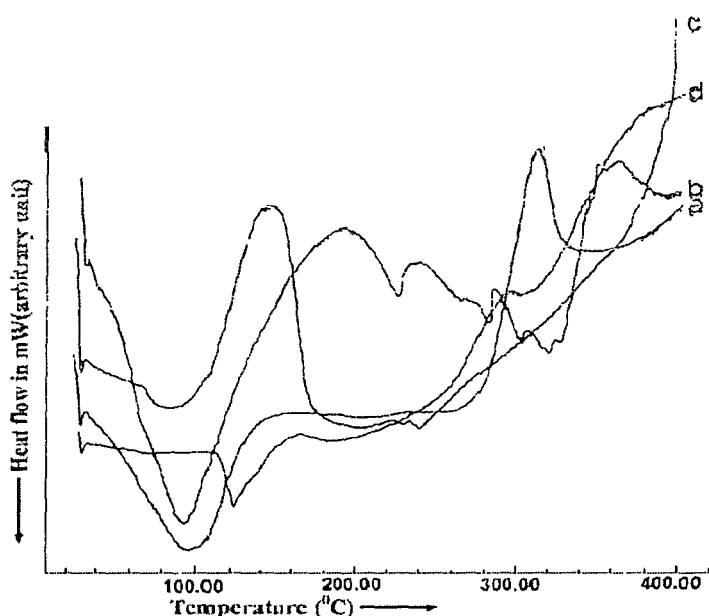
Fig 4.3.8. DSC thermograms of a) chitosan b) gelatin B c) oil   d) oil

containing microcapsules

4.3.8. Scanning Electron Microscopy Study

SEM micrographs of genipin crosslinked chitosan-gelatin microcapsules having

different percentage of oil content are shown in Fig.4.3.9. At low oil loading (Fig.4.3.9a),

the surface of the microcapsules appeared smooth compared to those of microcapsules

prepared at higher oil loading (Fig.4.3.9 b, c). At higher oil loading, a bursting look was

observed due to the presence of large percentage of oil. Similar observations were

reported in the literature [13]. The surface of the microcapsules became more irregular as

the percentage of oil loading increased. Fig. 4.3.9d shows the micrograph of the

microcapsules after release of substantial amount of oil. The surface of the microcapsules

contained a significant number of pinholes (arrow marked). These pinholes might be

formed due to the release of oil by diffusion. Moreover, on physical verification, the

microcapsules prepared at higher oil loading appeared oily and agglomerated where as those prepared at low oil loading appeared dry and powdery.
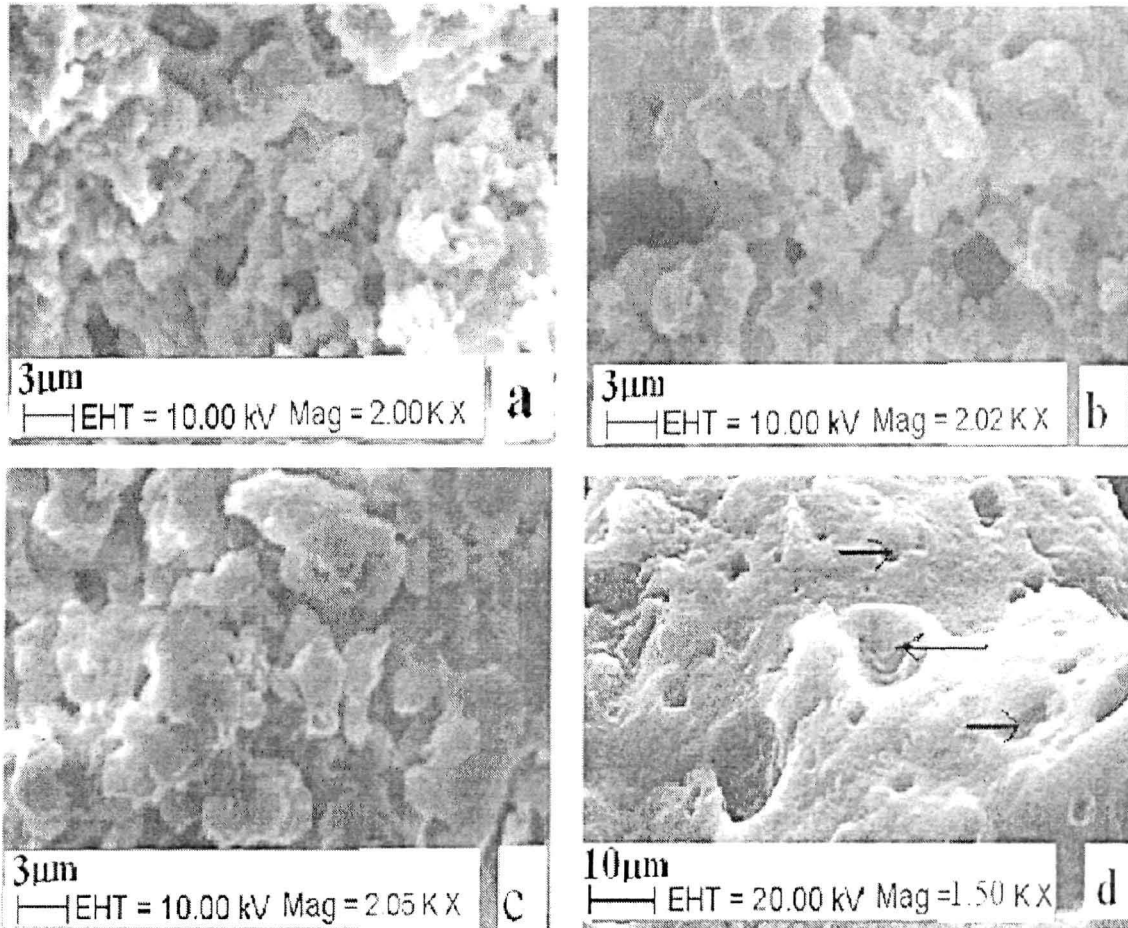
Fig.4.3.9. Scanning electron micrographs of microcapsules prepared with oil load (%) a) 154.37 b) 308.75   c) 617.02 d) 154.37(after oil release)

4.3.9. Laboratory Evaluation of Mosquito Repellency of Microcapsules

Table 4.3.5 shows the repellent properties of different prepared formulations. It was observed that the protection time varied between 0-3 h. The formulation (A) did not provide any protection, as there was no active repellent agent. The formulation (shown in

G) based on DEET provided protection lasting for 2.5 h. The mean protection time for

formulation based on chitosan-gelatin microcapsules (C-F) was in the range 2.0-3.0 h

Table 4.10. Repellency of different mosquito repellent formulations

Repellents	Formulations	Mean protection time (h)
A	Petroleum jelly	0
B	Petroleum jelly + 20% ZLO	1.5
C	Petroleum jelly + 20% ZLO containing microcapsules (a)	2.5
D	Petroleum jelly + 20% ZLO containing microcapsules (b)	2.5
E	Petroleum jelly + 20% ZLO containing microcapsules (c)	3.0
F	Petroleum jelly + 20% ZLO containing microcapsules (d)	3.0
G	Petroleum jelly + 20% DEET	2.5

(a): CG(0.5/0.5),ZLO 4.0ml,Gp 0.1mmol; (b): CG(0.5/0.5),ZLO 6.0ml,Gp 0.1mmol

(c): CG(0.5/0.5),ZLO 8.0ml,Gp 0.1mmol; (d): CG(0.5/0.5),ZLO 8.0ml,Gp 0.2mmol

Similar level of oil content was maintained in all the formulations by using different amount of microcapsules. The highest protection time was shown by formulation E and F. The protection time followed the order: E, F > C, D, G >B >A. Formulations base on microcapsules prepared from higher oil loading produced better protection compared to those of formulations based on lower oil loading microcapsules. Formulations E and F where different level of crosslinker were used indicated that the

present level of crosslinker had minor role. The crosslinking efficiency remained same within the range of crosslinker used (0.1-0.2 mmol). Therefore the release rate as well as protection time would be same.

Formulations based on microcapsules prepared from higher oil loading had shown better protection time compared to those of formulation based on microcapsules prepared from lower oil loading. Thickness of the microcapsules was probably responsible for showing such results. Higher the oil loading, the lower was the thickness of the microcapsule wall. As a result release rate of oil would be fast and as a consequence higher would be protection time.

4.4. Degree of Deacetylation of Chitosan: Determination and Their influence on the Release Behavior of Essential Oil from Chitosan and Chitosan-Gelatin Complex Microcapsules

Introduction

The most important parameter for characterizing a given chitosan specimen is the degree of deacetylation (DDA). The degree of deacetylation (DDA) influences the physical, chemical and biological properties of chitosan, such as acid base and electrostatic characteristics, biodegradability, self aggregation, sorption properties, and the ability to chelate metal ions.

In this part, chitosans of varying degree of deacetylation (DDA) have been prepared and their DDA has been detemined by various techniques.The effect of DDA on

characteristics of ZLO encapsulated chitosan and chitosan-gelatin microcapsules have been reported.

Results and Discussion

4.4.1 Molecular weight of chitosan

The molecular weights of untreated as well as alkali treated samples were determined and are presented in Table 4.4 1. From the table, it was observed that alkali treatment produced lower molecular weight product compared to that of untreated ones. Furthermore, higher the duration of alkali treatment lower was the molecular weight of chitosan sample. With the alkali treatment, the number of amine groups ($-NH_2$) increased while the number of acetylamide ($-NHCOCH_3$) group decreased [18]. The higher the duration of alkali treatment, the higher was the formation of amine groups and as a result a decrease in molecular weight was observed. Besides this, higher concentration of alkali might help to depolymerise the chitosan causing a decrease in molecular weight [14,15].

Table 4.4.1. Molecular weight of untreated and alkali treated chitosan samples

Chitosan Samples	Intrinsic Viscosity, $[\eta]$ (dl/g)	Molecular Weight, Mw x 10^5 (g/mol)
Chitosan (supplied) (Untreated)	6.859	⊕ 9.96
Chitosan (4.0h alkali treated)	6.522	9.44
Chitosan (8.0h alkali treated)	6.099	8.78

4.4.2. Degree of deacetylation

The variation of degree of deacetylation (DDA) of various chitosan samples has been determined by applying different methods of analysis as discussed below. The degree of deacetylation in the original sample and samples treated with alkali for 4.0 h and 8.0 h were determined potentiometrically. The measured degree of deacetylation (DDA) were 54.6, 60.5 and 84.7 for untreated, 4 h treated and 8h treated samples respectively.

The IR spectra of untreated and treated chitosan samples (in powder and film form) were done and DDA was calculated using the standard equation. The ratio of absorbance of amide-I at 1655 cm^{-1} to that of hydroxyl group at 3450cm^{-1} in chitosan depends upon the degree of deacetylation in the chitosan [16]. The lower the absorption of amide-I group, the higher is the degree of deacetylation. The absorbance corresponding to amide-I group was found to decrease with the increase in the time of alkali treatment.

The degree of deacetylation of untreated and alkali treated chitosan samples were determined by elemental analyzer and using standard equation. The weight percent of carbon and nitrogen were used to calculate the degree of deacetylation. The results obtained by different methods are shown in Table 4.4.2. Treated samples showed higher DDA compared to untreated sample. DDA was found to increase with the increase of duration of alkali treatment. The reason for the observed change in DDA could be explained as earlier. The more the duration of alkali treatment, the more was the formation of amine groups and as a result an increase in DDA was observed. In all the methods, the calculated values of DDA were same except in the case of film samples. Film samples showed higher DDA values as compared to those measured by other analytical techniques. The reason for this was not clear. Similar type of results were obtained and reported in literature [17].

Table.4.4.2. Degree of deacetylation of various chitosan samples determined by different methods

Samples of chitosan	DDA determined by potentiometric method	DDA determined by IR method		DDA determined by elemental method	
		Powdery sample	Film sample	On the basis of wt% of C	On the basis of wt% of N
Sample-1 (Untreated)	54.60	55.80	60.40	55.80	55.66
Sample-2 (treated for 4 h)	60.50	61.30	65.80	61.30	60.85
Sample-3 (treated for 8 h)	84.70	85.40	89.70	85.30	85.85

4.4.3 Effect of Variation of DDA on Characteristics of Microcapsules

The effect of variation of DDA of chitosan samples on oil loading (%), oil content (%), encapsulation efficiency (%) and release rate are shown in Table 4.4.3 and Fig.4.4.1. In both chitosan and chitosan-gelatin complex microcapsules, release rate was found to increase with the increase in the degree of deacetylation. As per expectation, oil loading (%) was found to be remaining constant. As DDA increased, oil content (%) and encapsulation efficiency (%) of both type of microcapsules showed a decreasing trend. This might be attributed to the presence of free amine groups in the microcapsules. The higher the degree of deacetylation, the more was the primary amine group [17].
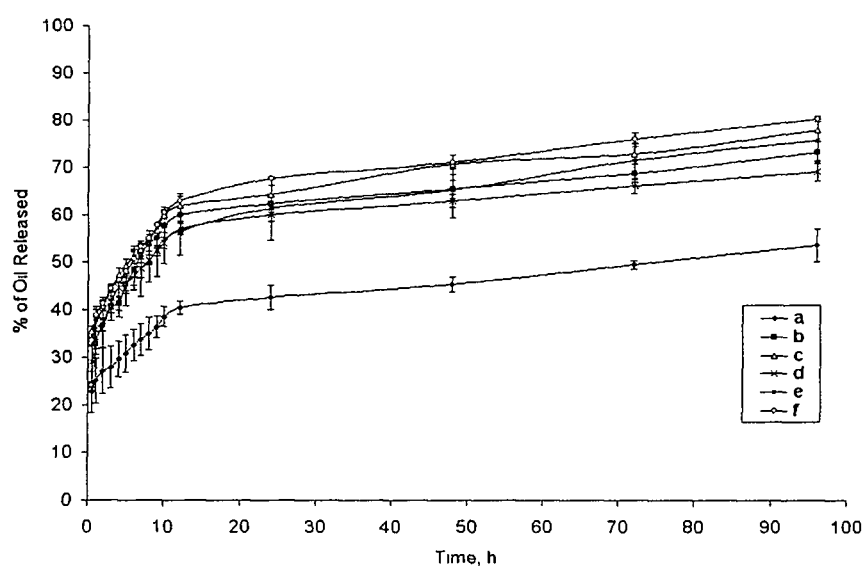
Fig.4.4.1 Effect of variation of DDA of chitosan on release of ZLO from microcapsules, (a) Ch (untreated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol, (b) Ch (4 h treated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol; (c) Ch (8 h treated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol (d) Ch (untreated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol; (e) Ch (4 h treated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol; (f) Ch (8 h treated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol

The crosslinking agent genipin, might not be sufficient to crosslink all the amine groups present in either chitosan / or chitosan gelatin complex. This resulted in the formation of a less compact wall of the microcapsules. Moreover the hydrophilicity of chitosan or chitosan gelatin complex increased due to presence of more amine groups. Both of these might be responsible for the observed trend. Similarly the reason for the lower encapsulation efficiency (%), oil content (%) and higher release rate of oil from microcapsules prepared by using a combination of chitosan and gelatin might be due to the presence of more amine groups in chitosan-gelatin complex compared to those of either untreated or treated chitosan. The observed encapsulation efficiency, oil content and release rate might be explained on the basis of crosslinking efficiency and hydrophilicity as discussed earlier. However, an opposite trend in the oil content, encapsulation efficiency and release rate of oil was observed when the microcapsules contained higher amount of crosslinker (0.3 mmol/ g of polymer) as shown in Fig.4.4.2. Higher amount of crosslinker and higher number of amine groups present in deacetylated chitosan and chitosan-gelatin complex microcapsules might form higher degree of crosslinking leading to an increase in oil content and encapsulation efficiency. As the degree of crosslinking increased, the microcapsule wall became denser, resulting in the decrease of diffusion rate of the oil through the microcapsule wall . Thus the release rate of oil from chitosan-gelatin / or chitosan microcapsule having higher DDA was found less compared to those of either untreated or lower   deacetylated chitosan microcapsules.
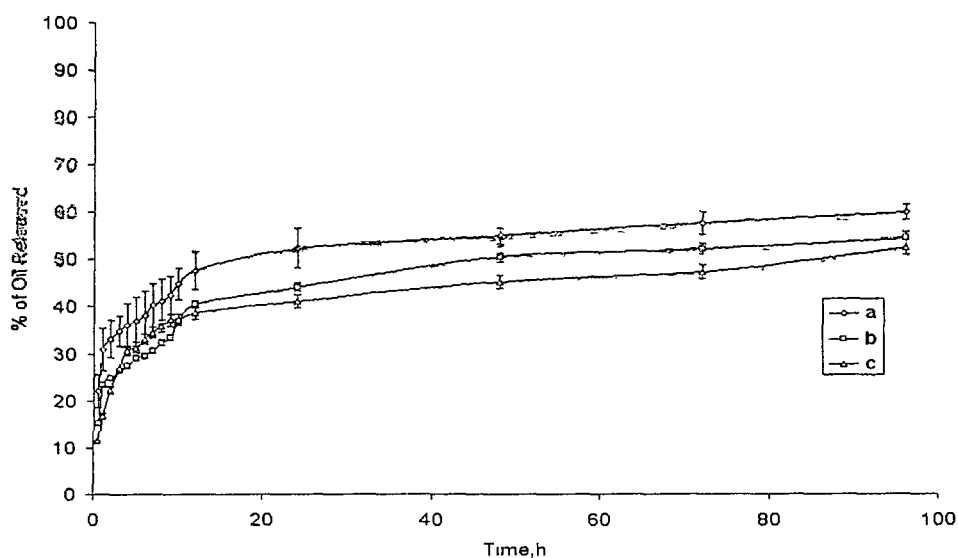
Fig. 4.4.2. Effect of variation of DDA of chitosan on release of ZLO from chitosan-gelatin microcapsules containing higher amount of crosslinking agent; (a) Ch (untreated) 1.0 g, ZLO 4.0ml, Gp 0.3 mmol; (b) Ch (4 h treated) 1.0 g, ZLO 4.0ml, Gp 0.3 mmol; (c) Ch (8 h treated) 1.0 g, ZLO 4.0ml, Gp 0.3 mmol.

4.4.4. Swelling of Microcapsules

The percentage of swelling of the microcapsules against time is presented in Fig.4.4.3. With the increase in the degree of deacetylation, swelling (%) of both chitosan and chitosan-gelatin microcapsules increased. Higher degree of deacetylation produced higher number of primary amine groups [17]. The number of free amine groups was more in chitosan-gelatin complex. The amount of cross-linking agent used might not be able to crosslink all the amine groups. As a result more and more free amine groups would be available as DDA increased. These amine groups increased the hydrophilicity and thereby swelling.
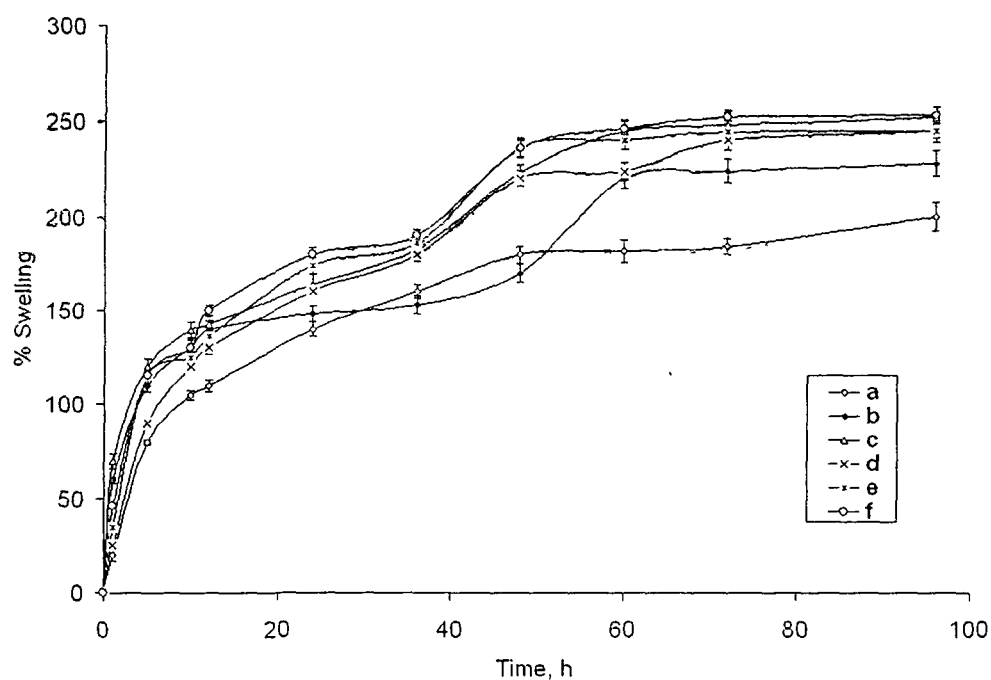
Fig 4.4.3. Swelling of microcapsules in water (a) Ch (untreated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol; (b) Ch(4 h treated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol; (c) Ch (8 h treated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol; (d) Ch(untreated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol; (e) Ch(4 h treated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol; (f) Ch(8 h treated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol

Table.4.4.3. Microcapsule properties with various chitosan samples [Chitosan and / or chitosan-gelatin mixture: 1.0 g; ZLO: 4.0 ml; Genipin: 0.1-0.3 mmol/g of polymer; water: 100ml; Temperature: 40±1.0° C]

Sample particulars				Oil load (%)	Oil Content (%)	Encapsulation efficiency (%)
Chitosan type	Chitosan : gelatin	Oil (ml)	Genipin (mmol)			
Untreated	1: 0	4.0	0.1	343.67	62.6± 2.1	80.8± 1.20
4 h treated	1:0	4.0	0.1	343.67	44.07±1.2	56.89±1.4
8 h treated	1:0	4.0	0.1	343.67	42.2± 1.35	54.5 ±1.5
Untreated	1:1	4.0	0.1	343.67	37.59± 0.07	48.53±1.0
4 h treated	1:1	4.0	0.1	343.67	36.4±0.8	46.9±0.7
8 h treated	1:1	4.0	0.1	343.67	30.7±1.2	39.6±0.67
Untreated	1:1	4.0	0.3	343.67	51.30±1.02	66.22±1.31
4 h treated	1:1	4.0	0 3	343.67	55.3± 0.14	71.4±0.18
8 h treated	1:1	4.0	0.3	343 67	58 2±0.15	75.19±0.2
Gelatin A*	0:1	4.0	0.1	343.67	37.66±3.39	48.6±4.38

* Gelatin B did not produce coacervation

4.4.5. Scanning Electron microscopic study

The morphological investigations of deacetylated chitosan and chitosan-gelatin microcapsules having similar oil loading and cross linker concentration were done by scanning electron microscope. Fig 4.4.4 (a and b) represents the micrographs of microcapsules prepared from low and high degree of deacetylated chitosan respectively. There was no remarkable difference in the smoothness of the surface of the microcapsules. Fig.4.4.4(c and d) shows the micrographs of chitosan-gelatin microcapsules prepared from gelatin- chitosan (low DDA) and gelatin-chitosan (high DDA) complexes. The surface of microcapsules prepared from gelatin and chitosan (high DDA) were found smoother compared to those of prepared from gelatin- chitosan (low DDA). Both the degree of deacetylation and crosslinking had an effect on surface smoothness. The lower the degree of deacetylation, the higher was the surface roughness [18]. In all the cases, similar amount of crosslinker was used. In highly deacetylated chitosan-gelatin microcapsules, the number of free amine groups would be more compared to microcapsules prepared from low DDA chitosan-gelatin complex. These amine groups on crosslinking with genipin might produce smoother surface observed in high DDA chitosan-gelatin microcapsules.
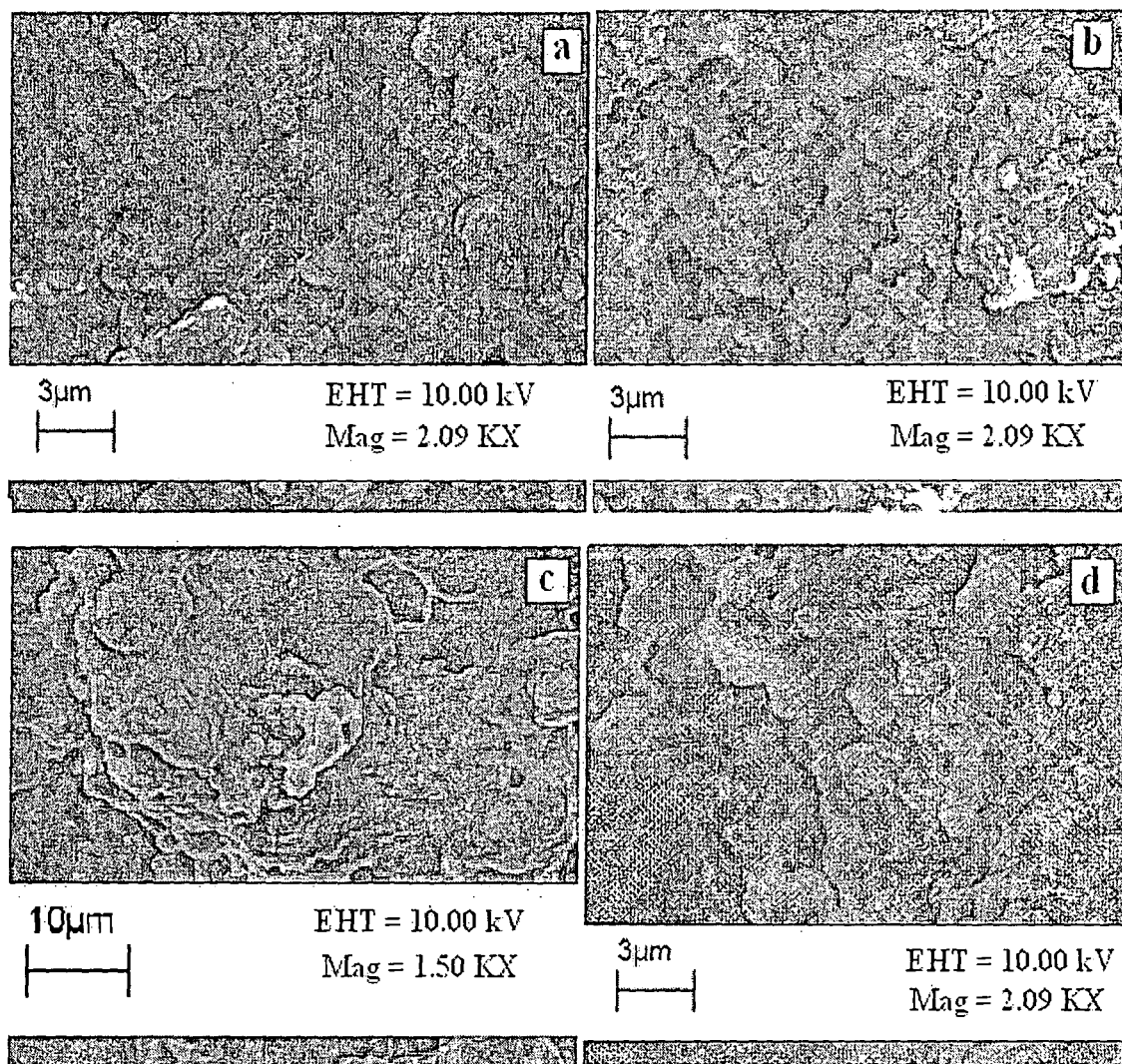
Figure 4.4.4. Scanning electron micrographs of chitosan microcapsules at oil load (%) (343.67); (a) Ch (4 h treated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol; (b) Ch (8 h treated) 1.0 g, ZLO 4.0ml, Gp 0.1 mmol (c) Ch (4 h treated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol; (d) Ch (8 h treated)/G (1:1) 1.0g, ZLO 4.0ml, Gp 0.1mmol

4.5.Controlled Release of Urea from Chitosan Microspheres prepared by Emulsification and Crosslinking Method

Introduction

In this part of work, urea has been microencapsulated in crosslinked chitosan microspheres. The crosslinking agents used for crosslinking of chitosan is a naturally occurring material namely, genipin. The effect of various parameters such as effect of polymer concentration, urea loading and genipin concentration on encapsulation efficiency, urea content in the microspheres and the release profile of the urea from the microspheres have been reported.

Results and Discussion

4.5.1. Effect of variation of urea loading

The effect of urea loading on microsphere behavior is shown in Table 4.5.1 and Fig.4.5.1. With the increase in urea loading, the % urea content of the chitosan/urea microspheres increased while the % entrapment efficiency decreased throughout the range of urea concentration studied. A possible explanation for the lower entrapment efficiency at higher urea loading might be due to the presence of higher percentage of urea on the surface of microspheres that was washed away during isolation. At higher urea loading, the amount of chitosan might not be sufficient to hold all the urea and as a result some urea might exist on the surface during cross-linking. On the other hand, with increasing the urea loading the release rate also increased. This can be explained as: when

chitosan/urea microspheres were put in water, urea began to release due to concentration gradient by diffusion into water. Microspheres with higher urea loading produced higher concentration gradient compared to those having lower urea content. This resulted in higher release rate of urea from chitosan/urea microspheres with higher urea loading. Similar types of result were reported by Bajpai et.al. [19].

Table 4.5.1. Characteristics of urea containing chitosan microspheres prepared

Chitosan (g)	Urea (g)	Genipin (mmol/g of polym.)	% of urea loading	Actual urea Content (%)	Entrapment Efficiency (%)
0.5	0.25	0.5	44.9	29.75 (31)*	96
0.5	0.5	0.5	89.85	44.96 (47.32)	95
0.5	1.5	0.5	269.54	65.64 (72.94)	90
0.75	0.5	0.5	59.88	36.33 (37.45)	97
0.85	0.5	0.5	52.85	33.88 (34.58)	98
1.0	0.5	0.5	44.9	30.68 (31)	99
0.5	1.0	0.1	195.6	57.56 (66.16)	87
0.5	1.0	0.25	189.3	57.28 (65.43)	87.5
0.5	1.0	0.75	171.01	55.08 (63.1)	87.3

* Values in bracket are theoretical urea content
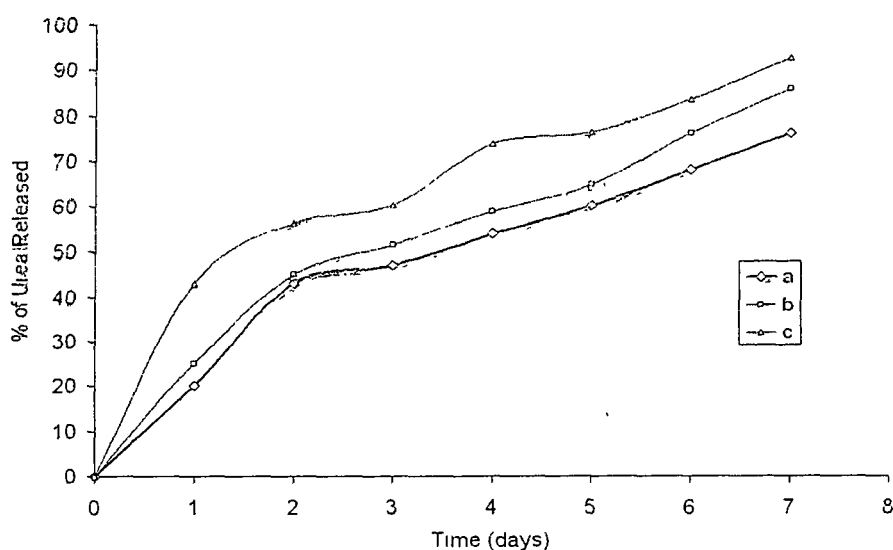
Fig.4.5.1. Effect of variation of urea loading on release rate from microspheres

(a) Chitosan 0.5 g, urea 0.25 g, genipin 0.5 mmol/ g of polymer;

(b) Chitosan 0.5 g, urea 0.5 g, genipin 0.5 mmol/ g of polymer;

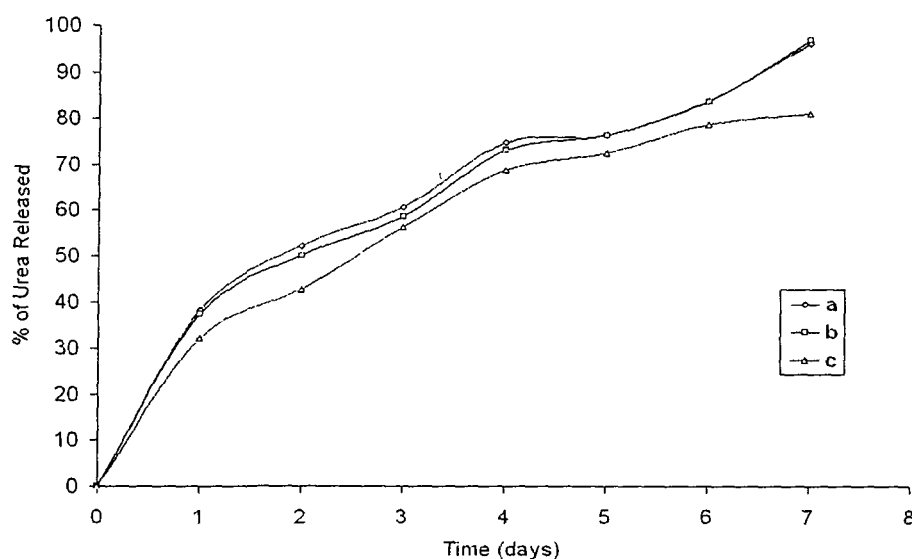(c) Chitosan 0.5 g, urea 1.5 g, genipin 0.5 mmol/ g of polymer

4.5.2. Effect of variation of chitosan concentration

The effect of variation of chitosan concentration on urea content (%), entrapment efficiency (%) and release rate are shown in Table 4.14 and Figure 4.5.2, respectively. From Table 4.5.1 it was observed that urea loading (%) and urea content (%) decreased while entrapment efficiency (%) increased with the increase in the amount of chitosan. The observed trend for urea loading and urea content were as per expectation. Again, at higher polymer concentration, the amount of polymer was sufficient to encapsulate the urea resulting in higher entrapment efficency. The release rate was found to decrease with the increase in the concentration of chitosan. The higher the chitosan concentration, the

higher was the thickness of the microsphere wall. Hence the diffusional path for urea would be longer which resulted in a decrease of release rate.
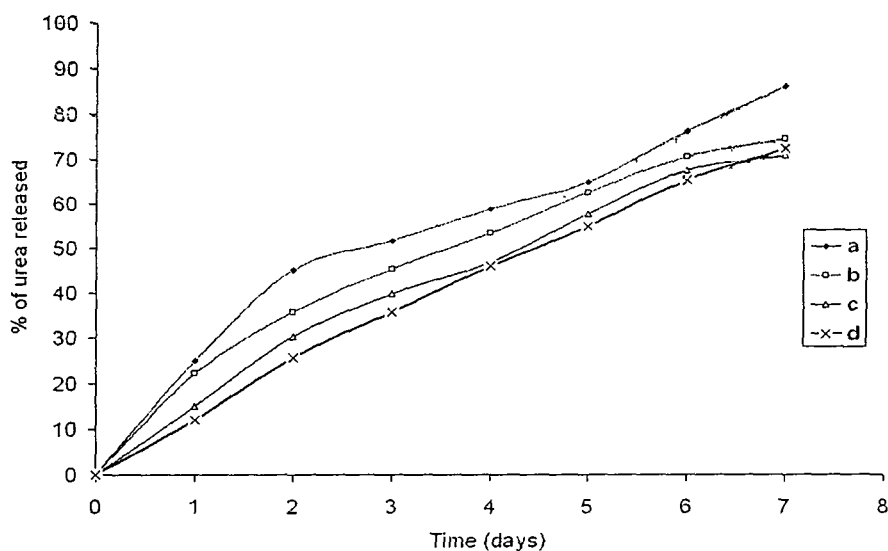
Fig.4.5.2. Effect of variation of % of chitosan on the release rate from microspheres

(a) Chitosan 0.5 g, urea 0.5 g, genipin 0.5 mmol/ g of polymer;

(b) Chitosan 0.75 g, urea 0.5 g, genipin 0.5 mmol/ g of polymer

(c) Chitosan 0.85 g, urea 0.5 g, genipin 0.5 mmol/ g of polymer;

(d) Chitosan 1.0 g, urea 0.5 g, genipin 0.5 mmol/ g of polymer

4.5.3. Effect of variation of cross-linker concentration

The effect of variation of cross-linker concentration on entrapment efficiency (%), urea content (%) and release rate are shown in Table 4.5.1 and Figure 4.5.3, respectively. The urea loading (%), urea content (%) and entrapment efficiency (%) were found to decrease with the increase in the concentration of genipin, a naturally occurring crosslinker. The trend of urea loading was as per expectation. The reason for lower urea content and entrapment efficiency might be due to the reduction in entrapment volume

inside the microspheres caused by cross-linking. With the increase in the concentration of crosslinker, the molecular distance between crosslinks decreased which in turn would reduce the entrapment volume for urea inside the microspheres. Similar results were reported by Kumber and his coworkers [20] while studying the effect of crosslinking agent on the behaviour of dichlorofenac sodium encapsulated chitosan microspheres.

The release rate was found to decrease as the crosslinker concentration increased. As degree of crosslinking increased, the microsphere wall became denser and hence swelling as well as release rate decreased. The lower percentage of urea content might also be responsible for the decrease in release rate

Fig. 4.5.3. Effect of variation of cross-linker concentration on release rate

    (a) Chitosan 0.5 g, urea 1.0 g, genipin 0.1 mmol/ g of polymer;

    (b) Chitosan 0.5 g, urea 1.0 g, genipin 0.25 mmol/ g of polymer;

    (c) Chitosan 0.5 g, urea 1.0 g, genipin 0.75 mmol/ g of polymer.

4.5.4. Effect of variation of temperature on release profile

The effect of variation of temperature of the release medium on the release rate from chitosan/urea microsphere is shown in Figure.4.5.4.The temperature of the release medium were choosen as 298,303 and 308 K. From Fig.4, it was observed that all of the curves were almost similar in pattern and the temperature had a considerable effect on the release of urea. The higher the temperature, the higher was the urea release rate. When the temperature increased, the rate of water absorption by chitosan/urea microspheres would increase, and then, the exchange of free water between microspheres and the solution would increase. This would result in the increase of release rate of urea Similar type of results were reported in literature [21, 22].
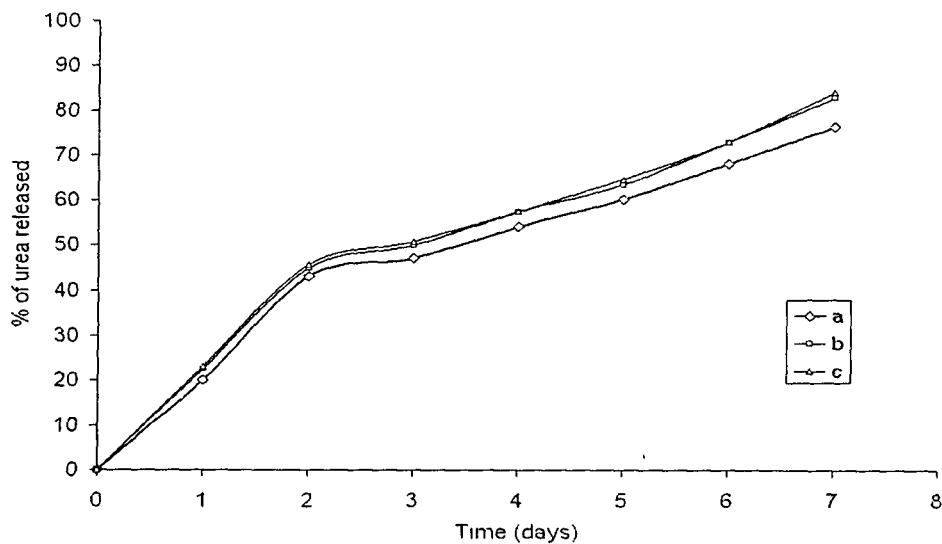
Fig.4.5.4. Effect of variation of temperature on release rate [Chitosan 0.5 g, urea 0.25 g, genipin 0.5 mmol/ g of polymer]: (a) 25°C; (b) 30° C; (c) 35°C

4.5.5. Water Uptake by microspheres

The water uptake (%) at equilibrium of the microspheres, which were emptied before swelling in distilled water, is shown in Table 4.5.2. It was observed from Table 4.5.2 that the variables chitosan concentration, urea loading during microsphere preparation and cross-linker concentration had remarkable effect on the water uptake capacity (%) of the microspheres. The higher the concentration of chitosan in the microsphere, the higher was the percentage of water uptake. This might attributed to the hydrophilic nature of the chitosan molecules where a large number of groups like $-NH_2$, $-OH$ was present. These functional groups could attract the water molecules and thus might increase the water uptake (%). Similarly the water uptake (%) was found to decrease as the amount of crosslinker increased. This could be due to the formation of more compact wall of the microspheres and as a result the penetration of water molecules became difficult. The water uptake (%) of the emptied microspheres prepared with different urea loading was found to increase as the amount of urea increased. A possible explanation for this was as follows: The volume occupied by the urea in the chitosan microspheres increased with the increase in the loading of urea. The void spaces after removal of urea would be available for absorption of water. The void spaces would be more in the highly urea loaded chitosan microspheres compared to low urea loaded chitosan microspheres. This in turn would help to absorb more water.

Table 4.5.2. Water Uptake (%) at equilibrium of the chitosan microspheres

Chitosan (g)	Urea (g)	Genipin (mmol/g of polymer)	% Water Uptake (%) at equilibrium
0.5	0.25	0.5	112.3 ±1.2
0.5	0.5	0.5	115.6 ± 1.4
0.5	1.5	0.5	126.4 ± 0.5
0.75	0.5	0.5	130.6 ± 1.75
0.85	0.5	0.5	132.2 ± 2.3
1.0	0.5	0.5	142.6 ± 0.75
0.5	1.0	0.1	164.2 ± 1.25
0.5	1.0	0.25	144.2 ± 2.26
0.5	1.0	0.75	78.5 ± 1.55

4.5.6. FTIR spectroscopy study

FTIR spectra of chitosan, urea and urea loaded microspheres are shown in Fig. 4.5.5 The spectrum of chitosan showed a strong amide characteristics peak at 1629 cm^{-1}. In urea, the peaks appeared at 1620 and 1461 cm^{-1} were due to amide II bending and C-N stretching. The peaks observed between 3350-3440 cm^{-1} were due to the $-CONH_2$ group. In the urea loaded microspheres, a sharp peak due to C-N stretching appeared at 1457 cm^{-} ₁ indicated the presence of urea in the microspheres. The position of other peaks did not shifted much. This suggested that there was no significant interaction between urea and chitosan.
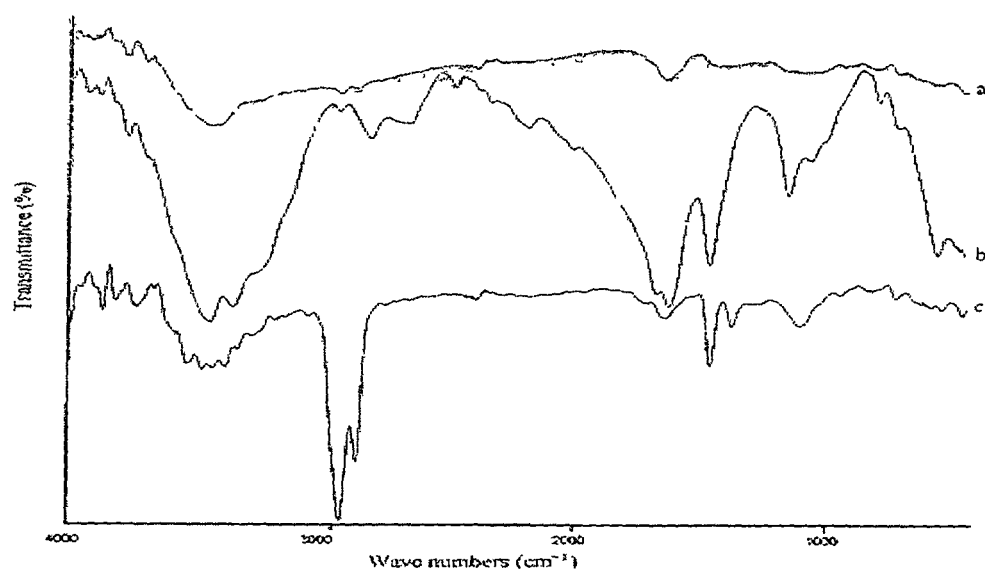
Fig.4.5.5. FTIR spectra of a) chitosan b) urea and c) urea loaded microsphere

4.5.7. Scanning Electron Microscopic study

Fig.4.5.6 shows the scanning electron micrograph of unloaded chitosan and urea loaded chitosan microspheres The study showed that the microspheres had almost spherical geometry with rough surface. In urea-loaded microspheres, some unentrapped urea particles were seen to adhere on the surface of the microspheres. The loaded microspheres had peak and valleys on their surface which also indicated that urea was entrapped to a larger extent near the surface of the microspheres.
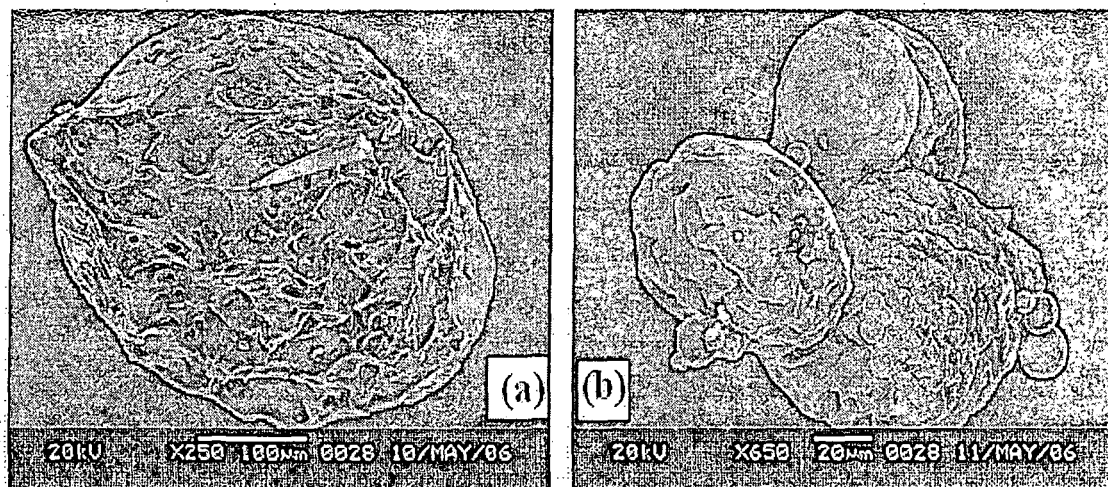
Fig.4.5.6. Scanning electron micrograph of chitosan/ urea microspheres with urea loading

(%) (a) 0 (b) 59.88

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CHAPTER V
SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1. Summary and Conclusions

The salient features that come out of the present study could be summarized as follows.

In the present experimental work, it was found that *Zanthoxylum limonella oil* (ZLO) could be encapsulated successfully inside glutaraldehyde or genipin cross-linked gelatin or chitosan having different degree of deacetylation (DDA) and gelatin-chitosan microcapsules. The microcapsules were prepared by salting out as well as varying pH of the polymer solution (i.e., complex coacervation technique). The minimum temperature at which phase separation occurred was 40°C.

In the case of coacervation by salting out method, the ratios of polymer to sodium sulphate were 1: 10 and 1: 5 for gelatin and gelatin-chitosan complex respectively. In the case of coacervation by variation of pH, the optimum ratio of gelatin to chitosan and pH were 1: 10 and 5.9 respectively.

The oil loading (%), oil content (%), encapsulation efficiency (%) and release rate of oil were dependent on various factors like amount of oil, concentration of polymer and crosslinker, pH of the reaction medium and degree of deacetylation of chitosan.

In general, encapsulation efficiency decreased as oil load increased. Similarly encapsulation efficiency was found to increase when concentration of polymer (gelatin or gelatin-chitosan) or cross-linker (glutaraldehyde, genipin) increased. The decrease in encapsulation efficiency at higher oil load might be due to the lowering in efficiency of the stirrer, which resulted in the formation of larger oil droplets. The amount of gelatin or gelatin-chitosan might not be sufficient to encapsulate all the oil droplets. Few oil droplets might exist without encapsulation and got lost during isolation. At higher polymer concentration, the availability of the polymer was high to encapsulate oil droplets and thereby efficiency increased. Higher crosslinker concentration (glutaraldehyde / or genipin) increased the cross-linking which improved the oil retention capacity.

Gelatin based microcapsules prepared from higher reaction medium pH medium and higher stirrer speed showed higher encapsulation efficiency. At lower pH, the amino groups of gelatin could get protonated and cause repulsion among gelatin chains. Higher pH decreased the repulsion and favored for cross-linking between gelatin and glutaraldehyde. This would help to increase the encapsulation efficiency. Higher stirrer speed resulted in the formation of higher percentage of smaller microcapsules and would enhance encapsulation efficiency.

The higher the degree of deacetylation, the lower was the molecular weight. The number of amine groups increased while the number of deacetylamide groups decreased due to alkali treatment and as a result a decrease in molecular weight was observed.

In chitosan-gelatin based microcapsules, the encapsulation efficiency increased with the increase in the concentration of chitosan in chitosan-gelatin complex. Higher the

concentration of chitosan, higher was the viscosity of the medium. The increase in viscosity resulted in the formation of large oil droplets and therefore more amount of oil could be encapsulated with the same amount of polymer.

The oil content and encapsulation efficiency decreased as the DDA increased. The higher the DDA, the more was the amino groups. The crosslinker genipin might not be sufficient to crosslink all the amine groups present in deacetylated chitosan / or its complex with gelatin and as a result less compact wall of microcapsules formed. Both hydrophilicity (due to amine groups) and lower cross-linking efficiency might be responsible for the observed low encapsulation efficiency.

Again, the oil content and encapsulation efficiency increased as the degree of cross-linking of chitosan / or its complex with gelatin increased. The microcapsule wall became denser due to cross-linking which resulted in an increase in encapsulation efficiency.

The faster or slower release rate of oil by the microcapsules prepared by varying different conditions could be explained on the basis of either decrease or increase in the thickness of microcapsule wall. Higher oil loading decreased the thickness of microcapsule wall whereas higher concentration of polymer and cross-linker increased the thickness of the wall. This in turn would control the release rate.

The higher the temperature of release environment, the higher the release rate was. This might be due to the increased solubility as well as higher diffusion rate of the core material to the environment caused by the temperature. The higher the percentage chitosan in chitosan-gelatin complex, the slower was the release rate of oil. Higher number of primary amine groups in chitosan could react with genipin to form sufficient

crosslink bridges, which helped to reduce the release rate. At lower level of crosslinker concentration, the release rate of oil from deacetylated chitosan / or deacetylated chitosan-gelatin matrix was found to decrease as DDA increased. At higher level of crosslinker concentration, the opposite trend was observed. This could be explained by considering the cross-linking efficiency and hydrophilicity of deacetylated chitosan as explained earlier. Swelling of both chitosan and chitosan-gelatin microcapsules increased as degree of deacetylation increased.

FTIR spectroscopy indicated that there was no significant interaction between gelatin or gelatin-chitosan with ZLO. But an interaction between chitosan and gelatin was observed during complex formation.

Thermal stability of chitosan-gelatin microcapsules (as suggested by thermogravimetric analysis) was found to improve with the increase in the percentage of chitosan in the complex. DSC results suggested that a low compatibility in thermal properties existed in the relation between oil and gelatin or chitosan or gelatin-chitosan complex.

SEM micrographs of gelatin / or gelatin-chitosan microcapsules prepared at higher oil loading appeared oily, agglomerated and having a bursting look compared to those of microcapsules prepared at lower oil loading. The surface of the microcapsules prepared from highly deacetylated chitosan was smooth compared to those prepared from lower deacetylated chitosan. Higher stirrer speed produced higher proportion of smaller and better shaped gelatin microcapsules.

Microcapsules with higher oil content provided better repellent activity compared to microcapsules of lower oil content. Formulations based on chitosan-gelatin

microcapsules showed better protection time in comparison to formulations based on gelatin based microcapsules. The optimized repellent formulation was found to be either comparable or more to that of standard one.

Encapsulation of urea was performed in chitosan microspheres by emulsification followed by cross-linking. The microspheres were prepared by varying different parameters like the concentration of chitosan, urea and cross-linker. Higher amount of chitosan (1.0g) and cross-linker concentration (0.75 mmol/g of chitosan) produced an entrapment efficiency (%) of 99 and 78.5 respectively. The release rate was found to be dependent on the concentration of urea, chitosan, cross-linker and temperature of the release medium. The higher temperature of the release medium enhanced the release rate. Water uptake (%) was found to increase as the concentration of urea, chitosan increased and cross-linker decreased. The surface of the urea-loaded microspheres appeared coarser and rough compared to that of unloaded microspheres as revealed by scanning electron microscopy study.

5.2. Future Scope

> ZLO was found to be an effective repellent when encapsulated within cross-linked polymer matrix. Similarly, the release of urea was also found to be controlled if encapsulated within polymer matrix. However, the present investigation is restricted to laboratory scale only. Further field evaluation is needed for commercialization of the products.

➤ The controlled release mosquito repellent formulations were evaluated in petroleum gelly. Other base materials can also be tried because it was reported that different base materials provide different efficacy of the active materials.

➤ The urea containing chitosan microspheres can be coated multiple times with other materials to further extend its release profile.

➤ The main obstacle to the wider use of controlled release product will remain the high cost of these materials compared to those of conventional products. The use of cheaper natural polymer waste materials such as sawdust, park, starch, baggase, rice husk lignin and pine-kraft lignin seems to be a viable way to provide the loading of bioactive species. Other possible trends to reduce cost are to increase weight efficiency by increasing the content of the active ingredients and to use the polymer itself in a positive way rather than as an inert vehicle. One possibility is to make the polymer backbone that has a dual function as an active ingredient and as a carrier for the bioactive agent.

➤ The particle size, thickness of polymer coating, crosslinking density, loading percentage, etc. have to be taken into account for designing a particular controlled release formulation. As A. Kundo said *"Microencapsulation is like the work of a clothing designer. He select the pattern, cuts the cloth, and sews the garment in due consideration of the desires and age of his customer plus the locale and climate where the garments to be worn. By analogy, in microencapsulation, capsules are designed and prepared to meet al the requirements in due consideration of the properties of the core material, intended use of the product, and the environment of storage."*

Publications

Papers Published

1. "Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of Zanthoxylum limonlla oil(ZLO) using salting-out method." **M.R.Hussain** and T.K.Maji, *Journal of Microencapsulation* 25(6), 414 (2008).

2. "Microencapsulation of Zanthoxylum limonella oil in glutaraldyhyde crosslinked gelatin for mosquito repellent application." T.K.Maji, I.Baruah, S.Dube and **M.R.Hussain**. *Bioresource Technology* 98, 840 (2007).

3. Microencapsulation of *Zanthoxylum limonella oil* (ZLO) In Genipin Crosslinked Chitosan-Gelatin Complex For Mosquito Repellent Application. T.K.Maji and **M.R.Hussain**, *Journal of Applied Polymer Science, 111, 779(2009)*.

Papers Communicated

1. "Degree of Deacetylation of Chitosan: Determination and Their influence on the release behaviour of essential oil from chitosan and chitosan-gelatin complex microspheres." **M. R. Hussain** and T. K. Maji.

2. "Controlled Release of Urea from Chitosan Microspheres prepared by Emulsification and Crosslinking Method." **M. R. Hussain** and T. K. Maji.

3. "Development and evaluation of controlled release mosquito repellent formulations based gelatin and chitosan-gelatin microcapsules." **M. R. Hussain** and T. K. Maji.

Microencapsulation of *Zanthoxylum limonella* Oil (ZLO) in Genipin Crosslinked Chitosan–Gelatin Complex for Mosquito Repellent Application

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ABSTRACT: Essential oil containing chitosan gelatin complex microcapsules crosslinked with genipin were prepared by complex coacervation process. The effects of various parameters such as oil loading, ratio of chitosan to gelatin, degree of crosslinking on oil content, encapsulation efficiency, and the release rate of the essential oil were studied. Scanning electron microscopy study indicated that the surface of the microcapsules were more irregular as the amount of oil loading increased. Thermal stability of microcapsules improved with the increase in the amount of chitosan in chitosan–gelatin matrix as revealed by thermogravimetric analysis. FT-IR spectroscopy and differential scanning calorimetry study indicated that there was no significant interaction between chitosan–gelatin complex and oil. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111. 779–785, 2009

Key words: chitosan; gelatin; essential oil; microencapsulation; genipin; mosquito repellent

INTRODUCTION

Recently, considerable efforts are made worldwide to promote the use of environmentally friendly and biodegradable natural insecticides and repellents. A large number of essential oils have been evaluated and found to possess mosquito repellency against various mosquito vectors.[1-7] The essential oil obtained from *Zanthoxylum limonella* (ZLO) has been found to possess mosquito repellent properties against different mosquito vectors.[8] However, the repellency of these plant based products is lower both in efficacy and duration than those of synthetic repellents. Furthermore, essential oils are subjected to environmental deterioration by heat, humidity, light, and oxygen.[9]

Controlled release by microencapsulation seems to be the best way to protect essential oil from environmental damage and thus securing a long shelf-life.[10] The vast majority of publications on microencapsulated repellents are patents.[11] Coacervation,[12-17] molecular inclusion,[18] and spray drying[9,19] techniques are generally used for microencapsulation.

Varieties of crosslinking agents like glutaraldehyde, formaldehyde, epoxy compounds[20-22] are reported to be employed for improving the controlled release behavior. These crosslinking agents can cause physiological toxicity. Therefore, a system is looked for which can produce product having either very less or nil toxicity. Genipin, a natural crosslinker, can react spontaneously with amino acids or proteins. Its toxicity is much less than glutaraldehyde.[23] Chitosan, gelatin, and genipin are naturally occurring materials and have attracted much attention from scientists all over the world. The whole system will be fully biodegradable. Genipin crosslinked alginate-chitosan microcapsule for live cell encapsulation was reported by Chen et al.[24] Chen et al.[25] investigated the fluorogenic characteristics of chitosan–genipin reaction for microencapsulation purposes.

The present work is aimed at to produce chitosan–gelatin complex microcapsules containing ZLO by complex coacervation technique using the natural crosslinker, genipin. Efforts have also been made to study the release characteristics of oil from microcapsules prepared under different conditions

EXPERIMENTAL

Materials

Gelatin type B from Bovine skin with a bloom strength ~ 225 and chitosan with a medium molecular weight with Brookfield viscosity ~ 200 cps were purchased from Sigma-Aldrich (USA). Sodium hydroxide (E. Merck, Mumbai, India), glacial acetic acid (E. Merck, India), Tween 80 (E. Merck), Genipin (Mol. wt. 226.22) (Challenge Bioproducts Co.,

Taiwan), and silicone oil (Ranbaxy Fine Chemicals, Delhi, India) were used as such received. The core material, essential oil from ZLO, was extracted in our laboratory. DDI (double-distilled deionized) water was used throughout the study. Other reagents used were of analytical grade.

Extraction of essential oil

The seeds of ZLO, a big tree available in Tezpur, were collected and shed dried. Essential oil was obtained by steam distillation of the seeds. The oil obtained was separated from the aqueous phase and dried by treating with anhydrous sodium sulfate. The dried oil was transferred into a dark glass bottle and kept inside the refrigerator at low temperature for subsequent use.

Microencapsulation procedure

To a beaker, certain amount of 2% (w/v) chitosan solution previously made in 1% (v/v) aqueous acetic acid and 2% (w/v) aqueous gelatin solution were taken. Total amount of polymer was kept constant at 1 g. The mixture of polymer solution was stirred by mechanical stirrer under high agitation after adding one drop of silicon antifoaming agent at 40°C. The temperature was maintained at 40°C. To this, essential oil (1–4 mL) was added under high agitation to form an emulsion. Using $0.1N$ NaOH the pH of the emulsion was brought to the range of 5.4–5.9 to attain the maximum coacervation. Once the coacervation took place with the formation of microcapsules, the system was brought to room temperature ($\sim 30°C$) to harden the microcapsules. The crosslinking of the polymer capsule was achieved by slow addition of certain amount of genipin (0.05–0.5 mmol/g of polymer) solution (0.5% w/v aq. solution). The temperature of the vessel was then raised to 40°C and stirring was continued for about 3–4 h in order to complete the crosslinking reaction. The vessel was then cooled to room temperature. The microcapsules were filtered, washed with 0.3% Tween 80 surfactant solution, dried and stored inside a refrigerator in a glass ampule.

Measurements

Calibration curve of oil

A calibration curve is required for the determination of release rate of oil from the microcapsules. It was found that 1 g of oil could be easily dissolved in 100 mL of water containing 0.3 g Tween 80.

A known concentration of essential oil in DDI water containing 0.3 wt % Tween 80 was scanned in the range of 200–400 nm by using UV visible spectrophotometer. For ZLO having concentration in the

range 0.005 to 0.1 g/100 mL, a sharp peak at 256 nm was noticed. The absorbance values at 256 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of ZLO was obtained by knowing the absorbance value

Encapsulation efficiency, oil content, and oil load

A known amount of accurately weighed microcapsules was grounded in a crucible, transferred with precaution to a volumetric flask containing a known amount of 0.3 wt % aqueous Tween 80 solution, and kept for about 3 days with continuous stirring to ensure complete extraction of oil in Tween 80 solution The encapsulation efficiency (%), oil content (%), and oil loading (%) were calculated by using the calibration curve and the following formulae

$$\text{Encapsulation efficiency } (\%) = w_1/w_2 \times 100$$

$$\text{Oil content } (\%) = w_1/w \times 100$$

$$\text{Oil load } (\%) = w_2/w_3 \times 100$$

where w, weight of microcapsules; w_1, actual amount of oil encapsulated in a known amount of microcapsules; w_2, amount of oil introduced in the same amount of microcapsules; and w_3, total amount of polymer used including crosslinker.

Oil release studies

Oil release studies of encapsulated oil were done by using UV–visible spectrophotometer (UV-2001 Hitachi). A known quantity of microcapsules was placed into a known volume of 0.3 wt % Tween 80 surfactant solution. The microcapsule-Tween 80 mixture was magnetically stirred at a constant rate and the temperature throughout was maintained at 30°C (room temperature). An aliquot sample of known volume (5 mL) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 256 nm for the determination of cumulative amount of oil release up to a time t. Each determination was carried out in triplicate. To maintain a constant volume, 5 mL of 0.3 wt % Tween 80 solution was returned to the container.

Scanning electron microscopy study

The samples were deposited on a brass holder and sputtered with gold. Surface characteristics of the microcapsules were studied using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 10–20 kV and at room temperature.

Thermal properties study

Thermal properties of chitosan, gelatin, ZLO, and ZLO containing microcapsules were evaluated by employing thermogravimetric analyzer (TGA) and differential scanning calorimeter (DSC). TGA study was carried out using TGA (model TA 50, shimadzu) at a heating rate of 10°C/min up to 600°C. DSC study was done in a differential scanning calorimeter (model DSC-60, shimadzu) at a heating rate of 10°C/min upto 400°C. Both the studies were done under nitrogen atmosphere.

Fourier transform infrared (FTIR) study

FTIR spectra were recorded using KBr pellet in a Nicolet (model Impact-410) spectrophotometer. Microcapsules, chitosan, gelatin, and ZLO were each separately finely grounded with KBr and FTIR spectra were recorded in the range of 4000–400 cm^{-1}.

RESULTS AND DISCUSSION

Pure gelatin B solution was scanned between 450 and 600 nm at different pH using UV spectrophotometer. The % transmittances studied in the above wavelength were found to follow more or less similar trend at different pH. For chitosan, the % transmittance at the above scanned wavelength remained unchanged up to a certain pH (~ 6.00), beyond that the % transmittance decreased due to precipitation.

Chitosan–gelatin mixture of different ratios showed the trend similar to those of chitosan. However, in the case of both chitosan and chitosan/gelatin mixture, the maximum absorption occurred at lower wavelength. Therefore all the successive measurements were done at 450 nm and reported.

To optimize the coacervation behavior, the study of phase separation behavior is essential. This was determined by measuring the coacervate yield as well as turbidity.

Gelatin and chitosan solutions were mixed at different ratio (1 : 1 to 1 : 40) at room temperature under stirring condition. The pH of the solution, prepared at different ratio, was varied from 5.0 to 6.0. In this pH range, no precipitation of chitosan occurred and also it was above the isoelectric point of gelatin. Turbidity would appear due to the formation of coacervate particles. The change in transmittance due to turbidity was monitored using UV spectrophotometer at 450 nm. The pH at which maximum absorption noticed was recorded. The coacervate yield was measured at different pH by decanting the supernatant and drying the coacervate phase. The optimum ratio of gelatin–chitosan and pH at which maximum coacervation observed were 1 : 10 and 5.9, respectively. Similar results were reported by Lopez and Bodmeier.[26]

Oil release studies

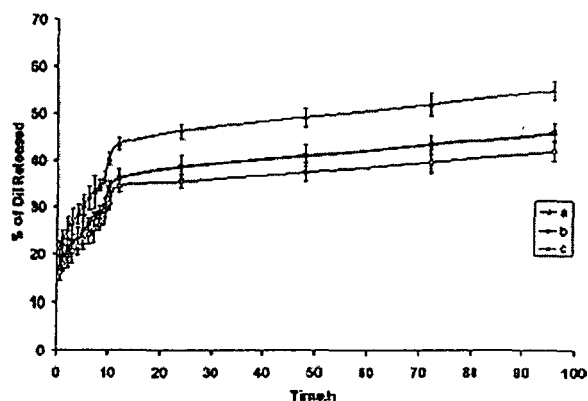### Effect of variation of oil loading on release rate

The effect of variation of oil loading on encapsulation efficiency and release rate for 1 : 1 chitosan–gelatin microcapsules are presented in Table I and Figure 1. The more the oil-load, the higher was the release rate. The lower in encapsulation efficiency might be due to the higher % of oil loss during isolation of microcapsules.

At low oil load, small oil droplets were formed as the dispersive force of the stirrer was more effective. The % of chitosan–gelatin mixture was enough to encapsulate properly the oil droplets. With the increase in oil-load, the dispersive force of the stirrer became less efficient which resulted in the formation of large oil droplets. At this stage, chitosan–gelatin mixture could be able to encapsulate the large oil droplets only at the expense of decrease in thickness

TABLE I
Effect of Variation of Oil Loading, Chitosan to Gelatin Ratio, and Genipin
Concentration on the Behavior of Microcapsules

Sample particulars					
Chitosan (gm) : Gelatin (gm)	Genipin (mmol)	ZLO (mL)	Oil load (%)	Oil content (%)	Encapsulation efficiency (%)
0.25 · 1	0.05	4	308.75	26.45 ± 1.20	34 14 ± 1.74
0 66 · 1	0 05	4	308 75	25.15 ± 0 57	32.47 ± 1.07
2 . 1	0.05	4	308 75	30 65 ± 0.54	39 56 ± 1.74
4 . 1	0 05	4	308 75	37.65 ± 0 75	48.60 ± 1.14
1 · 1	0.05	1	77.20	22.32 ± 0.15	48.31 ± 0.84
1 : 1	0.05	2	154 37	23.68 ± 0 51	44.3 ± 0.35
1 : 1 ·	0.05	3	231.56	28.0 ± 0.12	32.87 ± 0.96
1 : 1 ·	0.10	4	280.27	42.0 ± 0.45	54.22 ± 1.12
1 : 1	0.20	4	236.60	45 34 ± 0 87	58.53 ± 1.36
1 : 1	0 50	4	161.26	46.50 ± 0.64	60.05 ± 0.89

Total polymer = 1 g; genipin = (0.05–0.5 mmol/gm of polymer; oil = (1–4 mL); water = 100 mL; Temperature = (40 ± 1)°C.

Figure 1 Effect of variation of oil loading on release profile. (a) Polymer 1.0 gm, crosslinker 0.05 mmol, ZLO 1.0 mL; (b) polymer 1.0 gm, crosslinker 0.05 mmol, ZLO 2.0 mL; (c) polymer 1.0 gm, crosslinker 0 05 mmol, ZLO 3.0 mL
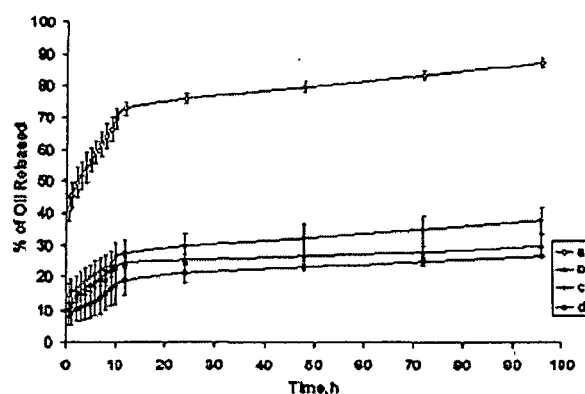

Figure 2 Effect of variation of chitosan to gelatin ratio on release profile. Total polymer 1.0 gm, ratio of chitosan to gelatin in (a) 0.25 · 1; (b) 0.66 : 1; (c) 2 : 1; (d) 4 : 1, ZLO 4 0 mL; crosslinker 0.05 mmol.

of microcapsule wall. Besides this, the amount of chitosan–gelatin mixture might not be sufficient to encapsulate all the oil droplets. Some oil droplets might present without encapsulation. These oil droplets might get exhausted during recovery of microcapsules. As wall thickness decreased, the diffusional path for the oil became short[27,28] which resulted in an increase of release rate.

Again oil content (%) was found to increase with the increase in the % of oil load. At low oil load, many of the microcapsules probably contained few oil droplets indicating that there was an abundance of encapsulating polymer for the oil present. As oil load (%) increased, the number of oil droplets in the microcapsules increased which resulted in an increase in oil content.

Effect of variation of chitosan/gelatin ratio on release rate

The effect of variation of chitosan–gelatin ratio on oil loading, encapsulation efficiency and release rate are shown in Table I and Figure 2. The release rate of oil was governed by the % of chitosan present in the chitosan–gelatin mixture. With the increase in the concentration of chitosan in chitosan–gelatin mixture, the release rate was found to decrease. Again an increase in the viscosity of the chitosan–gelatin mixture was noticed with the increase in the concentration of chitosan.

The higher viscosity might decrease the dispersive force of the stirrer. As a result large oil droplets were formed. The decrease in surface area could be responsible for the decrease in release rate. Moreover, chitosan has more average moieties of primary amine groups than gelatin. Chitosan could react with genipin to form sufficient crosslink bridges compared with gelatin. This might also play a role in reduction of release rate. Similar observations were reported by Kim et al [29]

during the study of the release behavior of triclosan encapsulated within chitosan–gelatin microcapsules.

Both oil content (%) and encapsulation efficiency were also found to increase with the increase in the chitosan concentration. As explained earlier, the increase in viscosity of the medium resulted in the formation of large oil droplets. These large oil droplets had a tendency to coalesce at higher oil load to form further large oil droplets and therefore more oil could be encapsulated with the same amount of encapsulating material.

Effect of variation of concentration of genipin on release rate

Results showing the oil load (%), oil content (%) and encapsulation efficiency are shown in Table I. The release profile of the oil is shown in Figure 3. The trend shown by both oil loading and oil content was as per expectation. Encapsulation efficiency increased
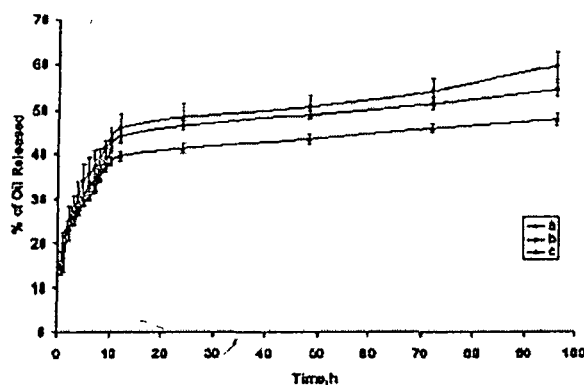
Figure 3 Effect of variation of crosslinker concentration on release profile. (a) Polymer 1.0 gm, crosslinker 0.1 mmol, ZLO 4.0 mL; (b) polymer 1.0 gm, crosslinker 0.2 mmol, ZLO 4.0 mL, (c) polymer 1.0 gm, crosslinker 0.5 mmol, ZLO 4.0 mL.
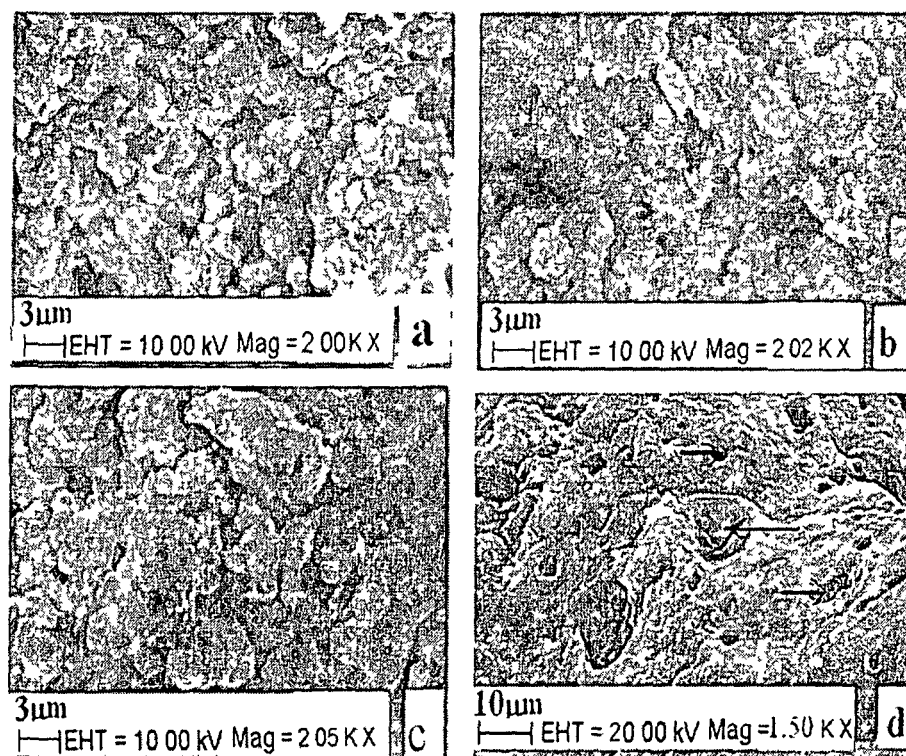
Figure 4 Scanning electron micrographs of microcapsules prepared with oil load (%) (a) 154 37, (b) 308 75, (c) 617 02, (d) 154 37 (after oil release)

with the increase in genipin concentration The concentration of genipin was varied from 0 1 to 0 50 mmol/g of polymer mixture The increased efficiency was due to the higher oil retention capacity of the microcapsules caused by the formation of crosslinking The crosslinking reaction took place between genipin, gelatin, and chitosan The release rate of oil was found to decrease as the % of genipin increased The microcapsule wall became compact as degree of crosslinking increased This resulted in the decrease of diffusion rate through the microcapsule wall Similar findings were cited in the literature [10,30]

Scanning electron microscopy study

Scanning electron microscopy (SEM) micrographs of genipin crosslinked chitosan–gelatin microcapsules having different percentage of oil content are shown in Figure 4 At low oil loading [Fig 4(a)], the surface of the microcapsules appeared smooth compared with those of microcapsules prepared at higher oil loading [Fig 4(b,c)] At higher oil loading, a bursting look was observed due to the presence of large percentage of oil Similar observations were reported in the literature [10,14] The surface of the microcapsules became more irregular as the percentage of oil loading increased Figure 4(d) shows the micrograph of the microcapsules after release of substantial amount

of oil The surface of the microcapsules contained a significant number of pin holes (arrow marked) These pin holes might be formed due to the release of oil by diffusion Moreover, on physical verification, the microcapsules prepared at higher oil loading appeared oily and agglomerated where as those prepared at low oil loading appeared dry and powdery

Thermogravimetric analysis

Table II shows the initial decomposition temperature (T_i) and residual weight (RW, %) of virgin polymers (chitosan and gelatin), ZLO and ZLO containing

TABLE II
Thermal Analytical Data for Virgin Polymer and Oil Containing Microcapsules

Sample particulars			
Gelatin (gm) Chitosan (gm)	Oil (mL)	T_i(°C)	RW (%) at 600 °C
1 0	–	236	8 75
1 0 5	4 0	175	9 33
1 1	4 0	190	23 06
0 1	–	273	37 74
–	Oil[a]	–	–

T_i, Initial decomposition temperature, RW, residual weight
[a] Started decomposing from the very beginning

TABLE III
Temperature of Decomposition (T_D) at Different Weight Loss (%) of Virgin Polymer and
Oil Containing Microcapsules

Sample particulars		Temperature of decomposition (T_D) (°C) at different weight loss (%)				
Gelatin (gm) : Chitosan (gm)	Oil (mL)	20	40	60	70	80
1 : 0	–	286	331	394	438	529
1 : 0.5	4	236	300	354	370	446
1 · 1	4	200	302	366	448	–
0 : 1	–	294	320 ·	543	–	–
–	Oil	90	115	155	–	228

microcapsules. Both the Ti (°C) and RW (%) were found to increase with the increase in chitosan concentration in the chitosan–gelatin mixture. The decomposition of ZLO started at an early stage and there was no residue at 600°C.

Temperature of decomposition (T_D) values of ZLO/chitosan/gelatin microcapsules, chitosan, gelatin and oil at different weight loss (%) are shown in Table III. T_D values for the microcapsules increased with the increase in the % of chitosan in the microcapsules. This observed high values might be due to the decreasing chance of elimination of small molecules like NH_3, CO_2, etc. with the formation of crosslinking by genipin. Gelatin contains lower % of lysine and arginine residues as primary amine groups. Chitosan contains glucosamine unit in larger percentage. Genipin could react with the primary amine group of gelatin and glucosamine unit of chi-

tosan. The reaction rate of chitosan and genipin was reported more compared with that of gelatin.[31] So chitosan could form more crosslink bridges compared with that of gelatin and thereby would lead to more thermally stable microcapsules.

The difference in T_i values for various samples could be explained on the basis of their difference in rate of decomposition. The crosslinking reaction of genipin with chitosan was higher compared with that of gelatin as per explanation given above. Moreover oil decomposed at fast rate. Both of these influenced the rate of decomposition and were responsible for different T_i values.

FTIR study

FTIR spectra of chitosan, gelatin, ZLO, and chitosan/gelatin microcapsules containing ZLO were recorded and presented in Figure 5. The spectrum of chitosan displayed a strong amide characteristic peak at 1632 cm^{-1}. Similarly gelatin spectrum also
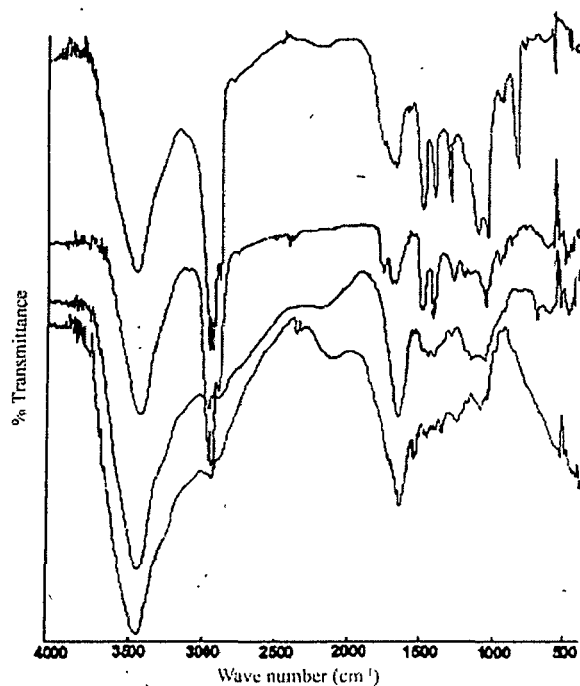
Figure 5   FTIR spectra of (a) gelatin B, (b) chitosan, (c) oil, (d) oil containing microcapsules.

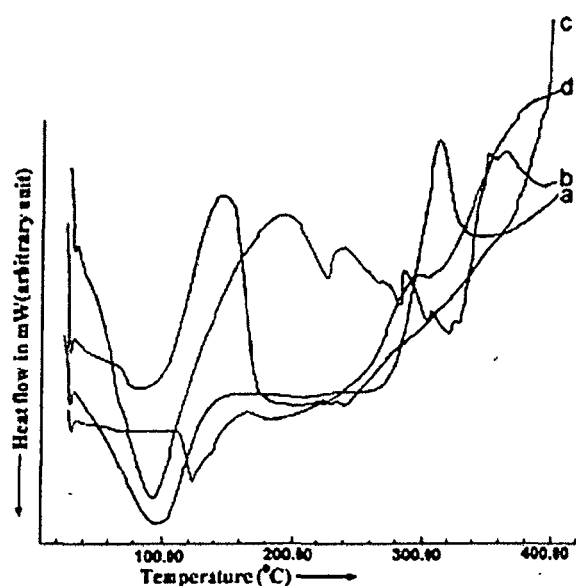Figure 6   DSC thermograms of (a) chitosan, (b) gelatin B, (c) oil, (d) oil containing microcapsules.

showed an amino band at 1547 cm^{-1} and carbonyl peak at 1624 cm^{-1} In ZLO, the peaks appeared between 1638 and 1720 cm^{-1} were due to carbonyl stretching band Besides this, the other notable peaks appeared at 1457 cm^{-1} and 1378 cm^{-1} were due to CH_2 asymmetric deformation and CH_2 symmetric deformation In the microcapsules, the carbonyl band shifted to 1641 cm^{-1} indicating an interaction between chitosan and gelatin complex The position of these peaks remained almost unchanged when compared with that of spectrum of ZLO The position of other peaks which were due to CH_2 asymmetric deformation was also remained unchanged This suggested that there was no significant interaction between ZLO and chitosan gelatin complex

Differential scanning calorimetry study

The DSC thermogram of pure chitosan (a), pure gelatin B (b), oil (c), and oil loaded chitosan/gelatin microcapsules (d) are presented in Figure 6 Pure chitosan showed peaks at 98°C, 271°C, and 340°C, respectively Pure gelatin B showed peaks at 95°C and some multiple peaks in the temperature range 226–323°C Pure oil showed a peak at 90°C and another broad peak having average peak temperature at 200°C Oil encapsulated chitosan/gelatin microcapsules showed a sharp peak at 120°C and another two peaks (in shoulder form) having average peak temperature at 240°C and 320°C The peaks appeared in the temperature range 95–98°C were due to the removal of moisture The position of one peak appeared in the thermogram (not shown) of physical mixture of chitosan/gelatin/oil at 95°C was found to disappeared and a new peak appeared (shown at 120°C) when genipin was used (cross-linked samples) The position of other two peaks in the thermogram of physical mixture remained almost unchanged irrespective of addition of genipin The peaks found at 240°C and 320°C in cross-linked oil loaded microcapsules were mainly due to the decomposition of oil and chitosan–gelatin complex respectively The position of these peaks exhibited in both the thermograms of physical mixture and crosslinked microcapsules suggested that a low compatibility in thermal properties existed in the relation between oil and gelatin-chitosan complex

CONCLUSIONS

The oil from ZLO can be encapsulated successfully in the chitosan–gelatin matrix using genipin as cross-linker The release rate of oil depends on oil content, crosslinking density, polymer concentration, etc The release rate increases with the increase in the oil loading The higher the percentage of genipin, the lower is the release rate The release rate has also been found

to decrease as the concentration of chitosan in the chitosan–gelatin mixture increases SEM study shows that the surface of the microcapsules became irregular due to presence of oil Thermal stability has been found to be improved with the increase in the percentage of chitosan in the chitosan/gelatin matrix A low compatibility in thermal properties in the relation between oil, gelatin, and chitosan exists as revealed by DSC study FTIR study shows that there is no remarkable interaction between polymer and oil

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30 Dinarvand, R, Mahmoodi, S, Farboud, E, Salehi, M, Artyabi, F Acta Pharm 2005, 55, 57
31 Mi, F L Biomacromolecules 2005, 6, 975

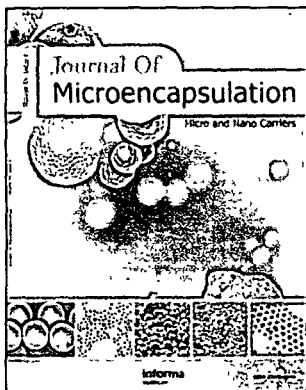## Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of Zanthoxylum limonella oil (ZLO) using salting-out method

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Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of *Zanthoxylum limonella oil (ZLO)* using salting-out method

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Abstract

Zanthoxylum limonella oil (ZLO) containing chitosan-gelatin complex microcapsules cross-linked with genipin, a cross-linker of natural origin, have been prepared by a complex coacervation process using the salting-out method. The effects of various parameters such as oil loading, degree of cross-linking, ratio of chitosan to gelatin, etc. on oil content, encapsulation efficiency and the release rate of ZLO have been studied. FT-IR spectroscopy has been used to understand the interaction between the polymers and oil. Scanning electron microscopy (SEM) has been employed to study the morphology of the prepared microcapsules.

Keywords: *Chitosan, gelatin, ZLO, genipin, release studies*

Introduction

For many chemists, an effective alternative to DEET (*N*, *N*-diethyl-*m*-toluamide) for personal protection against mosquitoes and biting flies is the holy grail (Isman 2006). In spite of five decades of research, no chemical has been found that provides the degree of protection against biting mosquitoes or persistence on human skin afforded by DEET. Concerns with the safety of DEET, especially to children (Fradin 1998), have resulted in the introduction of several plant oils as natural alternatives.

Plant essential oils, commonly used as fragrances and flavouring agents for foods and beverages, were recommended as an alternative source for insect control (Isman, 1999). Essential oil derived compounds can be applied to humans in a similar way to other conventional insecticides and they have little or no harmful effects (Hadfield-Law 2000, Mumcuoglu et al. 2002). The promising essential oils with repellent activity are derived from a large number of plants including *Cymbopogan* Spp. (Ansari and Razdan 1995), *Mentha Piperita* (Ansari et al. 2000), *Ocium* spp. (Tawatsin et al. 2001), *Zanthoxylum Limonella*

(Das et al. 2003), Osage orange and catnip (Peterson and Coats 2001), *Zanthoxylum piperitum* (Pitasawat et al. 2007) and *Eucalyptus maculata citriodon* (Collins and Brady 1993). However, the repellency of these essential oils is commonly lower in both efficacy and duration than that of synthetic repellents, principally DEET. Furthermore, essential oils undergo environmental deterioration by heat, humidity, light and oxygen (Edris and Bergnstahl 2001).

Microencapsulation of essential oils by polymeric materials is expected to slow down the release of essential oils and guarantee more protection against atmospheric conditions, thus providing them a longer shelf-life (Rosenberg et al. 1990, Maji et al. 2007). Another reason for microencapsulation is that the process enables conversion of liquid ingredients into fine powders—products with new properties.

Microencapsulation is a technique whereby liquid droplets or solid particles are packed into continuous individual shells. The shells or walls as they are called are designed to protect the encapsulated material (core) from factors that may cause deterioration. By a different approach, the wall is designed to permit

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controlled release of the encapsulated material (core) under desired conditions.

The microcapsule wall material can be formulated by using a wide variety of materials including natural and synthetic polymers. The selection of the microcapsule wall material is of the utmost importance, as it constitutes the main critical point for providing stability and cost efficiency to the system 'and must be compatible with the microencapsulating technique followed (Pedroza-Islas et al. 2002). Furthermore, the wall material must release the encapsulated material into the final product during the application.

Numerous techniques for microencapsulation for various core materials have been reported in the literature. Out of the various techniques available in the literature, the coacervation process (both complex and simple coacervation) for encapsulation has been extensively used. This study focused on coacervation which is based on a controlled phase separation induced by macromolecule desolvation. If two oppositely charged polyelectrolytes are simultaneously involved, the process is called complex coacervation (Bodmeier et al. 1996, Palmieri et al. 1996, Kim et al. 2001, Peniche et al. 2003, Tammishetti and Thimma 2003, Xing et al. 2004) and in simple coacervation the desolvation of one polymer is induced by salt addition, pH, temperature modification, etc. (Bachtsi and Kiparissides 1996, Mauguet et al. 2002, Maji et al. 2007). In complex coacervation, electrostatic interactions between two oppositely charged polyions play the major role.

Chitosan is a hydrophilic, biodegradable and bio-compatible positively charged polysaccharide of low toxicity, which in recent years has found applications in cosmetic, biotechnology and drug delivery systems. This polymer has mucoadhesive properties due to its positive charges at neutral pH that enable an electro-static interaction with mucous or a negatively charged mucosal surface. On the other hand, gelatin is an abundant protein and it is derived from collagen. There are two types of gelatin, namely Type A and Type B. Type A is positively charged below pH 8.0 and type B is negatively charged above pH 5.0. Gelatin is surface active and it is used as an emulsifier for various oils. Thus, gelatin is able to be an ideal candidate for the wall material of microcapsules. Again in order to improve the controlled release behaviour, varieties of cross-linking agents are used. Most of the cross-linking agents are synthetic and not free from problems caused by physiological toxicity. Genipin, a natural cross-linker, whose cytotoxicity, feasibility and biocompatibility have well been studied are reported (Sung et al. 1999, Mi et al. 2000). It is 10 000 times less toxic than glutaraldehyde (Sung et al. 1999). In the present investigation, a modified complex coacervation method was used to produce *zanthoxylum limoriella* oil (ZLO) containing chitosan-gelatin microcapsules cross-linked by genipin, a natural cross-linking agent. To the best of the authors' knowledge, genipin cross-linked

gelatin-chitosan microcapsules containing ZLO by using salting-out technique has not been investigated to date. This communication preparea and reports the effect of different parameters on release characteristics of oil.

Materials and methods

Materials

Gelatin (G) type B (from bovine skin, 225 bloom) and chitosan (C) (medium molecular weight, viscosity 200 cps) for microcapsule wall material were purchased from Sigma-Aldrich Inc. (USA). Anhydrous sodium sulphate, Tween 80, acetic acid and sodium hydroxide were obtained from E. Merck (India). Essential oil ZLO, the core material, was extracted and purified in the laboratory and used. The Genipin (Mw = 226.23) was purchased from Challenge Bioproducts Co. (Taichung, Taiwan). All other reagents and solvents used were of analytical grade.

Coacervation behaviour study

The study of phase separation behaviour of aqueous solution of chitosan-gelatin mixture in the presence of sodium sulphate solution is required in order to optimize the coacervation process. The coacervation process depends on several factors like polymer-to-salt ratio, temperature, etc.

Aqueous solution (0.025% w/v) of chitosan in 1% (v/v) acetic acid and 0.04% (w/v) aqueous solution of gelatin in deionized water were prepared. The solution of chitosan and gelatin were mixed at different ratios (1.0:0.50–2.0) at room temperature (~30°C) under stirring condition. Now a predetermined amount of aqueous sodium sulphate solution (20% w/v) was added to each polymer mixture containing chitosan and gelatin at different ratios at room temperature. The ratio of total polymer-to-sodium sulphate was varied from 1:2–1:30. The temperature was varied from 30–50°C. The minimum temperature and polymer–salt ratio at which clear phase separation occurred were recorded.

Preparation of microcapsules

Chitosan flakes (2.50 g) were dissolved in 100 ml of 1% (w/v) acetic acid solution by stirring overnight in a conical flask until a clear solution was obtained. Gelatin solution was prepared by swelling 4.0 g of gelatin in (type B) in 100 ml double distilled cold water followed by heating until the appearance of a clear solution. Variable amounts of gelatin and chitosan solution were taken in a beaker at room temperature (~30°C) so that the weight ratios of chitosan to gelatin were 0/1, 0.33/0.67, 0.5/0.5, 0.67/0.33 and 1/0. To this mixture, a drop of silicon anti-foaming agent and essential oil (1–8 ml) were added under high agitation

by mechanical stirring to form an emulsion. Initially the coacervation of chitosan was brought about by gradual addition of aqueous sodium sulphate solution (20% w/v) for ~2–2.5 h. In this stage ZLO encapsulated chitosan particles/microcapsules were formed. The pH of the entire mass of the beaker was then brought between 7.0–8.0 to induce interaction between chitosan, gelatin (type B) and genipin. The cross-linking of the chitosan-gelatin microcapsule was achieved by addition of a certain amount of aqueous genipin solution (0.1–0.3 mmol g^{-1} of polymer). The temperature of the vessel was maintained between 40–50°C and stirring was continued for another 3 h. The vessel was then cooled to room temperature. The microcapsules were filtered, washed initially with 0.3% Tween 80 solution to remove excess oil adhered to the surface and finally with double distilled water, dried and kept in a storage vial.

Measurements

A known concentration of essential oil in distilled water containing 0.3% Tween 80 was scanned in the range of 200–400 nm by using a UV-visible spectrophotometer. For ZLO having concentration in the range 0.005–0.10 gm/100 ml, a sharp peak at 256 nm was noticed. The absorbance values at 256 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of ZLO was obtained by knowing the absorbance value.

Encapsulation efficiency, oil content and oil load

A known amount of accurately weighed microcapsules were crushed using a mortar and carefully taken in a volumetric flask containing a known amount of 0.3% aqueous Tween 80 solution and kept for sufficient time under continuous stirring condition for ensuring complete extraction of oil from the microcapsules. The encapsulation efficiency (%), oil content (%) and oil loading (%) were calculated by using the calibration curve and the following formulae:

$$\text{Encapsulation efficiency (\%)} = w_1/w_2 \times 100$$
$$\text{Oil content (\%)} = w_1/w \times 100$$
$$\text{Oil load (\%)} = w_2/w_3 \times 100$$

where w = weight of microcapsules, w_1 = actual amount of oil encapsulated in a known amount of microcapsules, w_2 = amount of oil introduced in the same amount of microcapsules and w_3 = total amount of polymer used including cross-linker. Each measurement was performed in triplicate and average ±SD value was taken.

Oil release studies

Oil release studies of encapsulated oil were evaluated using a UV-visible spectrophotometer (UV–2001 Hitachi). A known quantity of microcapsules was placed directly into a known volume of 0.3% Tween 80 surfactant solution. The microcapsule– Tween 80 mixture was magnetically stirred at a constant rate and the temperature throughout was maintained at 30°C (room temperature). An aliquot sample of known volume (5 ml) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 256 nm for the determination of cumulative amount of oil release up to a time t. Each determination was carried out in triplicate. To maintain a constant volume, 5 ml of 0.3% Tween 80 solution was returned to the container.

Fourier transform infrared measurements

FT-IR spectral measurements were performed using a Nicolet (model impact-410, USA) spectrophotometer. Microcapsules were grounded finely with KBr and FT-IR spectra were taken in the range of 400–4000 cm^{-1}.

Scanning electron microscopy

Dry microcapsules were mounted on a metal stubs, sputtered with gold and viewed in a scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 15 kV and at room temperature.

Results and discussion

Phase separation behaviour

The phase separation behaviour of chitosan and gelatin (type B) were studied at first in order to get an idea regarding minimum temperature or polymer–salt ratio to be required individually. Gelatin (type B) solution did not produce any coacervate at any sodium sulphate concentration and temperature, whereas chitosan produced coacervate throughout the temperature range and salt ratio studied. In the case of the mixture of chitosan–gelatin, the minimum ratio of polymer mixture to salt and temperature at which clear phase separation observed were 1 : 5 and 40°C, respectively. Therefore, all the experiments for microencapsulation were done by maintaining the ratio of polymer-to-salt and temperature at 1 : 5 and 40°C, respectively.

Effect of variation of oil loading

The effect of variation of oil loading on the encapsulation efficiency, oil content and release rate is shown in Table I and Figure 1. With the increase of oil loading, the release rate of the oil from the chitosan gelatin microcapsules increased throughout the range of oil

Table 1. Effect of variation of oil loading, chitosan-to-gelatin ratio and genipin concentration on the behaviour of microcapsules (Chitosan/gelatin mixture: 1 gm; Genipin: 0.1–0.3 mmol gm⁻¹ of mixture, oil: 1–8 ml; water: 100 ml; temperature: 40 ± 1°C).

| Samples | | | | | |
Chitosan/gelatin (C/G)	Genipin	Oil	Oil load (%)	Oil content (%) ($M \pm SD$)	Encapsulation efficiency (%) ($M \pm SD$)
0.0/1.0*	0.1	4 0	—	—	—
0 33/0.67	0 1	4.0	⁓343.67	42.20 ± 1.67	48.36 ± 1.52
0.50/0.50	0.1	4.0	343.67	37 59 ± 0.07	48 53 ± 1 00
0.67/0.33	0:1	4.0	343.67	46.25 ± 2.36	52 65 ± 2 38
1.0/0.0	0.1	4.0	343.67	62.60 ± 2.10	80 80 ± 1 20
0.50/0.50	0.2	4.0	343 67	39.78 ± 1.52	51.36 ± 1.96
0.50/0.50	0 3	4.0	343.67	51.30 ± 1.02	66.22 ± 1.31
0 50/0.50	0.1	1.0	85.92	25 17 ± 0 34	54.46 ± 0.75
0 50/0.50	0.1	2.0	171.83	25.80 ± 1 01	40.82 ± 1.60
0.50/0.50	0 1	6 0	515.50	39.92 ± 0.20	47 67 ± 0.24
0.50/0.50	0.1	8 0	687.34	45.06 ± 3.03	51 60 ± 3.47

*Gelatin (type B) produced no coacervation.
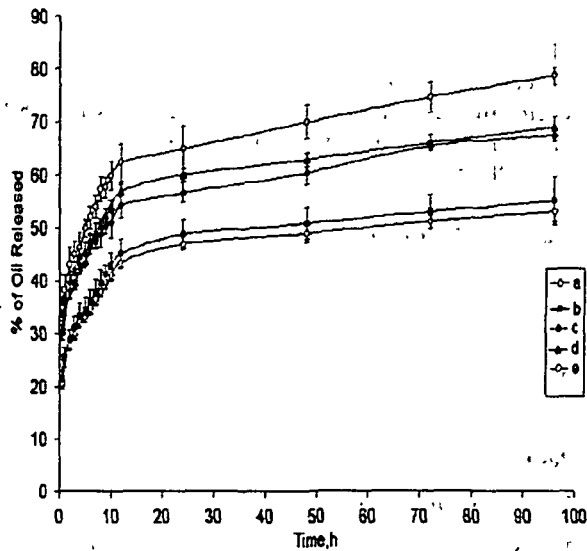
Figure 1. Effect of variation of oil loading on the release rate. (a) CG (0.5/0.5), ZLO 1.0 ml, Gp 0.1 mmol; (b) CG (0.5/0.5), ZLO 2.0 ml, Gp 0.1 mmol; (c) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.1 mmol; (d) CG (0.5/0.5), ZLO 6.0 ml, Gp 0.1 mmol; (e) CG (0.5/0.5), ZLO 8.0 ml, Gp 0.1 mmol.

concentration studied. The percentage oil content showed an increasing trend as expected. Encapsulation efficiency showed a decreasing trend initially and later almost levelled off. The level of decrease in encapsulation efficiency noticed was more when the amount of oil introduced was 2.0 ml. The reason was not clear. However, the reason for overall decrease in encapsulation efficiency might be explained as follows. At low oil loading, the dispersion of the oil into globules by the stirrer was more effective, therefore the oil vesicles were smaller. At this stage, the amount of polymer present in the system was enough to encapsulate properly the oil vesicles. However, as more oil was introduced, the dispersive force of the

stirrer became less efficient and larger oil vesicles were produced as a result. Also there was an increased tendency for the oil vesicles to coalesce at higher oil loads, so that the larger oil vesicles were formed and more oil could be encapsulated with the same amount of encapsulating material at the expense of decrease of thickness of microcapsule wall. At this time, the amount of polymer might not be sufficient for encapsulation of all oil vesicles. The chances of existing of some oil vesicles without encapsulation became more. The loss of these oil vesicles during isolation might cause a reduction in encapsulation efficiency. Both thickness of the wall and extent of cross-linking governed the release rate. In all these cases, it was assumed that the level of cross-linking remained almost same. Therefore, the thickness of the wall might play a predominant role for controlling the release rate. The increase in the oil load would result in a decrease of wall thickness, as explained earlier. The faster release rate of the microcapsule at higher loading might be due to decreased wall thickness of the microcapsule. With the decrease in wall thickness of the microcapsule, diffusional path for the oil release became short (Madan 1981, Senjokovic and Jalsenjak 1981) which resulted in an increase of release rate. Similar type of results was reported in the literature (Maji et al. 2007). Again with the increase in percentage oil load, the oil content (%) increased. At low oil load, many of the microcapsules have few or no oil vesicles in them, indicating that there was an abundance of the encapsulating polymer for the oil present. When the amount of the used oil increased, there was an increase in the number of oil vesicles in the microcapsules which resulted 'in' an increase of oil content.

Effect of variation of cross-linker concentration

The effect of variation of cross-linker concentration on encapsulation efficiency (%), oil content (%) and
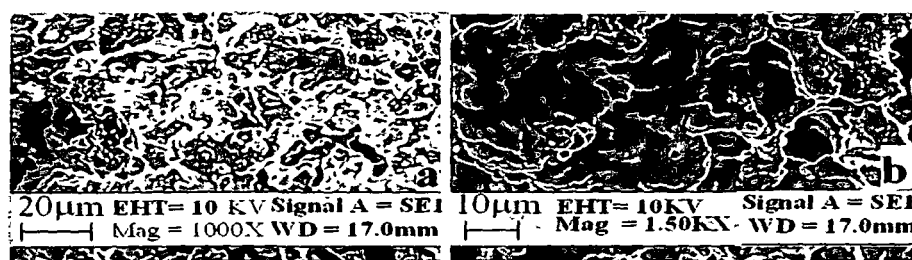
Figure 2. Scanning electron micrographs of microcapsules prepared with oil load (%) (a) 343.67 and (b) 687.34.
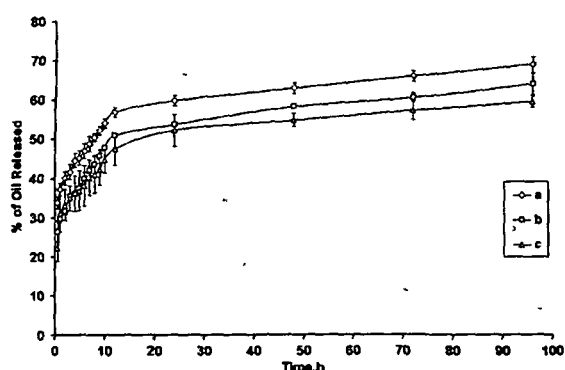

Figure 3. Effect of variation of cross-linker on the release rate. (a) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.1 mmol; (b) CG (0.5/0.5), ZLO 4 0 ml, Gp 0.2 mmol; (c) CG (0 5/0.5), ZLO 4.0 ml, Gp 0.3 mmol.

release rate are shown in Table I and Figure 2, respectively. The results found were as per expectation. The increased encapsulation efficiency (%) might be due to the improvement of oil retention capacity of the microcapsule caused by the reaction between cross-linking agent genipin and microcapsule wall material, chitosan and gelatin. An increase in the degree of cross-linking as expressed by molar concentration of genipin used, resulted in a decrease in oil release rate throughout the ' genipin concentration studied (0.1 mmol per gram of polymer-0.5 m mol per gram of polymer). As the degree of cross-linking of microcapsule wall material increased, the microcapsule wall became denser, resulting in the decrease . of diffusion rate of the oil through the microcapsule wall. Similar type of observations were reported in the literature (Bachtsi and Kipparissides 1996, Maji et al. 2007). The probable reactions between gelatin, chitosan and genipin are presented in Figure 3. ·

Effect of variation of chitosan-to-gelatin ratio

The related results are shown in Table I and· Figure 4. The release rate of oil from gelatin-chitosan microcapsules was dependent on the percentage of chitosan present in the 'mixture. The higher the percentage of chitosan in the chitosan-gelatin mixture, the lower was

the release rate. The lower release rate might be due to the formation of microcapsules having more compact wall. It was known that every glucosamine unit of chitosan could react with genipin, whereas only primary amine groups of lysine and arginine residues on gelatin could react with genipin. The lysine and arginine residues in gelatin are much less. On the other hand, chitosan has a more average number of primary amine groups than gelatin for the reaction with genipin (Mi 2005). The more the percentage of chitosan in the chitosan-gelatin mixture, the higher the reaction between chitosan and genipin. As a result, more cross-linking would take place. This would in turn form a more compact wall, resulting in a decrease of release rate. A decrease in the release rate was reported (Kim et al. 2006) in the literature during studying of release behaviour of triclosan encapsulated in chitosan–gelatin microcapsules.

FTIR study

Figure 5 shows the FTIR spectra of gelatin, chitosan, ZLO and ZLO containing chitosan-gelatin microcapsules. The spectrum of ZLO displayed peaks around 1673 and 1720 cm^{-1} which were due to carbonyl stretching band. The other peaks, which appeared at 1463 and 1383 cm^{-1}, were due to CH$_2$ assymetric and CH$_2$ symmetric deformation. The spectrum of chitosan showed an amide characteristic peak at 1633 cm^{-1}. Gelatin was characterized by its carbonyl peak and amino band appeared at 1624 and 1547 cm^{-1}, respectively. The shifting of carbonyl band to 1641 cm^{-1} indicated an interaction between gelatin and chitosan. The position of this peak did not alter when compared to that of spectrum of ZLO. The position of other peaks also were found to remain unchanged. This suggested that there was no significant interaction between chitosan-gelatin complex and ZLO.

Scanning electron microscopy study

Figure 6 shows the SEM photographs of microcapsules having different percentages of oil loading. At higher oil loading (Figure 6(b)), a bursting look was observed and it appeared more· compared to those of. microcapsules
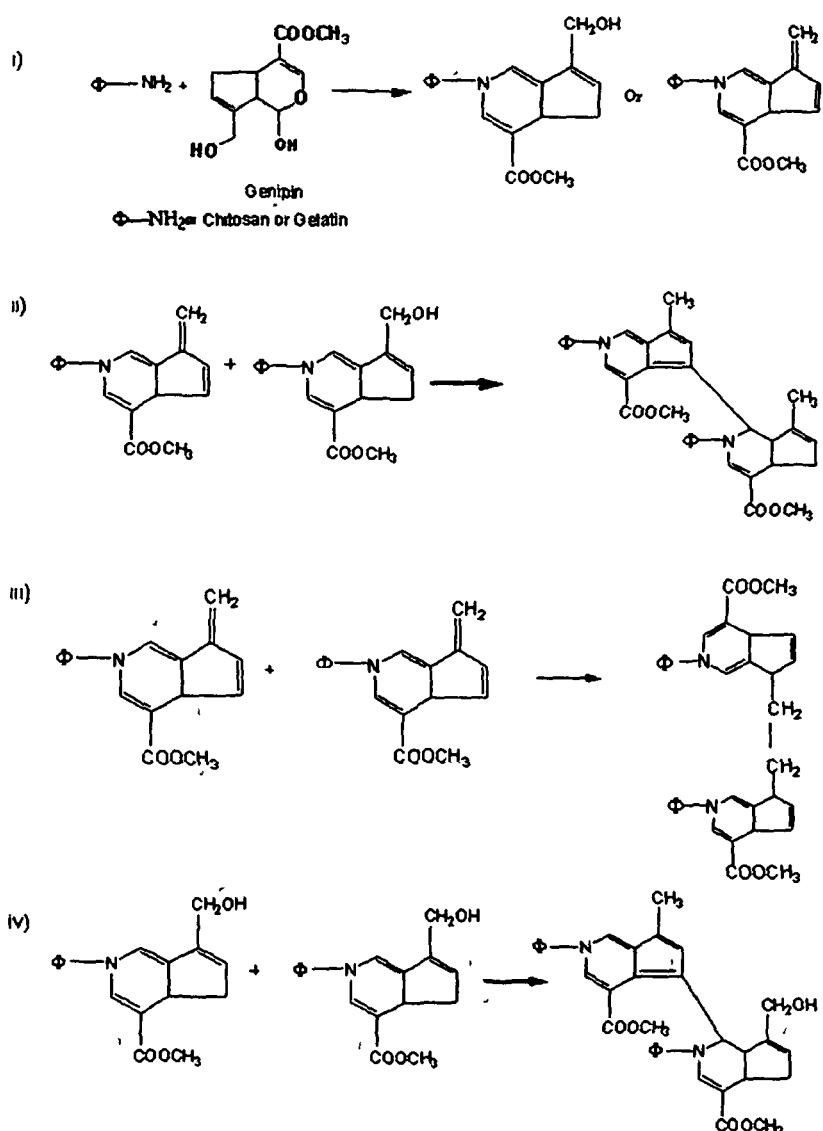
Figure 4   Reaction scheme between chitosan, gelatin and genipin
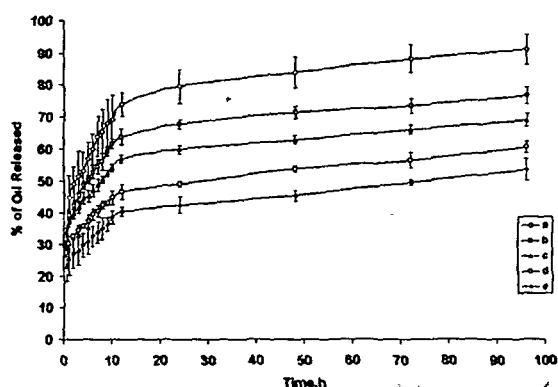

Figure 5   Effect of variation of chitosan-to-gelatin ratio on release rate (a) CG (0/1), ZLO 4 0 ml, Gp 0 1 mmol, (b) CG (0 33/0 67), ZLO 4 0 ml, Gp 0 1 mmol, (c) CG (0 5/0 5), ZLO 4 0 ml, Gp 0 1 mmol, (d) CG (0 67/0 33), ZLO 4 0 ml, Gp 0 1 mmol, (e) CG (1/0), ZLO 4 0 ml, Gp 0 1 mmol

prepared at low oil load (Figure 6(a)) Moreover, on physical examination, the surface of the microcapsules containing higher percentages of oil appeared more oily and agglomerated compared to those of microcapsules containing lower percentages of oil

Conclusion

The study showed that the entrapment of ZLO into chitosan-gelatin microcapsules could be achieved using the salting-out procedure The release of ZLO was found to be dependent on percentage of oil loading, cross-linking density and chitosan-gelatin ratio There was an interaction between chitosan and gelatin during the formation of complex, as evident by FTIR study The study also showed no significant interaction between oil and chitosan-gelatin matrix
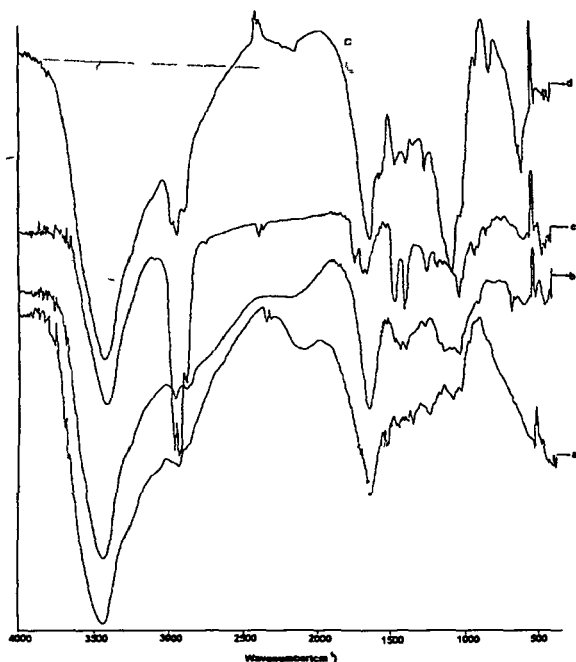
Figure 6   FTIR spectra of (a) gelatin B, (b) chitosan, (c) oil, and (d) oil containing microcapsules

SEM study showed the presence of oil on the microcapsule surface

Acknowledgement

Declaration of interest: The authors report no conflicts of interest The authors alone are responsible for the content and writing of the paper

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Microencapsulation of *Zanthoxylum limonella* oil (ZLO) in glutaraldehyde crosslinked gelatin for mosquito repellent application

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Abstract

Glutaraldehyde (GA) crosslinked gelatin (G) microcapsules containing *Zanthoxylum limonella* oil (ZLO) were prepared by coacervation technique. The effect of various parameters such as variation of oil-loading, gelatin concentration and degree of crosslinking on release rate of oil were studied. Scanning electron microscopy (SEM) was used to understand the surface characteristics of microcapsules. FTIR-results indicated the absence of any significant interaction between polymer and oil.
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Keywords: Microencapsulation; Essential oil; Crosslinker; Gelatin

1. Introduction

Use of essential oil is the subject matter of many investigations for in recent years due to its eco-friendly and biodegradable nature. Various essential oils used as mosquito repellent have been reported in the literature (Sukumar et al., 1991; Sharma et al., 1993; Quarles, 1996; Brown and Hebert, 1997; Ansari et al., 2000; Tawatsin et al., 2001; Prajapati et al., 2005). Volatile oil of *Zanthoxylum hamiltonianum* (timur) has been found to posses effective mosquito larvicidal properties (Nath et al., 1989).

Controlled release formulation seems to be the best choice for increasing the efficiency and minimization of environmental damage. Out of various techniques available in the literature, coacervation process for encapsulation has been extensively used. Gelatin (Rosenblat et al., 1989), polyvinyl alcohol (Bachtsi and Kipparissides, 1996) and various other polymers (Salib et al., 1986; Beyger and Nairn, 1986) have been employed for the production of microcapsules. The permeation characteristic of coacervated crosslinked gelatin-acacia membranes to various

active agents (Jalsenjak and Kondo, 1981; Nixon and Wang, 1989) have been reported in the literature. The release characteristics of ZLO in crosslinked gelatin have not been investigated to the best of our knowledge. The present investigation aimed at to study the release characteristic of oil containing microcapsules prepared under different conditions.

2. Methods

2.1. Materials

Gelatin (E. Merck, India), glutaraldehyde 25% w/v (E. Merck, Germany), anhydrous sodium sulphate (E. Merck, India), Tween 80 (S.d. fine chemicals, Mumbai) were used as received, without further purification. Essential oil (ZLO) was obtained from *Zanthoxylum limonella* plant as per description in the extraction section. Besides this, other reagents used were of analytical grade.

2.2. Essential oil extraction

Seeds of *Z. limonella* a big tree available in the Solmara area of Tezpur, were collected and shed dried for three to

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four days. Essential oil was obtained by steam distillation of the seeds. The oil obtained was separated from aqueous phase and dried by treatment with anhydrous sodium sulphate. The dried oil was transferred into a dark glass bottle and kept at 4 °C for subsequent use.

2.3. Phase separation behaviour of gelatin

A series of experiments were carried out to determine the cloud point temperature of a gelatin solution as a function of sodium sulphate concentration. Flask containing a certain amount of gelatin was immersed in a thermostatic water bath maintained at 5 °C. A predetermined amount of aqueous sodium sulphate solution (10%, w/v) was added to the flask under stirring condition and the temperature of the water bath was gradually raised. The temperature at which the onset of phase separation started was recorded.

2.4. Encapsulation procedure

In a reaction vessel, 4–10% (w/v) of an aqueous solution (50 ml) of gelatin was taken at 30 °C. To this, essential oil (3–15 ml) was added under high agitation to form an emulsion. The temperature of the vessel was then raised to 40 °C. Coacervation was brought by gradual addition of aqueous sodium sulphate solution (20% w/v) for about 90 min. The vessel was kept at this temperature for another 30 min. The temperature of the vessel was then brought down to about 5 °C. The crosslinking of the polymer capsule was achieved by slow addition of certain amount of glutaraldehyde (1–10 mmol/g of gelatin) solution, which consisted of methanol 16.67%, acetic acid 5%, sulphuric acid 0.17% and glutaraldehyde 25%. The temperature of the vessel was then raised to 40 °C and stirring was continued for about 3–4 h. The vessel was cooled to room temperature. The microcapsules were filtered, washed with 0.3% Tween 80 solution, dried and stored in a glass bottle.

2.5. Measurements

A known concentration of essential oil in distilled water containing 0.3% Tween 80 was scanned in the range of 200–400 nm by using UV visible spectrophotometer. For ZLO having concentration in the range 0.005–0.1 g/100 ml, a sharp peak at 256 nm was noticed. The absorbance values at 256 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of ZLO was obtained by knowing the absorbance value.

2.5.1. Encapsulation efficiency, oil content and oil load

A known amount of accurately weighed crushed microcapsules was taken in a volumetric flask containing a known amount of 0.3% aqueous Tween 80 solution and kept overnight with continuous stirring. The encapsulation efficiency (%), oil content (%) and oil loading (%) were cal-

culated by using the calibration curve and the following formulae:

Encapsulation efficiency (%) $= w_1/w_2 \times 100$

Oil content (%) $= w_1/w \times 100$

Oil load (%) $= w_2/w_3 \times 100$

where w = weight of microcapsules; w_1 = actual amount of oil encapsulated in a known amount of microcapsules; w_2 = amount of oil introduced in the same amount of microcapsules; w_3 = total amount of polymer used including crosslinker.

2.5.2. Oil release studies

A known quantity of microcapsules was placed into a known volume of 0.3% Tween 80 surfactant solution. The microcapsule—Tween 80 mixture was magnetically stirred at a constant rate and the temperature throughout was maintained at 30 °C. An aliquot (5 ml) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 256 nm (UV-2001 Hitachi) for the determination of cumulative amount of oil release up to a time t. Each determination was carried out in triplicate. To maintain a constant volume, 5 ml of 0.3% Tween 80 solution was returned to the container.

Microcapsules were grounded and FTIR spectra were recorded using KBr pellet in a Nicholet (model Impact-410) spectrophotometer. Surface characteristics of the microcapsules were studied using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 15 kV.

3. Results and discussion

The minimum temperature and ratio of gelatin to sodium sulphate at which phase separation occurred were 40 °C and 1:10 (data not shown). This was judged by the clear separation of gelatin in fine particle form from its aqueous solution. This temperature and ratio were maintained during preparation of microcapsules in the subsequent experiments. All experiments were carried out in triplicate and results presented were the average values.

3.1. Effect of variation of oil loading

The effect of variation of oil loading on the encapsulation efficiency and release rate is shown in Table 1 and Fig. 1. With the increase in oil loading, the release rate increased throughout the range of oil concentration studied. The encapsulation efficiency decreased while the % oil content and release rate increased. A possible explanation for the lower encapsulation efficiency at higher oil load might be due to the higher percentage of oil loss during isolation. At low oil load, the disperse force by the stirrer was more effective, causing the formation of smaller oil vesicles. The amount of gelatin present in the system was

Table 1

Effect of variation of oil loading, gelatin and glutaraldehyde concentration on the behaviour of microcapsules

Sample particulars			Oil load (%)	Oil content (%)	Encapsulation efficiency (%)
Gelatin	Glutaraldehyde	Oil			
3	10	5	74.10	42.90 ± 0.51	98.20 ± 1.13
3	10	7	106.42	49.69 ± 1.29	96.42 ± 2.50
3	10	10	148.30	50.26 ± 0.57	88.42 ± 1.07
3	10	13	192.60	51.60 ± 2.0	87.89 ± 3.04
3	10	15	222.30	59.82 ± 1.2	78.00 ± 1.73
2	5	7	295.60	55.90 ± 0.64	78.35 ± 0.89
3	5	7	142.00	51.16 ± 0.54	97.46 ± 1.02
5	5	7	79.95	29.90 ± 0.54	96.34 ± 1.74
3	1	7	193.82	38.98 ± 0.73	59.10 ± 1.11
3	2	7	177.00	41.80 ± 0.87	65.32 ± 1.36

Gelatin: (2–5 gm); glutaraldehyde: (1–10 mmol/gm of gelatin); oil: (5–15 ml); water: 50 ml; temperature: 30 °C.

Fig. 2. Effect of variation of gelatin concentration on the release rate: (a) G 2 g, GA 5 mmol, ZLO 7 ml; (b) G 3 g, GA 5 mmol, ZLO 7 ml; (c) G 5 g, GA 5 mmol, ZLO 7 ml.

3.2. Effect of variation of gelatin concentration

Table 1 shows the effect of variation of gelatin concentration. As expected, oil loading (%) and oil content (%) decreased as polymer content increased. Encapsulation efficiency (%) increased first and then leveled off. With the increase in polymer content, more and more gelatin would be available to encapsulate the oil vesicles and thereby efficiency would be increased. At certain polymer content, all the oil vesicles present would be encapsulated by the polymer. After that, excess polymer would be used to thicken the microcapsule wall, which resulted in leveling off the efficiency. Fig. 2 shows the release profile with the variation of gelatin concentration. The concentration of gelatin was varied from 2 to 5 g. The release rate decreased with increase in gelatin concentration. The increase in wall thickness of the microcapsule might be responsible for this type of behavior
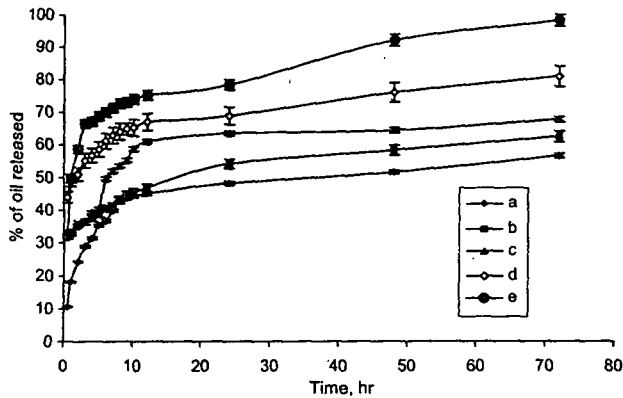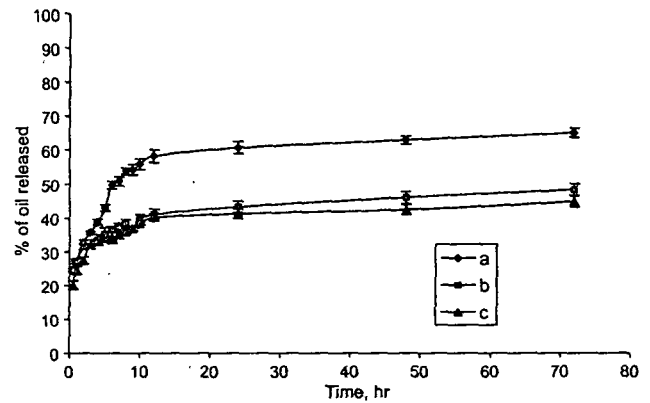

Fig. 1. Effect of variation of oil loading on the release rate: (a) G 3 g, GA 10 mmol, ZLO 5 ml; (b) G 3 g, GA 10 mmol, ZLO 7 ml; (c) G 3 g, GA 10 mmol, ZLO 10 ml; (d) G 3 g, GA 10 mmol, ZLO 13 ml; (e) G 3 g, GA 10 mmol, ZLO 15 ml.

sufficient to encapsulate the oil vesicles properly. At higher oil load, the dispersive force of the stirrer became less efficient and larger oil vesicles were produced as a result. At this stage, gelatin would try to encapsulate the larger oil vesicles at the expense of decrease of thickness of microcapsule. Also, the amount of gelatin might not be sufficient to encapsulate all the oil vesicles. Some of the oil vesicles might exist without encapsulation. These oil vesicles might get lost during isolation. The faster release rate might be due to the decrease of the thickness of the capsule wall. With decrease in wall thickness, diffusional path for the oil release became short (Senjokovic and Jalsenjak, 1981; Madan, 1981) which resulted in an increase of release rate. With increase in % oil load, the oil content (%) increased. At low oil load, many of the microcapsules probably contained few oil vesicles indicating that there was an abundance of the encapsulating polymer for the oil present. With the increase in oil load (%), the number of oil vesicles in the microcapsule increased and thereby resulted in an increase in oil content.
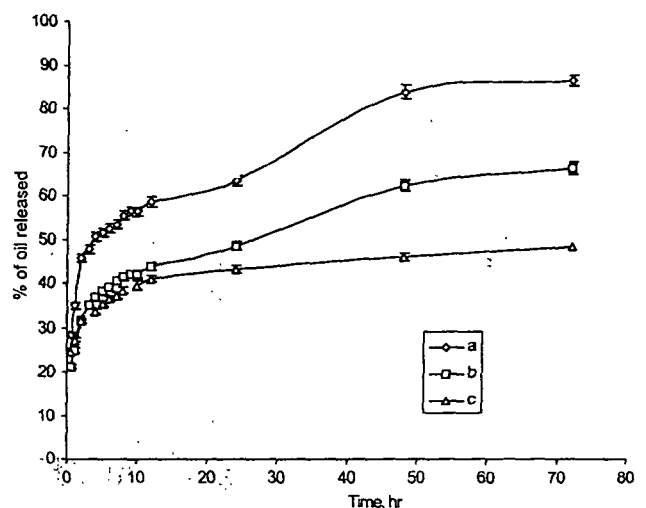

Fig. 3. Effect of variation of crosslinker on the release rate: (a) G 3 g, GA 1 mmol, ZLO 7 ml; (b) G 3 g, GA 2 mmol, ZLO 7 ml; (c) G 3 g, GA 5 mmol, ZLO 7 ml.

3.3. Effect of variation of glutaraldehyde concentration

The related results are shown in Table 1 and Fig. 3. The trend of oil loading (%) and oil content (%) shown in the table was as per expectation. The increased encapsulation efficiency (%) could be due to the improvement in oil retention capacity of the microcapsules caused by the reaction between gelatin and glutaraldehyde. An increase in the degree of crosslinking, as expressed by molar concentration of glutaraldehyde used, resulted in a significant decrease in oil release rate throughout the glutaraldehyde concentration studied (1 mmol/g of gelatin −5 m mol/g of gelatin). As degree of crosslinking of gelatin increased, the microcapsule wall became denser resulting in the decrease of diffusion rate of oil through the microcapsule wall. Similar types of observations were reported in the literature (Bachtsi and

Kipparissides, 1996; Raymond et al., 1990). Dinarvand et al. (2005) also investigated and reported the effect of crosslinker on the release rate of lactic acid from gelatin microspheres.

3.4. Scanning electron microscopic study

SEM photographs of glutaraldehyde crosslinked gelatin microcapsules of varying oil content are shown (Fig. 4). Microcapsules appeared to be made of spherical units linked to each other. The external surface appeared smooth at low oil loading indicating the formation of a continuous film by gelatin. At higher oil loading, a bursting look was observed. Chan et al. (2000) reported similar type of result while encapsulating two different type of oils in sodium alginate crosslinked matrix. The microcapsules prepared at
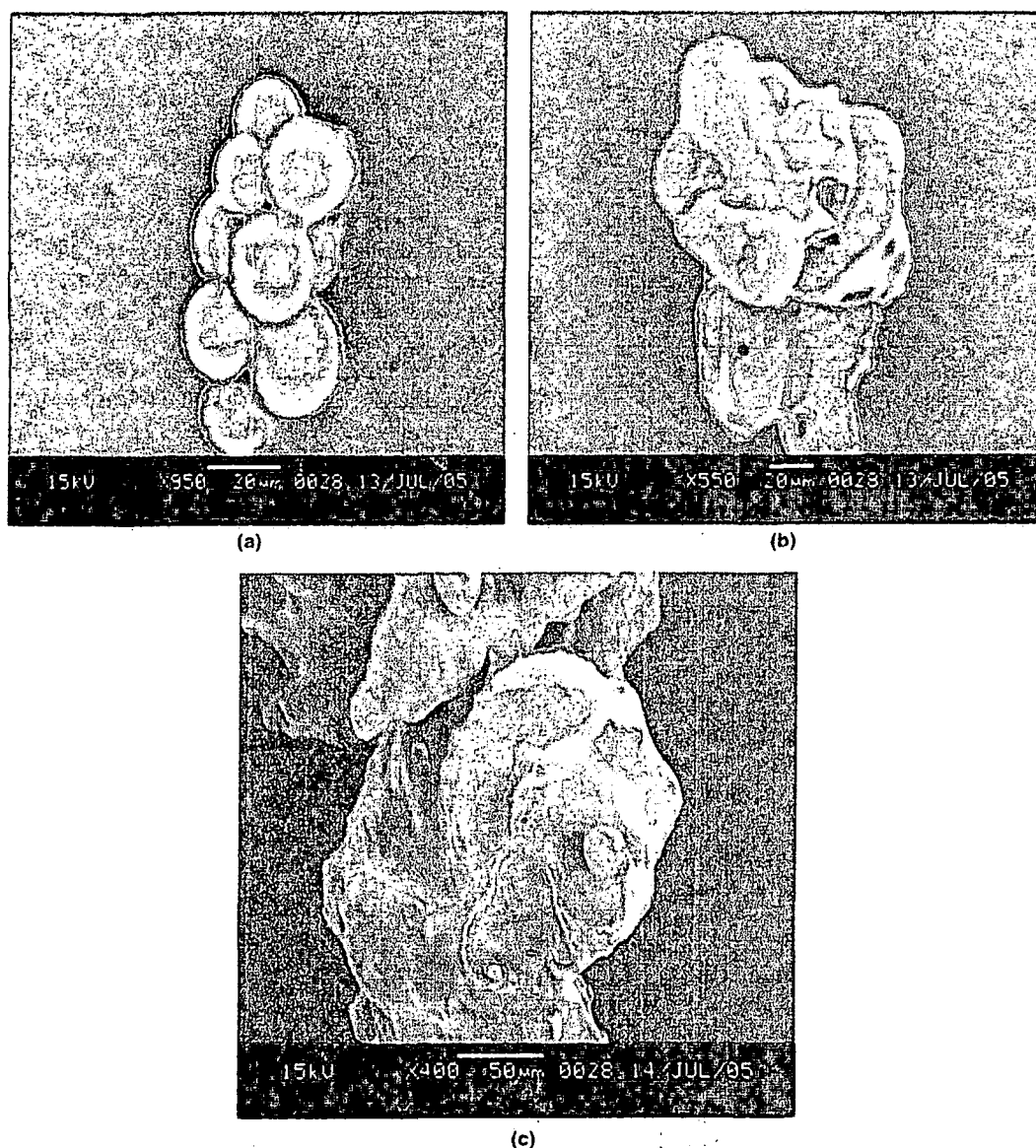

Fig. 4. Scanning electron microphotograph of microcapsules prepared with oil load: (%) (a) 144.7 (b) 200 (c) 438.8.

low oil loading, appeared dry and powdery on physical verification, whereas those prepared at higher oil loading appeared oily and agglomerated

3 5 FTIR study

FTIR spectra of ZLO, gelatin, physical mixture of ZLO, glutaraldehyde, gelatin and ZLO containing crosslinked gelatin microcapsules were recorded (spectra not shown) Physical mixture was prepared using the ratio of ZLO, glutaraldehyde and gelatin similar to those of ratio used in preparing ZLO containing crosslinked gelatin microcapsules In the spectra, the carbonyl stretching band of ZLO between $1637-1720\,cm^{-1}$ remained almost unchanged in the case of physical mixture as well as microcapsule The other notable peaks appeared at $1457\,70\,cm^{-1}$, $1377\,78\,cm^{-1}$, $1232\,73\,cm^{-1}$, $1167\,53\,cm^{-1}$ and $1019\,89\,cm^{-1}$, which were due to CH_2 asymmetric deformation, CH_2 symmetric deformation, C–N, C–C and C–O stretching vibration also remained almost unchanged in the physical mixture and microcapsules These results indicated the absence of any significant interaction between the ZLO and the gelatin polymer

4. Conclusion

It was concluded that oil from *Z limonella* could be encapsulated successfully within crosslinked gelatin microcapsule The release rate was dependent on oil content, crosslinking density and encapsulating polymer concentration

Acknowledgement

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