Chapter 5

Microencapsulation of anthocyanin-rich extract using double emulsion complex coacervation technique and its characterization

5.1. Introduction

Antioxidants such as anthocyanin are sustainable natural pigments found in leaves, roots, fruits, flowers of plants, and some cereal grains. Anthocyanins (ANCs) are the largest group of water-soluble flavonoids, profoundly colored materials, that impart red, purple, and blue colors [46]. These compounds are also used as food colorants; providing a brilliant red color to many foods. Consumers are increasingly interested in developing natural colorant alternatives to synthetic colors in food applications [38]. Again, artificial pigments are becoming unacceptable in food and beverages due to their negative health and environmental effects [17]. Fruit such as blackberries, and gooseberries comprise the natural source of antioxidants that effectively inhibit free radical formation [35]. They can either suppress or inhibit the oxidation of lipids, including fats, oils, and fatty acids [8]. Also, burgeoning studies have reported its importance in terms of nutritional and medicinal aspects. These compounds are very promising and are also known to possess a wide range of potential nutraceuticals and promote health characteristics such as anti-microbial, anti-cancer, antidiabetic, antiproliferative, anti-inflammatory activities, etc. [36]. Antioxidants are also used as a food component to maintain the lipid components from quality deterioration. Due to their multiple physiological functions, consumers demand has been addressed for commercialization for food fortification and its nutraceutical applications [37].

Functional components present in food (specially antioxidants) are non-conventional biomolecules possessing the extend to regulate one or more metabolic pathways in the body that results to health promotion [1]. They are also known to be available in rice varieties, which deliver many health-beneficial properties. It is also reported that rice bran contains several bioactive compounds which includes γ-oryzanol, vitamin E (α-tocopherol, γ-tocopherol, and δ-tocopherol) [43]. Colored rice such as black and other pigmented rice bran varieties are known to provide antioxidant activities [24, 26]. They contain significant amounts of phenolic acids such as vanillic acid, protocatechuic acid, chlorogenic acid, gallic acid, p-hydroxybenzoic acid, syringic acid, p-coumaric acid, ferulic acid, flavonoids (kaempferol) [24, 26]. They are also

known to contain color pigment ‘anthocyanins’, especially cyanidin and peonidin-3-glucoside [24, 26] that are known for *in vitro* inhibitory effect on the proliferation of cancer cell, oxidative stress protection on endothelial cells [25, 40]. Pigmented rice has also shown an *in vivo* effect to subsequently reduce the risk of atherosclerosis, total cholesterol, low-density cholesterol, and total triacylglycerol contents of rats [45]. Also, great attention and curiosity have been received due to its great physiological response to human health by acting as a nutraceutical [28]. There is also a need for promotion of this rice variety in terms of functional foods because of therapeutic benefits [7].

Despite their high antioxidant potential, these compounds have low stability and can degrade under various environmental and processing conditions (temperature, pH, light, oxygen). They have high reactivity with other compounds in the food matrix [3]. Anthocyanin degradation is also accompanied by change or loss of color, which can be due to the change in molecular structure [42]. The stability of these compounds is reduced by the hydroxylation process while methylation increases it [19]. Due to poor chemical stability of anthocyanin, the applications in food matrix are restricted [46]. One alternative for prolonging ANCs stability is the microencapsulation, which aims to entrap the active agent or component inside a coating material. It ensures component stability and physicochemical characteristics from external environmental conditions by developing modified wall materials, such as proteins and polysaccharides into micron structures [32]. A double emulsion W/O/W (water-in-oil-water) is a microencapsulation process that is used to trap hydrophilic active materials and slow their release. However, the process is unstable and in order to improve the dual emulsion’s stability, complex coacervation has been employed [21]. Complex coacervation is an associative phase separation process that produces a colloid-rich phase called coacervates by modifying the environment under controlled conditions such as ionic strength, pH, temperature, solubility, and so on [32]. The driving force accompanying this process is electrostatic interaction among the charged macromolecules present in the reaction medium [41]. Hence, introducing a protective method to preserve them with great curiosity has been given lots of attention in recent times.

Many studies have been carried out on the double emulsion encapsulation process [16, 20, 23]. These include complex coacervations of core materials black raspberry

anthocyanin and aspartame with the encapsulating agent's gelatin and gum arabic carried out for improvement of the double emulsion's stability along with freeze drying [30, 35]. Spray dried encapsulation have also been developed, characterized and evaluated for their kinetic degradation and color stability of bioactive compounds [5, 6, 12, 14]. The high value of encapsulation efficiency of ascorbic acid was observed in microencapsulation using double emulsion technique [9]. Microencapsulation of lycopene aimed to protect and control release has also been reported by Silva et al., [39]. Encapsulation of propolis extract by complex coacervation was also carried out using isolated soy protein and pectin as encapsulating agents [22]. For decades, this process has been carried out for its control release and stability enhancement in the pharmaceutical industries. Although many studies have been conducted on microencapsulation techniques, limited studies were reported on application of coacervation technique for microencapsulation of black rice bran anthocyanin technique applied in black rice bran anthocyanin.

Therefore, the present work aims to develop microcapsules with different formulations of active anthocyanin as core solution with varying composition of the wall materials gelatin and acacia gum, keeping in mind to provide the maximum encapsulation efficiency and analyzing their effect on the controlled release in foods. This work can provide, explore and stimulate new research on the microencapsulation technique; further utilize and incorporate it in food matrix.

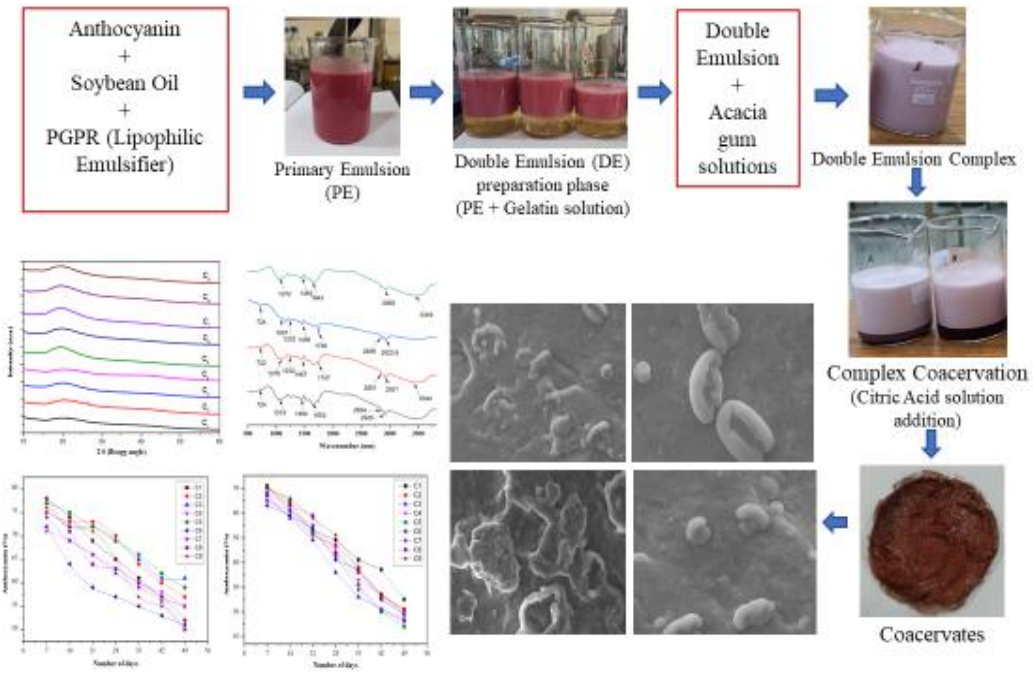


Fig. 5.1. Pictorial representation of Chapter 5

5.2. Materials and methods

5.2.1. Raw materials and chemicals

The black rice (*Oryza sativa L. indica*) was procured from the local farmer of Manipur, India. The rice was milled using a rice dehusker (APL-5KC30GF029T, Marathon, India) and manually cleaned for foreign particles. Bran was then obtained by milling the black rice in a rice polisher (NF366, Marathon, India). The milled rice bran was then passed through a 500 µm sieve to attain fine particles. The fine bran particles were packed in a zip lock and kept in refrigeration for further use for encapsulation.

Encapsulating agents such as gelatin (GE) and acacia gum (AC) were procured from Merck Specialities Pvt.Ltd., Mumbai, India. Citric acid monohydrate ($C_6H_8O_7.H_2O$, m.w. 210.14 g mol^{-1}; Cat. No. GRM229-500G) was obtained from HiMedia Laboratory, India. PGPR (polyglycerol polyricinoleate) and tween 80, procured from Lasenor India Pvt. Ltd, Nagpur, India, and HiMedia Laboratory, India, respectively; were used as emulsifiers. Soyabean oil (Dhara brand) was purchased from the nearby market of Tezpur University, Assam, India. All the chemicals and reagents required for experiments were of analytical grade. Double distilled water was used which was made using a distillation unit.

5.2.2. Extraction and concentration of black rice bran anthocyanin

The extraction of anthocyanin from black rice bran was carried out previously using Ultrasonic Homogenizer (U500, Takashi, Japan). The optimized condition was found to be 33.78 min and 234.01 W. The optimized parameters were selected based on maximizing the total anthocyanin content. For obtaining the anthocyanin content, extraction was carried out with slight modification as described by Shaddel et al., [35]. Black rice bran (20 g) was mixed with 250 mL of 70 % acidified ethanol (1 M HCl). The solution was sonicated under the optimized parameter. It was then kept in an orbital shaker at room temperature for 10 min. Centrifugation was done at 3500 rpm for 30 min and the extract was filtered using Whatman no. 1 filter paper. The retentate was again reextracted/washed with 50 mL of acidified ethanol and the obtained extracts were mixed. The solvent was removed and concentrated using a rotary evaporator (8703, Roteva) at 40 ˚C. N_2 gas was blown to the evaporated extracts to achieve concentrated extracts. The total extract volume was dried and concentrated

using a magnetic stirrer maintaining the temperature at 40 ˚C till the extract volume becomes 20-30 mL. The obtained extract was stored under refrigeration (4 ˚C) for further use.

5.2.3. Dual emulsion (W/O/W)

The primary emulsion (W/O) was produced according to the method described by Shaddel et al., [35] with some modifications and initial trials. 20 mL of concentrated black rice bran anthocyanin extract was mixed with 80 mL of soybean oil in presence of lipophilic emulsifier [PGPR 3 % (v/v)]. The primary emulsion was homogenized at 15,500 rpm for 4 min using Ultraturrax homogenizer (T25, IKA India Pvt. Ltd., Bengaluru, India).

To create a dual emulsion (W/O/W) process, gelation solutions (50 mL) containing a hydrophilic emulsifier (1 % Tween 80) was put on to the primary emulsion. The dual emulsions were produced at 13,500 rpm for 3 min using an Ultraturrax homogenizer. Both primary and dual emulsions stability were determined by visual observation and an optical microscope. Fig. 5.2 showed the double emulsion complex coacervation.

Fig. 5.2. Double emulsion complex coacervation

5.2.4. Complex coacervation process

In order to obtain the coacervates, acacia gum solutions (50 mL) were slowly added to the double emulsion at 40 ˚C with the aid of a magnetic stirrer. The pH of the solutions was found to be approximately 5.5. It was then adjusted to 3.5 by gradual addition of citric acid solution (1 M) with continuous stirring. The temperature was maintained at 40 ±2 ˚C during the coacervation process [9]. The final emulsion was

then cooled down slowly under refrigerated conditions (7 ˚C) and kept overnight to allow complete decantation. The supernatants were then discarded, and the precipitates (shown in Fig. 5.3) were freeze-dried in order to attain dried samples.

For developing of anthocyanin microcapsules, different combination of core solution (primary emulsion) and wall materials solution (gelatin and acacia gum) were used (Table 5.1). The coacervates thus obtained were ground to a fine powder and stored in a zip-lock bag in the desiccator for further analysis.

(a) (b) (c)

Fig. 5.3. Coacervates: (a) and (b) Coacervates after phase separation, (c) Freeze-dried coacervates

5.2.5. Characterization of the extracts using HPLC analysis

The anthocyanin compounds (cyanidin-3-glucoside and peonidin-3-glucoside) determination was conducted using the HPLC method described by Das et al., [11]. HPLC analysis of these compounds was performed using RP-HPLC (Waters Corporation, USA) with UV/ Vis Detector. The solvent used as mobile phase was a mixture of milli-Q-water, methanol and folic acid (75:20:5 v/v) with isocratic elution flow rate of 0.5 mL/min. Dual wavelength UV/Vis detector (SPD-10A, Shimadzu) analysis was used by connecting the HPLC pumps (Liquid Chromatography-10AT, Shimadzu) and column for the analysis. The HPLC analysis was performed at 22 ºC with column C18 with 5.0 µm (4.6 dia × 250) mm^2 at 530 nm. The standards used for the quantification were Cynanidin-3-glucoside (C3G) and Peonidin-3- glucoside (P3G).

The alpha tocopherol, alpha tocotrienol and β-Carotene for both extracts were also evaluated according to Samyor et al., [34]. The UV/Vis detector at two wavelengths (292 and 325 nm) was used. The solvent used as mobile phase was a mixture of

methanol and acetonitrile (15:85 v/v) maintaining the flow rate of 1 mL/min with isocratic mode. Identification and quantification of tocopherol, tocotrienol and β-Carotene were carried out and analyzed on the basis of peaks and compared with the standard curves.

5.2.6. Characterization

5.2.6.1. Moisture content

Moisture content of the coacervated microcapsules was determined according to the standard method of the AOAC 2010 [2].

5.2.6.2. Hygroscopicity

The hygroscopicity of the samples was measured according to the method described by Comunian et al., [9]. Approximately 1 g of each freeze-dried powder was measured in glass Petri plates and placed in a desiccator containing a saturated solution of sodium sulphate, Na_2SO_4 (RH 81 %) for a week. Then the hygroscopicity was calculated as gram of moisture absorbed per hundred grams of sample (%).

5.2.6.3. Solubility

The solubility of the microcapsules was determined according to the method described by Rocha-Selmi et al., [31]. 2 g of each sample were added in a conical flask. 100 mL distilled water were poured into it and stirred at 210 rpm for 30 min using a magnetic stirrer (MS-H280-Pro, Abdos). The solution was centrifuged at 3500 rpm for 5 min. The supernatant was then discarded and the precipitates were taken and kept in the hot air oven at 105 ˚C until they reached a constant weight. The final dry materials were brought back in a desiccator until they cooled down and the weights were measured. The percentage of total soluble matter (%) was determined from the difference in weight.

5.2.6.4. Encapsulation efficiency

Encapsulation efficiency was determined from the total ANCs (TANCs) and surface ANCs content (SANCs) of the microcapsules according to the method described by Shaddel et al., [36]. 0.2 g of freeze-dried microcapsules samples were measured and added to 1 mL distilled water in a centrifuge tube followed by the addition of 4 mL of ethanol. The microcapsule membrane was then disrupted by sonication (U500, Takashi, Japan) at 200 W for 5 min.

Again, for determining the surface ANCs content, the same process was carried out and 5 mL of ethanol was added directly followed by vortex for 30 s and centrifugation was done at 3000 rpm for 3 min. The filtered supernatants were used for the analysis [9].

The encapsulation efficiency (EE) was calculated using the Eq. (5.1).

$$\text{Encapsulation efficiency (\%)} = \left[\frac{(TANCs - SANCs)}{TANCs}\right] \times 100 \quad (5.1)$$

5.2.6.5. Surface morphology

The microstructure of the freeze-dried microcapsules powder was studied using scanning electron microscopy (SEM) equipment (JEOL JSM 6390 LV, Singapore). Dried microcapsules powder was sputtered and coated with platinum and the images was taken at an accelerating voltage of 5 kV and magnification of 1000× and 2000×. SEM images were obtained from the surface and a cross-section of the microcapsule powder.

5.2.6.6. FT-IR

The microcapsules loaded with black rice bran ANCs using gelatin and acacia as carrier agents were subjected to FT-IR spectroscopic analysis. The scanning range of the equipment was at 4000 to 450 cm^{-1} (Spectrum 100, PerkinElmer, USA).

5.2.6.7. Thermal stability

The onset temperature, melting point (Tm) and peak temperature of the nine different microcapsules were measured using a differential scanning calorimeter (Model DSC-60; Shimadzu, Tokyo, Japan) under an ultrahigh-purity nitrogen atmosphere. About 10-20 mg of each sample were measured and placed in hermetically sealed aluminum pans at a heating (or cooling) rate of 10 ˚C/min in the temperature range between -10 to 200 ˚C with liquid nitrogen cooling and N_2 inert gas. The reference was used using an empty pan.

5.2.6.8. XRD

The X-ray diffraction analysis for all the coacervated microcapsule was carried out using an X-ray diffractometer (D8 Focus, Bruker AXS, Germany). The range of diffraction angle was 10.00 to 60.00 ° 2θ. The total curve area and each peak were

analysed and the percentage of crystallinity was calculated using the formula given in Eq. 5.2.

$$\text{Crystallinity (\%)} = \frac{Area\ under\ peaks}{Total\ diffraction\ area\ above\ baseline} \times 100 \qquad (5.2)$$

5.2.6.9. Stability study of anthocyanin

The prepared microcapsules were sealed under vacuum and stored separately in an incubator and under refrigeration with a constant temperature of 37 ˚C and 7 ˚C respectively. The stability of the microcapsules was then determined by calculating the total ANC content of the freeze-dried microcapsules every 7 days for 7 weeks. Each analysis was carried out from the same sealed container, so that the remaining samples do not come in contact with light and oxygen.

5.2.7. Statistical analysis

All the analyses were carried out in triplicates independently and depicted as mean ± SD. The data were subjected to one way analysis of variance (ANOVA) and significant differences were evaluated between means by Duncan's multiple range tests at a significance level of $p \leq 0.05$ using statistical package using SPSS Statistics 20.

5.3. Results and discussion

5.3.1. Preliminary trials

Preliminary trials were conducted using different percentages of concentrated ANCs solution (20, 30 and 40 mL) with soybean oil for preparation of primary emulsion. Out of these primary emulsions prepared, 30 and 40-mL ANCs core solution (primary emulsion) showed unstable and provided low encapsulation efficiency (65.76 and 59.83 % respectively). However, the efficiency was more (76.47 %) and also provided stable emulsion in case of 20 mL ANCs core solution.

Despite the fact of differing core solution percentage, trials have also been carried out using different percentage of gelatin and acacia gum (1:1, 1:2, 1:3, 2:1, 2:2 and 2:3) using 20 mL ANCs core solution. Based on the trials, the encapsulation efficiency was observed between 69.54 to 81.24 % for the various ratios of gelatin and acacia gum, where the highest was found to be in ratio 1:1. However, some literatures suggested the ratio of the polymers to be used should be calculated depending on the charge of the polymers they carry, while others have shown that the ratio had little

influence on capsule formation [16]. Moreover, the process yields of the coacervates appeared to be influenced more by the pH for each ratio than the ratio itself [16]. The best yield was also reported to be observed at a pH below the gelatin isoelectric point (3.8 or 3.5 for type A and type B gelatin, respectively), using a gelatin–gum acacia system [4]. Therefore, based on the initial trials and literatures to be considered, the ratio of wall material solutions (gelatin and acacia gum) and the core solution to be employed was fixed at 1:1:0.5, 1:1:0.75 and 1:1:1 respectively. Nine formations of microcapsules were generated with different combinations as shown in Table 5.1.

Table 5.1. Physicochemical properties of coacervated microcapsules

Treatments	Core solution (mL)	Gelatin (% w/v)	Gum Acacia (% w/v)	Moisture content (%)	Hygroscopicity (%)	Encapsulation efficiency (%)	Solubility (%)
C_1	50	2.5	2.5	3.71 ± 0.20^{a}	38.41 ± 1.20^{e}	76.57 ± 1.09^{c}	11.95 ± 1.03^{a}
C_2	75	2.5	2.5	3.62 ± 0.31^{a}	32.26 ± 0.78^{c}	83.65 ± 1.33^{f}	12.67 ± 1.42^{bc}
C_3	100	2.5	2.5	4.49 ± 0.18^{bcd}	27.62 ± 1.15^{a}	78.78 ± 1.74^{cd}	14.33 ± 1.80^{bc}
C_4	50	5	5	3.92 ± 0.35^{ab}	37.51 ± 0.92^{e}	75.83 ± 1.72^{bc}	14.18 ± 1.83^{bc}
C_5	75	5	5	4.35 ± 0.21^{bc}	31.46 ± 0.90^{bc}	80.59 ± 1.11^{de}	14.54 ± 0.61^{bc}
C_6	100	5	5	4.56 ± 0.33^{cd}	30.04 ± 1.47^{b}	72.70 ± 1.30^{c}	15.92 ± 1.32^{c}
C_7	50	7.5	7.5	5.08 ± 0.72^{d}	34.67 ± 1.24^{d}	77.90 ± 1.90^{cd}	14.37 ± 1.40^{bc}
C_8	75	7.5	7.5	4.10 ± 0.57^{ab}	31.46 ± 0.94^{bc}	82.51 ± 2.88^{ef}	14.70 ± 1.12^{bc}
C_9	100	7.5	7.5	5.07 ± 0.13^{d}	30.76 ± 1.30^{bc}	73.32 ± 1.18^{ab}	15.45 ± 1.52^{c}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$)

5.3.2. Effect of hydrocolloids on microcapsules

Coacervation is an interaction between two oppositely charged polymers that forms a complex structure. Gelatin and acacia interactions during coacervation were analyzed under various pH conditions (3.0-4.5). Additionally, at different pHs, different percentages of polymers interaction cause turbidity in the emulsion complex [32]. The interaction between gelatin and acacia gum showed better results at pH 3.5 as the precipitates were of large amount and clear supernatant (approx. 50-60 g) was observed. For the polymer concentration (2.5 and 5 % w/v) with different amount of core solutions, greater phase separation was also observed with pH adjusted to 3.5. However, for the higher polymer concentration (7.5 % w/v), provided the little formation of microcapsules (20 g) even at lower pH (3), determined by negligible sediment and very high turbid supernatant. Similar results were also observed in Nori et al., [22].

The formation of coacervates is of two phases viz: 'rich in polymers' containing the precipitate coacervates and another called 'poor in coacervates' containing the solvents of the solution [22]. In order to promote the techniques efficiently i.e., to promote the greatest possible interaction between the colloids, three different pH values were employed. The coacervates thus obtained after the phase separation in each pH were extracted after the mass settlement, aspect of the supernatant. With the help of optical microscope, the presence and shape of the microcapsules were verified. The dried coacervates were frozen (4 º C), freeze dried and powdered for further analyzed.

5.3.3. Anthocyanin and vitamin

A comparison between UAE extract (Chapter 4) and concentrated extract was conducted in term of anthocyanin and vitamins. From Table 5.2 it was observed that after the removal of solvent (acidified ethanol), the anthocyanin compounds [cyanidin (C3G) and peonidin-3-D-glucoside (P3G)] and vitamins (α-Tocopherol, D-α-Tocotrienol and β-Carotene) increases by five folds as compared to the crude UAE extract. Similar observation was also reported by Das et al., [11]. Cyanidin-3-glucoside (101.73 µg/L) is the predominated anthocyanin as compared to peonidin-3-D-glucoside (2.12 µg/L). Several peaks were observed in the HPLC analysis at different wavelength (292 and 325 nm) for the vitamin analysis. However, peaks at 3.2, 3.5 and 2.9 were the major peaks identified as α-tocopherol, D-α-Tocotrienol and β-Carotene, respectively. The amount of α-tocopherol is

higher (762 µg/L) than β-Carotene and D-α-Tocotrienol. Similar results were also observed and could be compared according to Das et al., [11].

Table 5.2. Quantification of vitamins and anthocyanin compounds

Sl. No.	Compounds	Retention time (min)	UAE extract (µg/L)	Concentrated extract (µg/L)
1.	Cyanidin-3-glucoside	16.18	16.93	101.74
2.	Peonidin-3-D-glucoside	17.51	0.67	2.12
3.	α-Tocopherol	3.20	112.93	762.93
4.	D- α-Tocotrienol	3.50	135.30	208.3
5.	β-Carotene	2.90	99.42	179.71

5.3.4. Physicochemical characterization of the coacervated microcapsules

5.3.4.1. Moisture content

The moisture contents of the freeze dried coacervates were found to be considerably low ranging between 3.62±0.31 to 5.24±0.54 %. The moisture content obtained seemed to increase as increased in the core solution and wall materials and significantly differ in all the treatment ($p<0.05$). This can be related to reason due to increase in water content of the core solution. And, in order to prevent the agglomeration, low moisture contents are favorable to assure the stability of coacervates by preventing from caking. Retention of the active agent has been reduced by agglomeration and makes coacervates dispersion difficult when applied in food [39]. Low moisture contents also reduce the glass transition temperature thereby reducing the action of plasticizer [13, 31]. Moreover, due to the high hygroscopicity nature of the coacervates, it is more likely to affect the core ANCs.

5.3.4.2. Hygroscopicity

The hygroscopicity of the coacervates was found to be in the range 27.62±1.14 to 39.51±0.92 g water absorbed/100 g sample. As observed from Table 5.1, it can be seen that with increasing core solution, the hygroscopicity decreases, which can be due to an increase in the water content of the core solution. The values obtained were found to be similar to those obtained by Silva et al., [39] ranging from 33.4 to 36.8 g/100 g for lycopene microcapsules obtained using gelatin and pectin as the encapsulating agents. However, the hygroscopicity is relatively high as compared to the values obtained by Rocha-Selmi et

al., [31] ranging from 10.73 to 13.43 g/100 g for aspartame microcapsules obtained using gelatin and gum arabic as the encapsulating agents. The reason for high hygroscopicity could be due to the high hydrophilicity nature of the core ANCs indicating the necessity of good packaging and handling of microcapsules [36]. The samples have the capacity of moisture absorption from the environment which also suggested the material to be packed in water vapour impermeable containers, especially in a high humidity environment.

5.3.4.3. Solubility

The results showed low values of solubility ranging from 11.94±1.02 to 15.92±0.61 %, which are one of the characteristics of microcapsules obtained by the complex coacervation process. An encapsulation is successful if the retention of the core materials is achieved very high and minimum amounts of the core materials on the surface of powder particles [20]. The highest solubility (C_6) among nine coacervates was prepared using 100 mL core solution ANCs, 5 % GE and 5 % AC. It is also desirable to have lower solubility in order to provide a gradual, slow, and controlled release of the targeted core active materials for possibly prolonging the effect of ANCs which also act as pharmaceutical ingredients, absorbed at the intestine. Similar solubility values were also obtained by Rocha-Selmi et al., [31] ranging from 9.46 to 21.37 % for aspartame microencapsulation. Literature has also reported that a higher degree of the amorphous structure provides higher solubility of the powder in the water while lower solubility for crystalline state [36]. Again, higher solubility might be due to the hydrophilic nature of acacia being used as an encapsulating agent in the sample. Furthermore, as a hydrophilic agent, acacia has good controlled release in drug delivery systems [27]. Liu et al., [18] also reported that hydrophilic molecules are being absorbed on the surface by curcumin-based nanoparticles for enhancing the solubility of encapsulation.

5.3.4.4. Encapsulation efficiency

The essential criteria for a successful encapsulation process are the loading capacity and encapsulation efficiency. According to Mahdavi et al., [20], the efficiency is also known to be affected by a number of factors such as the properties of wall and core materials along with characteristics of emulsion and drying parameters (especially spray drying conditions such as inlet and outlet temperatures, feed flow rate, air flow and humidity, size of powder particles, etc.). Table 5.1 showed the encapsulation efficiency of the nine developed coacervated microcapsules which ranged from 72.70±1.31 to 83.65±1.13 %.

The obtained result was found higher than those obtained by Rocha-Selmi et al., [31] where the core solution was aspartame emulsified in soybean oil and the encapsulating materials were gelatin and gum acacia, and Silva et al., [39] when encapsulating oily dispersion of lycopene using complex coacervation technique with gelatin and pectin as the wall materials. The higher value of encapsulation efficiency indicates a significant quantity of active complex availability was found inside the microcapsules. It was also reported that lower encapsulation efficiency was observed in by Comunian et al., [9] where the encapsulating agents were gelatin and gum arabic and core solution ascorbic acid emulsified in corn oil. However, ANCs content on the surface of the coacervated microcapsules was found very less or negligible. The encapsulation efficiency of lutein was found to be 85.32±0.63 % where gelatin and gum arabic were used as wall materials [30]. Again, the retention capability of the encapsulated active core material depends on the utilization of different encapsulating agents [9]. The results thus obtained in the study for ANCs microencapsulation by complex coacervation process were similar to the encapsulation efficiency observed by Qv et al., [30]. The similar results obtained may be due to the same encapsulating agents used for microencapsulation process.

Moreover, it was observed that the encapsulation efficiency values were significantly high for the treatment (C_2, C_5 and C_8). The various ranges of encapsulation efficiency may be due to the different macromolecule species, core and the ratio of the wall materials [36].

5.3.5. Microstructure

It was observed that the micrographs of coacervated microcapsule powders formulated with a lower concentration of both the wall materials (gelatin and gum acacia) showed hard, round and agglomerated structures shown in Fig. 5.4. Again, uniformity of particle shape and distribution on either smooth or intact surfaces was observed as compared to the higher concentration treatment (C_7). The distribution of anthocyanin in the double emulsion followed by complex coacervation yielded microparticles that were irregular with non-characteristic shapes. These irregular shapes could be due to the method incorporated by means of surfactants or organic solvents [33]. The microcapsules were observed to be in the form of a reservoir where the wall material surround the core perfectly. These showed that greater protection of core could be observed in the developed microcapsules. The morphology of the coacervated microcapsules thus obtained were similar to those obtained microcapsules by spray dryer where the encapsulating material

was modified starch and anthocyanin as core a core material [10]. However, more uniform structures were observed by Das et al., [10] as compared to the developed microcapsules.

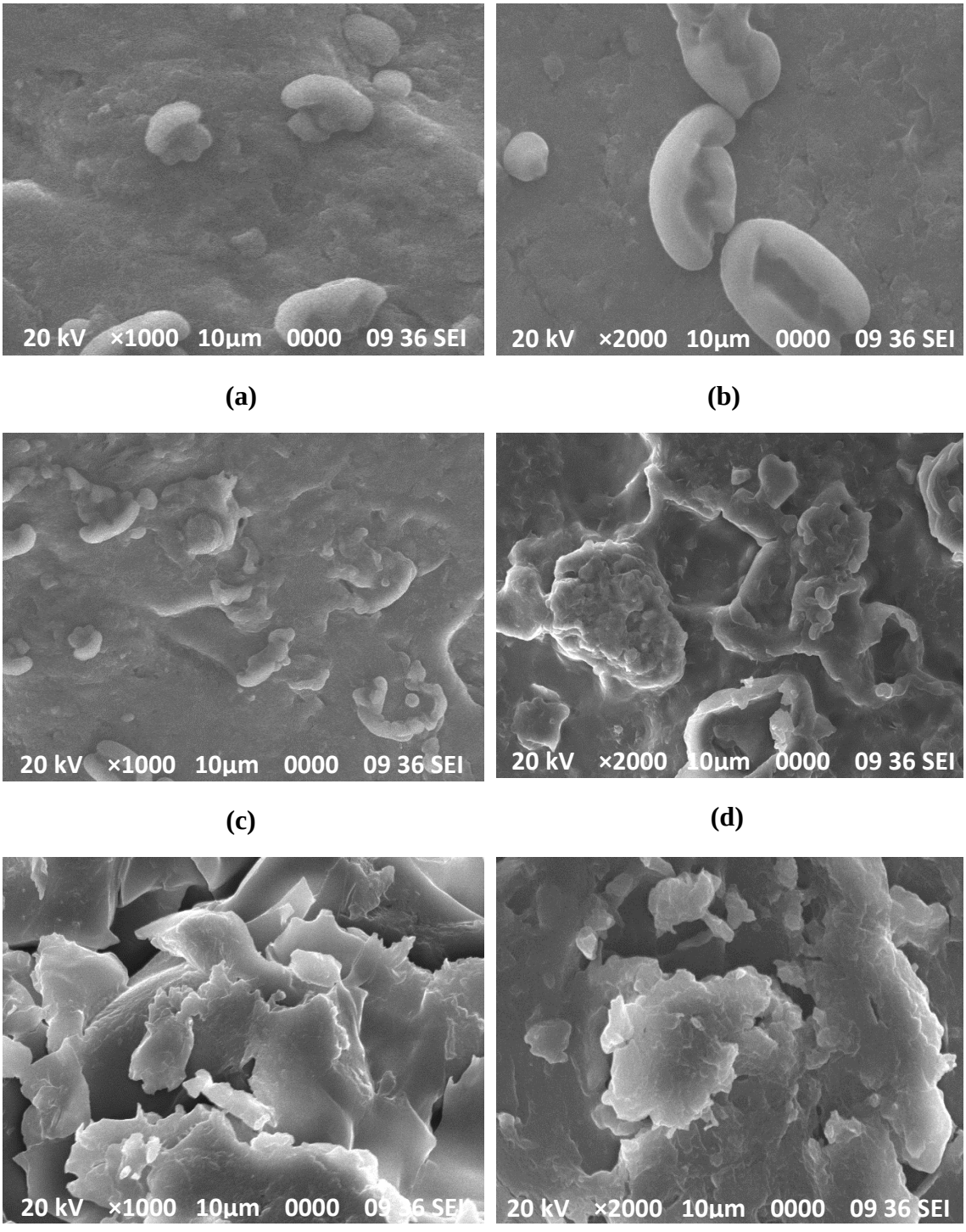


Fig. 5.4. SEM images of freeze-dried coacervated microcapsules (a) C_1 with 1000×; (b) C_1 with 2000×; (c) C_4 with 1000×; (d) C_4 with 2000× ; (e) C_7 with 1000× and (f) C_7 with 2000×.

concentration of wall materials, crystallinity of the coacervates increases which could be due to gelatin being a semi-crystalline biopolymer. Again, with increasing concentration of wall materials (treatment C_7), the surface seemed to crack and break. The reason can be due to the loosening of the coatings or the wall materials leading to the gradual diffusion of core particles. The penetration and release of active core particles from microcapsules into the substrate is one of the fundamentals which can be influenced by the morphologies of the microcapsules. Moreover, anthocyanins are hydrophillic compounds which found suitable with formulation of gum and gelatin as coating materials for polar matrix [20].

5.3.6. Infrared Spectroscopy of the coacervated microcapsule powders

The infrared spectra obtained for the formulations were similar and opted to show for only four spectra of coacervated encapsulates (C_1, C_2, C_4, and C_7) as shown in Fig. 5.5. The FT-IR spectra of the wall materials (gelatin and acacia gum) was also incorporated in the graph shown in Fig. 5.5 so that the spectra of the coacervates make more intuitive comparison. The absorption spectra between 3373-3422 cm^{-1} were the N-H stretching of amines and amides group which could be seen in the wall material (gelatin). In addition, the peaks between 3349-3344 cm^{-1} observed in the coacervates corresponds to gelatin polymer molecule due to the presence of an amine group (N-H stretching). The intensity of the peaks was observed to be the only difference between all formulations of the samples; related to either small or large amount of ingredient used in the combination. The absorbance of functional groups in the characteristic regions can be used as evidence for the presence of these groups in the molecular structure [9].

Acacia gum is a polysaccharide containing free carboxylic groups (O-H) with absorption spectra ranging 2500-3300 cm^{-1} attaching a negative charge molecule. The peaks between 2921-2937 cm^{-1} of the coacervates was attributed to the presence of strong and broad O-H stretching which can be free carboxylic group from the acacia gum shown in Fig. 5.5. Moreover, the characteristics of the peaks of amide can be seen at 1452 cm^{-1} of the spectra of microcapsule which also showed a medium C-H bending appearance. The aromatic compounds have also been shown to present in the sample formulations where the peaks at 1746-1747 cm^{-1} denotes C-H bending.

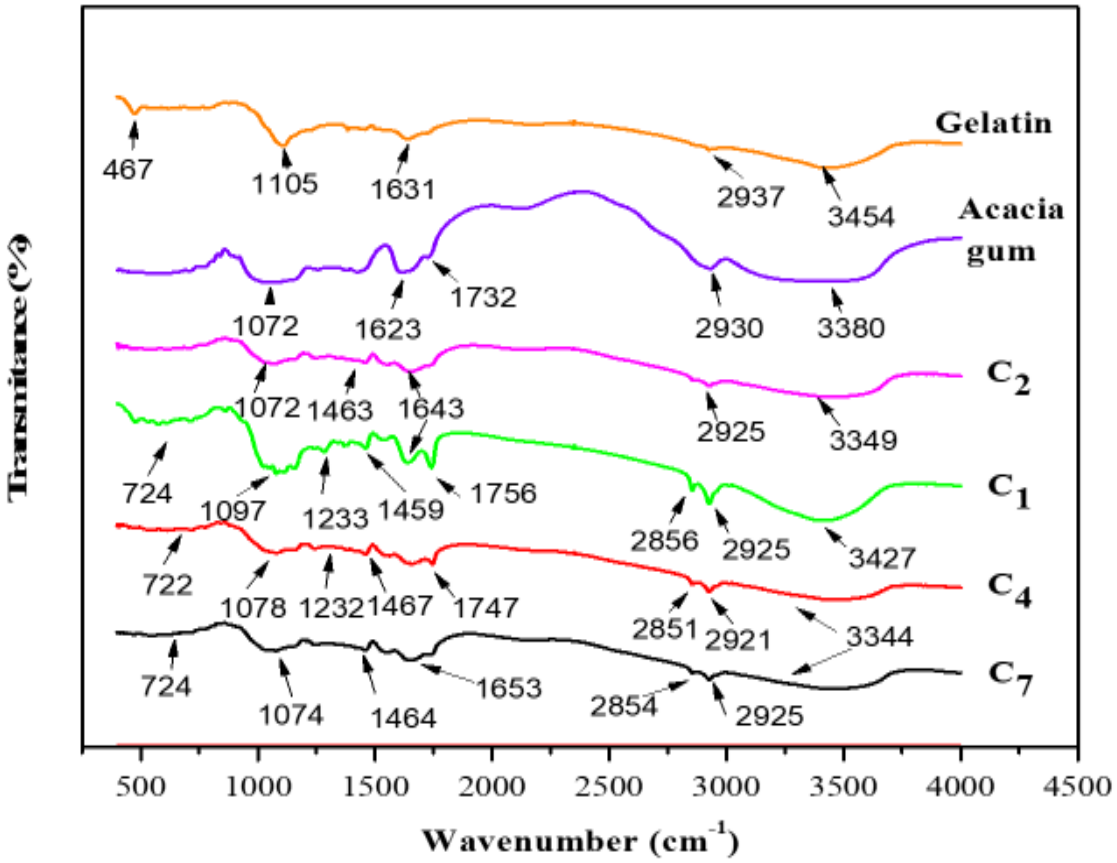


Fig. 5.5. FT-IR of coacervated microcapsule powder

5.3.7. XRD

Fig. 5.6. showed the XRD pattern for different formulations of coacervated microcapsules and the wall materials. All of the microcapsules had an amorphous structure with peaks ranging from 19.37 to 20.87 Θ. The XRD pattern also revealed that with increasing the concentration of the wall material (7.5 % w/v), the microcapsules were found to observed more crystallinity than the microcapsules with lower concentration polymer (2.5 % w/v). Treatment C_1 showed less crystallinity and higher amorphous region as observed from the XRD graph. Similar results were reported by Kanha et al., [15]. As already observed by Shaddel et al., [36], the wall materials gelatin and gum acacia provided a sharp crystalline and lower crystalline XRD peak between 2 Theta of 10 to 60. However, the formulation of the wall materials with the concentrated anthocyanin extract forming double emulsion coacervates changed the intensity of the XRD peaks from crystalline to amorphous structure. However, Shaddel et al., [36] had shown that the microcapsules exhibited a semi-crystalline structure in contrast to the amorphous structure. This indicated that

stability of microcapsules is attained not only at room temperature but also at higher temperature.

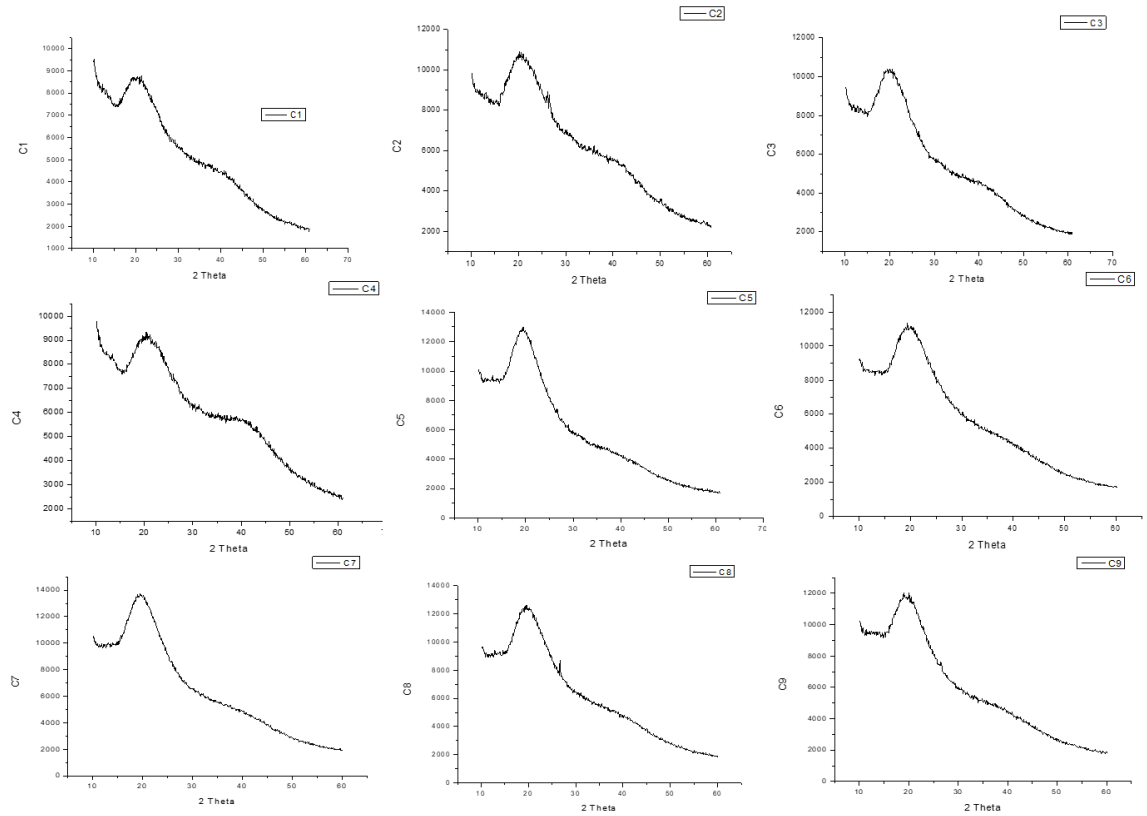


Fig. 5.6. XRD pattern of coacervated microcapsule powder

5.3.8. Thermal stability

Differential scanning calorimetry (DSC) is an analytical technique investigating the physicochemical properties of a sample using temperature as a function [29]. In addition, this analysis can also determine the shelf life and storage condition of the microcapsules during the process of incorporation in food. Again, the method also allows the simultaneous evaluation of physico-chemical alterations occurred within the samples that is affected by heat. Moreover, an exothermic peak rises downwards (decrease in enthalpy), while an endothermic peak goes in the opposite direction [9]. Generally, phase transitions, dehydration and reduction produce endothermic effects while oxidation, crystallization, and certain decomposition reactions produce exothermic effects. The study of glass transition of materials can also be observed in this technique as an endothermic stepwise change in the heat flow [33].

Table 5.3. Thermal properties of the coacervated microcapsules

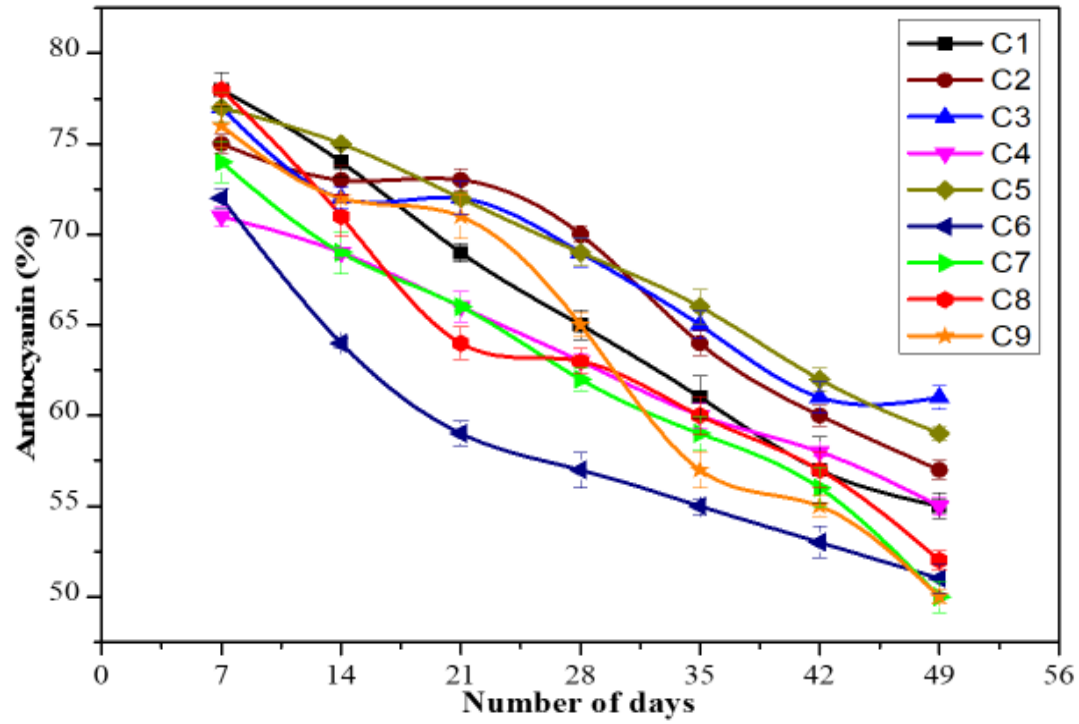
Treatments	Onset temperature (˚C)	Melting point (˚C)	Enthalpy (J/g)
C_1	34.9	85.0	452.7
C_2	49.4	90.4	390.7
C_3	41.7	92.2	410.5
C_4	59.9	93.4	530.5
C_5	47.3	93.5	462.7
C_6	38.1	83.7	469.5
C_7	39.3	91.8	482.3
C_8	50.0	97.6	456.8
C_9	34.3	94.2	424.7

The thermal degradation behaviour of microcapsules displayed endothermic reaction confirming the thermostability of the microcapsules where the peak ranges from 83.7 to 97.6 ˚C also corresponding to their melting point. Table 5.3 showed the differential scanning calorimetry of all microcapsule formulations. The table indicated that with increasing concentration of the polymers (gelatin and acacia gum), heat penetration decreases in the microcapsules thereby increasing heat stability. The result so obtained were found to be higher as observed by Shaddel et al., [35]. Glass transition temperature for the amorphous fraction, melting transition for the crystalline fraction and transition due to crystallization are the three main characteristics of the thermal transition of DSC thermograms. In general, crystalline structure has provided a profitable result that consisted of tightly packed molecules limiting their involvement with environmental factors such as moisture or water [44].

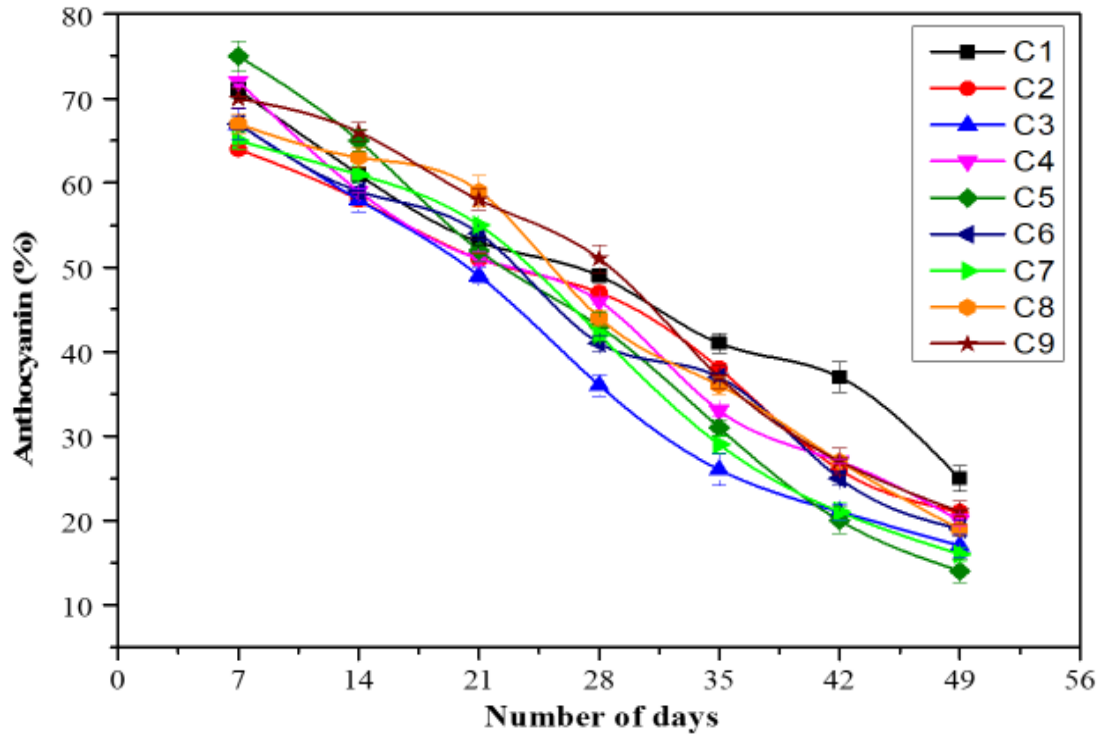
5.3.9. Stability study of anthocyanin

The stability of anthocyanin of all the coacervated microcapsules was determined and was plotted as a time function. The total anthocyanin content for all the microcapsules with different formulations was examined and observed at different storage conditions (Fig. 5.7) both under refrigeration (7 ˚C) and incubation temperature (37 ˚C). Parameters such as temperature under different storage conditions, water activity, O_2 concentration, thickness and shell permeability, core materials quality, minerals content in traces amount and food matrix control the stability of microcapsules obtained by coacervation technology

[44]. The results showed that the total ANCs content of all samples reduces with longer storage condition.



(a)



(b)

Fig. 5.7. Stability of anthocyanins in percentage under different temperature for 7 weeks (a) Anthocyanin degradation at 7 º C (b) Anthocyanin degradation at 37 º C

The Fig. 5.7 indicates that coacervated encapsulates showed a high stability for 7 ˚C storage condition while compared to the room temperature 37 ˚C for all microcapsules. Under refrigerated condition, the retention time gradually reduces at different rates with the increase in storage time (no. of days) for all formulations. These might be due to degradation of ANCs indicating the effect of temperature on the coacervated encapsulates. The molecular structure of anthocyanin actually shifts as there is change in environmental factors. It was observed that after 7 weeks storage under 37 ˚C, the encapsulates were degraded to 15-25 % while storage under 7 ˚C, the total ANCs of the encapsulates were found to be 50-55 %. Similar results were also observed in Shaddel et al., [35]. Lowest ANCs retention was observed in formulation C_5 for the encapsulates stored under 37 ˚C compared to the actual concentration of ANCs. At high temperature, the encapsulates maintained 15-25 % of the actual concentration of ANCs. On the other hand, encapsulates stored under low temperature showed high stability than those stored under high temperature. Again, polymer matrices tend to change their structural phase; from crystallinity to amorphous state, solid to molten state, glass state to rubbery state and sol to gel state. Hence, release profile of the product differs in each of transition states.

5.4. Conclusions

The utilization of the double emulsion technique preceding the complex coacervation process provided microcapsules successfully. The results so obtained for encapsulation efficiency of concentrated anthocyanin from black rice bran with high values indicated that encapsulation process was effective. The high hygroscopicity values suggested that the material should be packed in water vapour impermeable containers at high humidity. It is also desirable to have low solubility in order to provide a gradual, slow, and controlled release of the targeted core active materials for possibly prolonging the effect of the target material which also acts as pharmaceutical ingredients, absorbed at the intestine. The different treatments studied in this research indicated the possibilities to apply in food, for which they provided the highest encapsulation efficiency. In the future, research should be carried out to determine the controlled release from these microcapsules for their use in food applications.

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