

Chapter 4

Reinforcing 2D heterostructure to design efficient and sustainable film for food packaging

Flexible polymeric films are regularly used as packaging materials, but the key challenge is to improve the barrier properties in order to maintain freshness of the food. In this chapter, we will be discussing the development of two dimensional (2D) heterostructure based biodegradable film with active intriguing properties that can hold high potential for intelligent packaging. Excellent barrier properties and exceptional mechanical properties of the film have been achieved due to reinforcement of layered structure within the polymer matrix attributing torturous path in gas/water penetration.

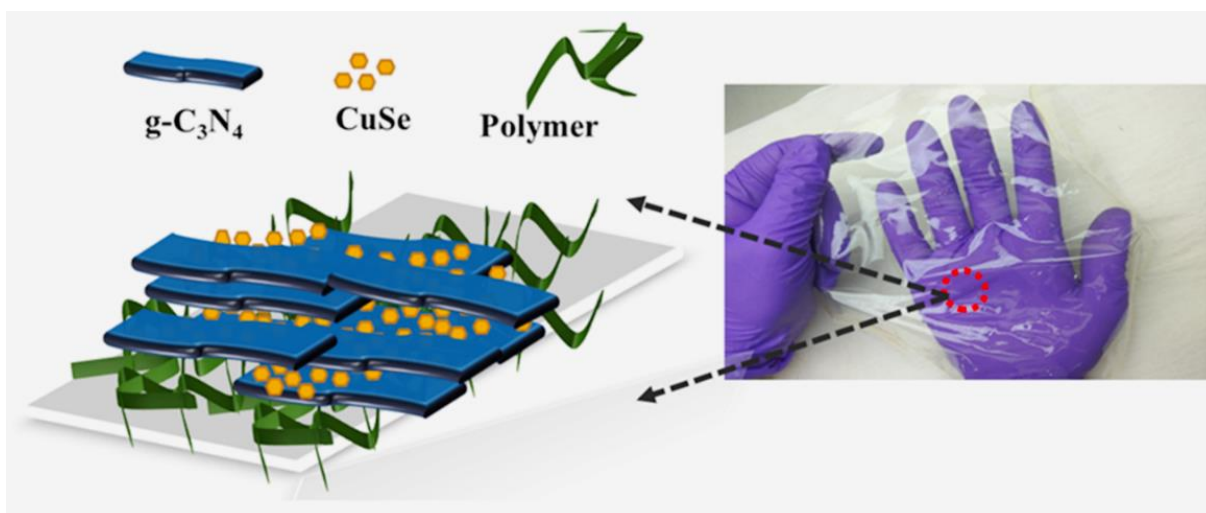


Figure 4.1: Schematic of the developed 2D heterostructure based composite film.

4.1 Introduction

Food packaging is concerned with the preservation and protection of food products without damaging its freshness and organoleptic. According to the estimation of Center for Disease Control and Prevention (CDC, 2016), every year 128,000 people are hospitalized, 48 million people get sick and 3,000 die due to consumption of food that contains pathogens [1]. The primary criteria of this study is to develop an alternative material with increased efficiencies and ability for impenetrable of noxious gases and particles. Here, we summarize the criteria of replacement of plastic using Two dimensional (2D) layered heterostructure materials as (1) advancing the utilization of parent polymers in food packaging (2) creating torturous path for water and oxygen permeation that can elongate the duration in food storage and preservation (3) antimicrobial packaging of food product by impeding the microorganism's growth and extending the product shelf-life (4) commitment towards food safety, quality and traceability (5) lastly easy biodegradability. It is worthwhile to mention that nearly 40% of the waste produced is conventional from packaging waste and market of global plastic production is predictable to grow 5% at compound annual growth rate (CAGR) by the end of 2022. Unfortunately, conventional polymers such as polyethylene and polypropylene cannot meet all the aforementioned requirements, as they persist for many years after disposal [2]. Recently blending two or more polymers has become an increasingly important technique for preparing new materials with multiple functionalities. Natural polymers such as chitosan have received a considerable attention because of their non-toxicity and biodegradable properties [3]. Due to presence of amino groups in repeating units and its biodegradability, it has been applied in diverse technological areas. PVA on the other hand is a water-soluble polymer with excellent chemical resistance, physical properties, excellent biocompatibility and good film forming properties [4]. Synergistic combination of PVA and chitosan produce a unique hybrid system with properties not present in either one individually [5]. In order to make it competitive with petroleum-derived polymers used in food packaging, moderate barrier properties are a concern.

However, one captivating method for improving these critical issues will be addition of Two Dimensional (2D) material into the host matrix. This strategy will help in reducing the permeability of water and oxygen within the system and will maintain the affluence of processing of parent polymer [6]. Thus, the primary function of 2D layered fillers as packaging material will be to achieve the benefits of improved food quality and safety with prolong shelf life. Graphitic carbon nitride (g-C₃N₄) nanosheets have been receiving intense attention owing to their many fascinating advantages such as non-toxicity,[7] easy accessibility,[8] excellent thermal stability, [9] and outstanding mechanical property [10]. These superior properties reveal the potential of g-C₃N₄ based on its ability to produce electron-hole pairs upon visible illumination and thus generate highly reactive oxygen species (ROS) [11-12]. But due to limited ROS generation ability, it has been only rarely used as an antibacterial agent.

In this present work, 2D nanoflakes with antibacterial property was chemically tailored with graphitic C₃N₄ to maximize their interaction allowing enhancement in antimicrobial property. Looking into the environmental hardship regarding non-biodegradable plastics, we have developed here a biodegradable packaging film by incorporating 2D g-C₃N₄@CuSe heterostructure into PVA/Chitosan matrix and investigated the shelf life of the stored food (as shown in **Figure 4.1**). This excellent nanofillers as barrier can prevent the food stuff from direct contact with air and moisture and can prolong its shelf life. In addition to this, mechanical, thermal, biocompatibility and antimicrobial properties were studied together with the detailed physicochemical characterization of the film. More importantly, biodegradability of the film has been studied for a sustainable packaging ecosystem.

4.2 Methods and Methodology

4.2.1 Chemicals and materials used

Precursors like, Selenium dioxide, Oleylamine, Copper (II) acetate, monohydrate, Urea (CH₄N₂O), Ethanol (C₂H₆O), Poly vinyl alcohol (PVA), Chitosan, chemicals

with 99.97% purity from Merck were used during synthesis. Double distilled water was used for washing purposes during the synthesis process. The experiments were repeated for three times to achieve the uncertainties in the results.

4.3 Experimental synthesis

4.3.1 Synthesis of 2D/2D g-C₃N₄@CuSe heterostructure

The synthesis of nanofilm was carried out in two step procedure. During the initial stage, 2D g-C₃N₄ was prepared by pyrolysis method and 2D CuSe nanoflakes were prepared by microwave method. The composite of g-C₃N₄@CuSe was prepared by considering an optimized ratio of 1:1 g-C₃N₄ and CuSe in 20 ml DMF solvent. After 1hr of rigorous mechanical stirring, the solution was transferred into a Teflon-lined stainless-steel autoclave, which was sealed at 200°C for 24 h. Then, the solution was centrifuged to obtain the precipitate. Later on, the precipitate was washed with ethanol and dried at 60°C. After drying, the sample was embedded within polymer matrix for film preparation.

4.3.2 Synthesis of composite film embedding 2D/2D heterostructure within polymer matrix

For the preparation of 2D/2D heterostructure based composite film, 5 wt. % PVA in distilled water solution was prepared by heating the solution at 80°C for 2 hr. Again, 5 wt. % of chitosan with 1% acetic acid solution was prepared separately by dissolving chitosan in distilled water at ambient temperature within 10hrs stirring. Then PVA and Chitosan (v/v %) solution were mixed together and sonicated for 15 min to form homogeneous solution. 1% glycerol is added into the solution as a plasticizer. 2D/2D g-C₃N₄@CuSe (at three different concentrations 0.25 wt. %, 0.50 wt. % and 0.70 wt. %) was suspended in 20 mL of water along with 0.9% saline solution and the mixture was subjected to sonication at room temperature for 5 hours.

For the preparation of film, the mixture of well dispersed g-C₃N₄@CuSe with PVA and chitosan solution was subjected to stirring for 1hr to get transparent homogeneous solution. Finally, the homogeneous solution was casted on glass

plate and heated for 8hrs at 70°C temperature. After drying, the film was peeled off and used in experimental food items packaging.

4.4 Results and discussion

4.4.1 Structural and Surface Morphology

Morphology of the film is an important characteristic for its application. **Figure 4.2 (a and b)** identifies the cross-sectional area and surface morphology of 2D heterostructure based composite film. FESEM images of the film shows homogeneous and smooth surface structure without pores and cracks. Cross section area of the film identifies the uniform and homogeneous distribution of layered material inside the host matrix with quality of interlayer adhesion within the sheets. Uniformity in the surface is due to modification of the 2D heterostructure by functional groups of PVA which are electrostatically compatible allowing wide penetration of polymer between the layers of g-C₃N₄.

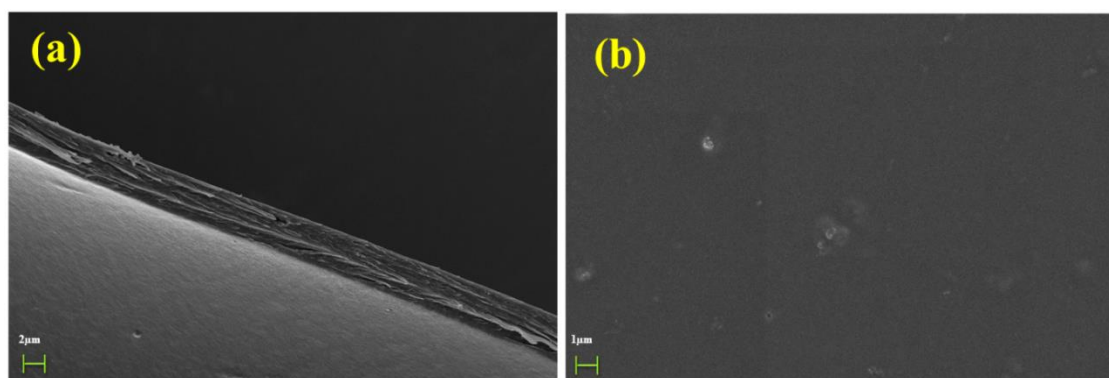


Figure 4.2: Represents surface morphology of the developed heterostructure based packaging film. **(a)** FESEM images of the cross-sectional and **(b)** top view of the film. The uniformity of heterostructure into the polymer can be easily identified from the cross-sectional image.

Furthermore, thickness and roughness of the films were measured using AFM topographic images. AFM image (**Figure 4.3 a-c**) indicates that heterostructure material was not agglomerated within the PVA matrix. This suggests the superior compatibility between 2D heterostructure nanofillers and host polymer, and

uniform dispersion of 2D nanofillers within the polymer matrix. Further investigations reveal that the prepared film was visually homogeneous with no cracks, scratches, bubbles, or visible phase separation justifying with FESEM images. **Figure 4.3 (a and c)** show an average height of 11.7 nm thick layer of the film with an RMS roughness of 1.5 nm.

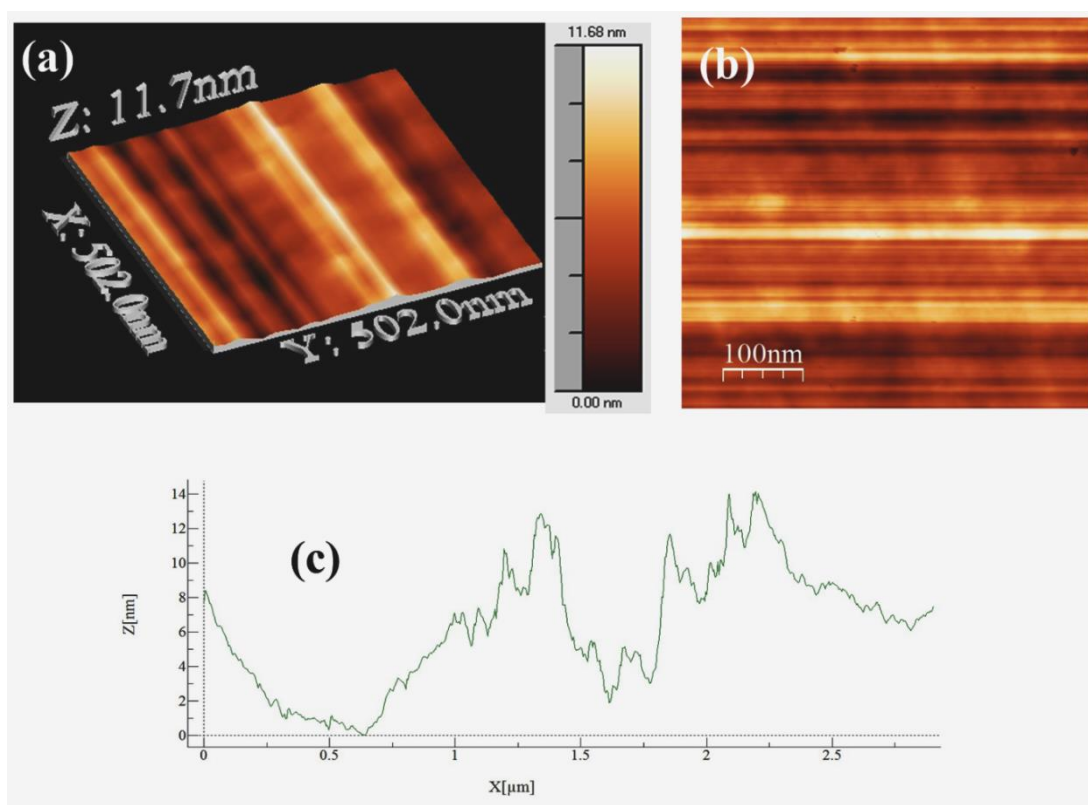


Figure 4.3: AFM images of (a) Surface topology (b) phase profile (c) height profile of the developed film.

4.4.2 Thermal properties

Further to identify the thermal stability of the film, thermo-gravimetric Analysis (TGA) as shown in **Figure 4.4** of the samples were executed. **Figure 4.4 (b)** shows the TGA plot for pristine g-C₃N₄. At the initial stage, loss of weight in the sample in the range from 60°C to 160°C might be due to evaporation of water or some other volatile impurities adsorbed in the sample. In the temperature range from around 160°C to 450°C there was no loss of weight of the sample. This means that the sample is thermally stable up to about 450°C. A further increase in temperature up to 600°C results in total degradation of the sample. In case of heterostructure

(shown in **Figure 4.4.(a)**), there was an initial loss of weight in the sample in range from 60°C to 160°C which was due to evaporation of water or some other volatile impurities adsorbed in the sample. In the temperature range from 160°C to 350°C there was no loss of weight from the sample which shows the thermal stability of the sample. After 350°C, there was a gradual loss of mass in the sample which was due to the degradation of g-C₃N₄, releasing Nitrogen gas from the sample. Around 580°C to 660°C, the degradation of CuSe takes place, resulting the formation of Cu₂Se.

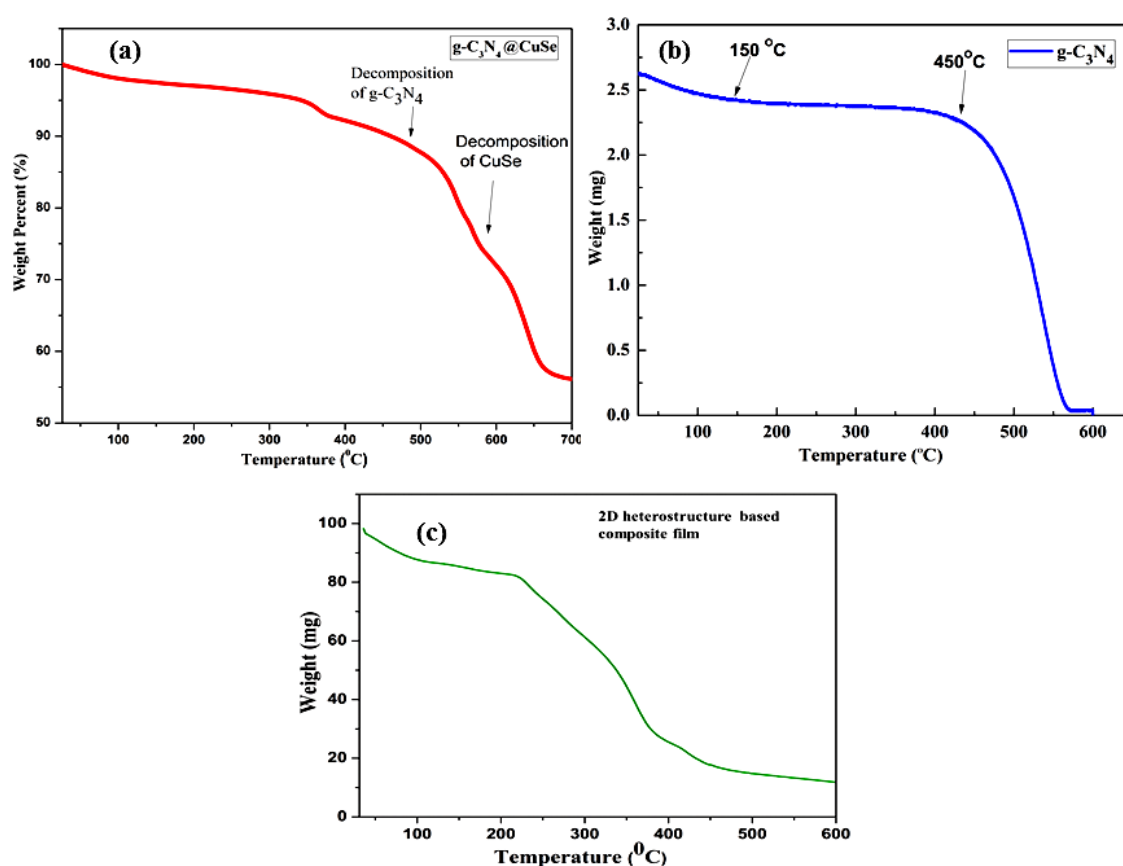


Figure 4.4: Thermogravimetric analysis (TGA) curves (a) 2D/2D heterostructure, (b) g-C₃N₄, and (c) 2D/2D biodegradable film.

In case of the developed film (as shown in **Figure 4.4 (c)**), TGA identifies slight loss of weight in the sample in the range from 70°C to 100°C due to evaporation of water or some other volatile impurities adsorbed in the sample. There was no weight-loss of the sample in the temperature range 100°C to 215°C showing thermal stability of the sample up to about 215°C. An increase in temperature up to

390°C results in significant weight loss due to degradation of PVA and chitosan polymers [13]. At 450°C, thermal degradation of g-C₃N₄ starts evolving nitrogen gas and resulting carbon powder in the pan as residue. This demonstrates that enhancement in thermal stability of the polymer was due to presence of 2D heterostructure as nanofillers.

4.4.3 Barrier properties

To evaluate effectiveness of the film, water vapour transmittance rate (WVTR) was carried out by cutting and placing the samples as a cap on top of a beaker containing water, shown in **Figure 4.5 a**. Further the bottle was kept at 35°C for 24 hours in a chamber and the system weight loss was calculated using the formula $WVTR = \frac{\Delta W}{A \cdot t}$ where ΔW is the loss of weight in gm, A is the cross-sectional area and t is time in hours. [14] The WVTR of PVA/chitosan film before incorporation of nanofillers is found to be 580 gm/m² hr. Incorporation of 2D heterostructure nanomaterial within the polymer matrix decreases the WVTR rate up to 368 gm/m² hr shown in **Figure 4.5 b**. It seems that the water vapour transmission rate (WVTR) of PVA/chitosan film is higher in comparison to 2D heterostructure based film which can be attributed to hydrophobicity of the surface of nanomaterials compared to pristine polymer film. Presence of 2D nanofillers hinders the diffusion of water resulting increase in diffusion length, which further delivers higher potential energy barrier and large mass transfer resistance across the film [15]. More compact lamellar structure of the 2D g-C₃N₄ materials i.e., sheet structure with large dimension is resulting more constricted interlayer spacing. At the same time, increased hydrophobicity reduces the wettability of the film. Essentially, these factors combined together increase the potential energy barrier and greatly resists water molecules, permeating across the film leading towards a reduction of 1.57 times in its value [**Appendix A.2**]. Thus, low rate of WVTR unlocks a wide range of applications for 2D incorporated film for packaging, particularly in a significantly humid environment. Regardless, presence of 2D nanofillers rendered the barrier by increasing hydrophobicity and greater resistance towards water vapour penetration. Also, 2D g-C₃N₄@CuSe was assembled in PVA/chitosan

matrix solution where the polymer acts as a binder to secure the heterostructure morphology and property. This architecture is specifically designed to address several issues. As the pristine polymer film has shown good mechanical and physical properties but possesses only moderate barrier property toward water and gas molecules. Addition of small amount of 2D heterostructure material into polymer matrix advances the functionality of the film.

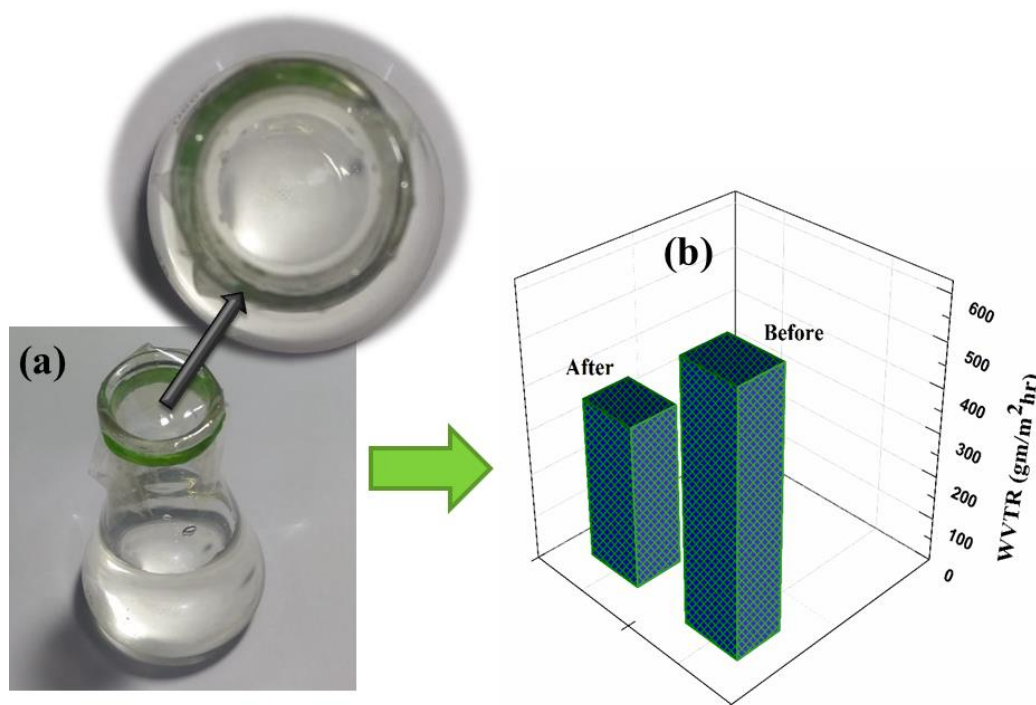


Figure 4.5: Barrier properties (a) real time image of Water vapour transmittance rate (b) 3D representation of WVTR before and after nanomaterial incorporation into host matrix.

4.4.4 Mechanical properties

Flexible nature is one of the desirable features for food packaging film to protect the food material intact. **Figure 4.6 (a-c)**, shows the stress-strain curves of the prepared 2D heterostructure based film and its flexibility. Both the tensile strength and Young's Modulus are significantly improved with little addition of 2D material as nanofillers in the polymer epoxy. Tensile strength shown in **Figure 4.6 (a)**, of bare PVA/Chitosan film is found to be 24.68 MPa, whereas addition of optimized trace amount of 2D material into the film improves the tensile strength up to 59.08 MPa, which is more than twice compared to its pristine form. This

behaviour can be explained taking recourse to homogeneous dispersion of 2D g-C₃N₄@CuSe in polymer matrix and causes superior load transfer from the matrix to reinforcement. The good adhesion between the host polymer and 2D nanofillers likely enables improved toughness. The Young's modulus of PVA/chitosan film without incorporation of 2D nanofillers is 401 MPa, however it is found to be 2316 MPa for 2D heterostructure based film (shown in **Figure 4.6 b**). The attribution of high young's modulus in the 2D heterostructure based film is due to better and homogeneous dispersion of nanofillers within the host polymer, which not only forms effective adhesion but also overwhelms the polymer chain mobility under applied load. This increment in mechanical properties was attributed due to the chemical interaction of 2D material with polymer chains, [16] which reduces the risk of abrupt mechanical failure.

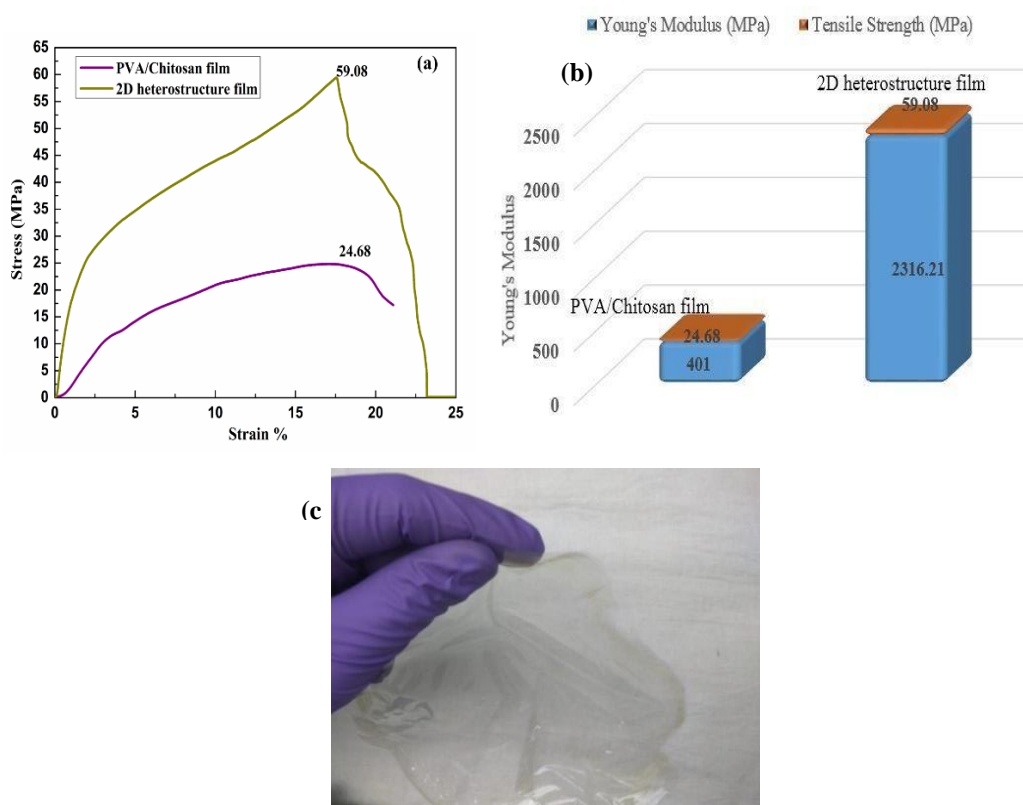


Figure 4.6: (a) Tensile strength and (b) Young's Modulus of PVA/chitosan and 2D heterostructure based film, (c) Flexibility of the film.

4.4.5 Contact Angle

Figure 4.7 identifies the contact angle (CA) of 2D heterostructure based film as 87.6° denoting hydrophilic nature of the film. Due to presence of large number of highly polar hydroxyl groups in the PVA system, the CA of pristine PVA/Chitosan film was 46.8° . Hydrophilic behaviour indicates highly cross-linked PVA structure with the layered material, hindering the diffusion of water molecules within the polymer network causing decreased wettability [17]. Addition of layered material reduces the hydroxyl groups in the surface resulting increase in contact angle.

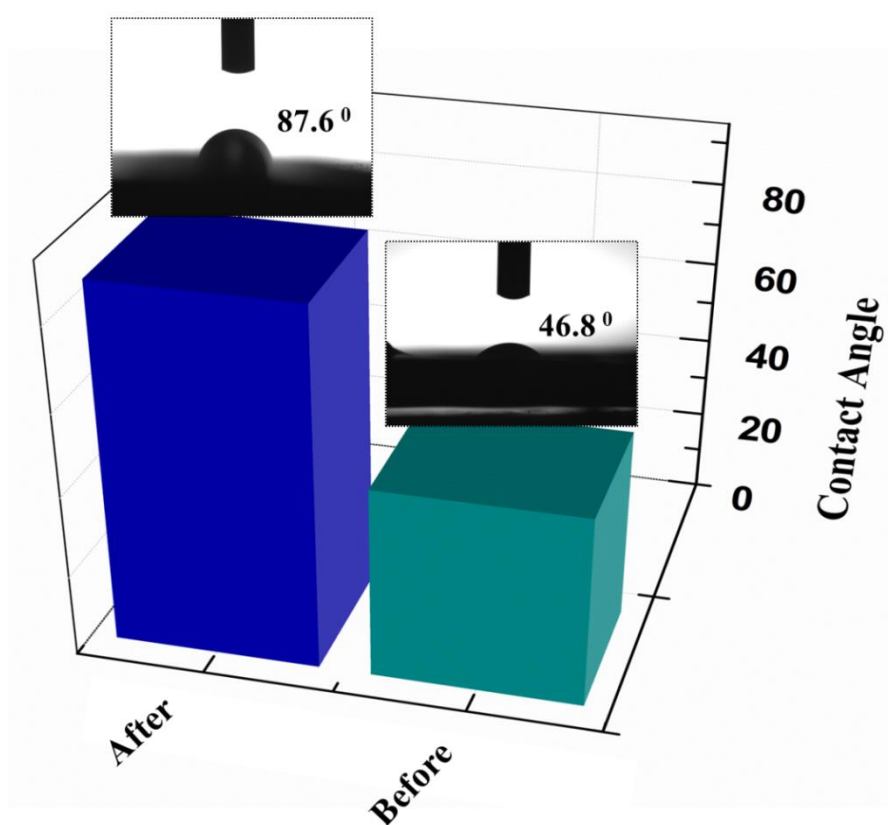


Figure 4.7: 3D representation of contact angle measurement of PVA/chitosan film before and after inculcating with 2D/2D heterostructure.

4.4.6 Antimicrobial properties

Recently addition of antimicrobial properties into the food packaging system has received considerable attention. Antimicrobial systems are particularly effective in case of 2D systems because of its high surface to volume ratio that enhance the surface reactivity of nanosized antimicrobial agents, making them able to restrict the growth of microorganisms more effectively compared to counterparts of other dimension. At first, a piece of 2D heterostructure film (1cm×1cm) was taken and placed on a bacteria culture plate to evaluate the zone of inhibition. The plates are incubated at 37°C for 24 h and the inhibition zone is measured. Antibiotic Gentamicin was used as positive control. Antimicrobial study has been performed taking different Gram positive (*Staphylococcus aureus* and *Bacillus cereus*) **Figure 4.8 (c, d)** and Gram negative bacteria (*Pseudomonas aeruginosa* and *Pseudomonas salmonella*) **Figure 4.8 (a, b)**. The antibacterial activity of the heterostructure film is evaluated by analysing the diameter of these inhibition zones. Presence of zone of inhibition (shown in **Figure 4.8 e**) during the test proves the effective antimicrobial behaviour of the sample. Antimicrobial study is performed using various concentrations of g-C₃N₄@CuSe nanomaterial (0.25wt%, 0.50 wt% and 0.75 wt %) and further zone of inhibitions are measured. The pristine film without incorporation of 2D CuSe flakes does not show significant zone of inhibition. Bacteria are protected by a layer called cell wall composed of polysaccharides and peptides. The thickness of this layer varies due to them. Cu materials have been found to generate reactive oxygen species (ROS). Also, selenium compounds are studied for their antioxidant properties [18]. Moreover, selenium compound coordination with other metal ions has shown additional antioxidant mechanism. Combination of selenium with metal ions complements to ROS scavenging and anticipated as an additional antioxidant mechanism [19]. Addition of CuSe into the pristine film shows increment in zone of inhibition. This phenomenon is mainly due to the diffusion of metal ions linked to the intercalated/exfoliated sheets of 2D graphitic carbon nitride by ionic bonds, towards the culture medium. Once released, they interact with negatively charged membrane surfaces of bacteria, which hinder the metabolism causing a destabilization of the bacterial cell wall. As

a result, significant decrease in nutrient exchange occurs between the bacterial cells and the external environment which leads towards the radical death of the bacteria.

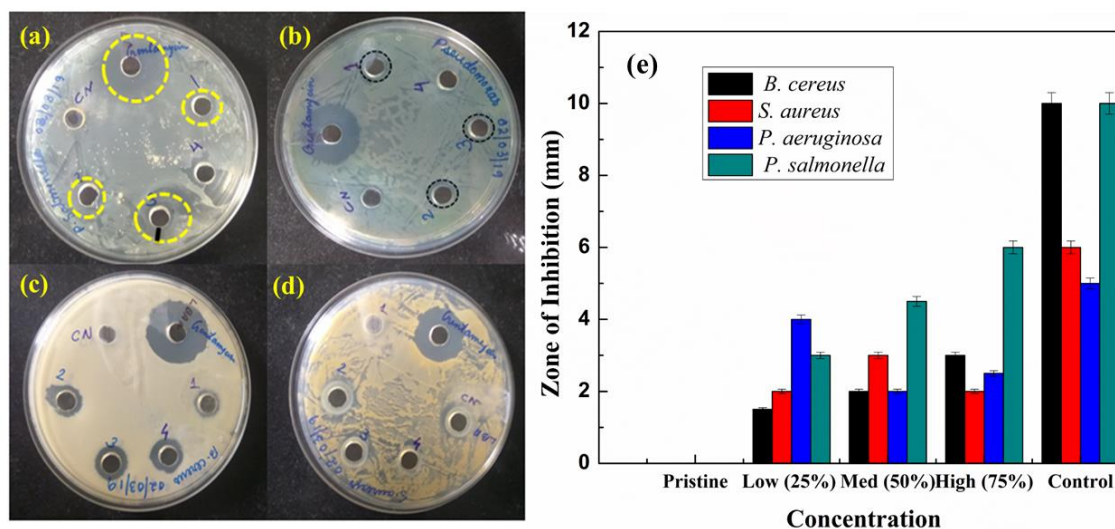


Figure 4.8: Effect of CuSe as antimicrobial property studies on 2D $g\text{-C}_3\text{N}_4\text{@CuSe}$ heterostructure film (a, b) Gram negative bacteria and (c, d) Gram positive and (e) zone of inhibition of the respective concentration. It can be seen in (a-d) that without presence of CuSe within the pristine $g\text{-C}_3\text{N}_4$ leads towards no antimicrobial property as a result no zone of inhibition occurs.

4.4.7 Ion Migration Analysis

Despite all the benefits offered by 2D heterostructure based film, there is always a potential risk of ion migration from the packaging material into the foods and its impact on human being. Therefore, it is important to evaluate the migration of nanomaterials from film to the packed food before use. Consequently, a prior in-depth experiment was carried out to investigate the migration effect of nanomaterials from nanocomposites to the packed food. Thus, atomic absorption spectrometry (AAS) characterization has been conducted to check the migration of these nanomaterials from the film to the packed food. At first, a small piece of each film, before and after food packaging are cut into a square strip of $2 \times 2 \text{ cm}^2$ area and immersed in 50 mL of slightly acidified aqueous medium. The film after food

packaging is obtained from the packaging material after storing food inside it for short term and long term. After homogeneously dissolved, AAS analysis is conducted to verify the presence of elements (Cu metal) in the film. This AAS experiment has been conducted to check the activity of the nanomaterials over the food items that were packed using the developed film for several days. For 2D heterostructure materials and its potential application in food packaging field, it must fulfil the standards required by the government health safety policies. **Figure 4.9** shows the concentration of nanomaterials released after coming in contact with the food. The results show that the concentration of nanomaterials present within the film before and after food packaging is same, even for a long duration. Thus, the presence of Cu ion is steady in both the cases ensuring no toxicity due to ion migration over long period from the film to the food.

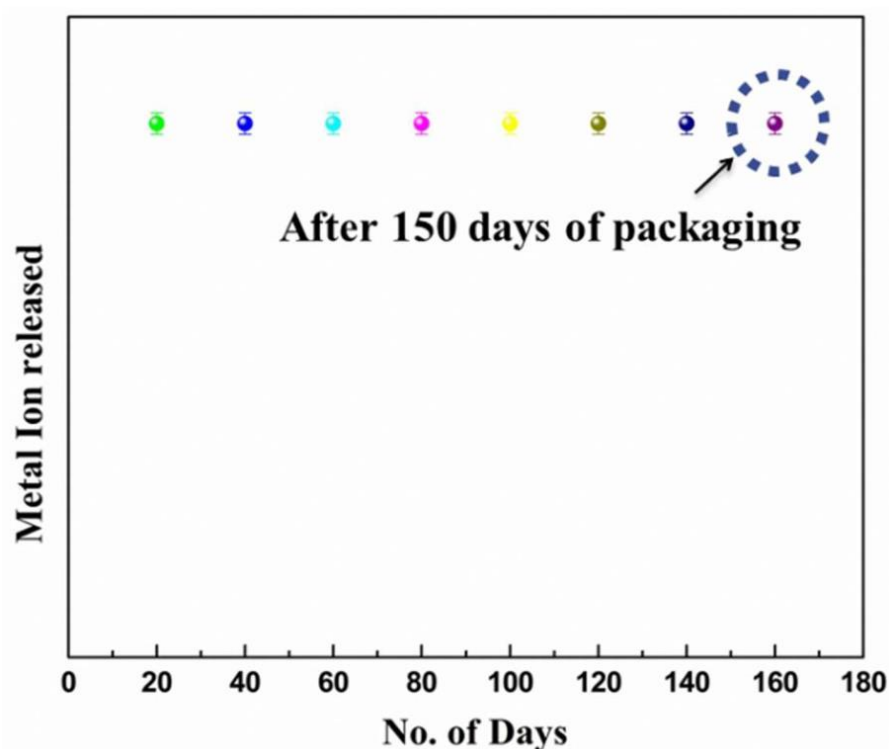


Figure 4.9: Investigation of Ion migration of the film after 150 days. The developed heterostructure film does not release any ion when the food items are in contact with it.

4.4.8 Degradation phenomena

The biodegradability of this 2D heterostructure based film will be an important parameter to be assessed for its successful application for packaging material. To test the degradability of the prepared 2D heterostructure film, a piece of film was buried into sterilized soil where earth worms are present. The buried film has been removed from the soil after finite time interval, washed by deionized water, dried at 60 °C until the moisture and water content get completely evaporated. The degradation percentage of the film is calculated by the formula;

$$I_D = \left(1 - \frac{W_f}{W_o}\right) * 100$$

where W_o is the initial and W_f is the weight of the buried film. A film of 20 cm × 20 cm dimension takes over a period exceedingly more than 90 days for undergoing complete degradation. Compared to the materials for plastic preparation, PVA and chitosan polymers can degrade quickly in soil due to easy dissolution. The delay in decomposition of the film is due to presence of 2D nanomaterials which develops a compact structure with PVA and chitosan, deferring the degradation time [20]. Further the colour of the film after buried in the soil changes due to long exposure time in the soil. The original colour of the film is transparent, but the colour gets changed during the degradation process with a sign weight loss indicating continuous degradation of the film in the soil (shown in **Figure 4.10**).

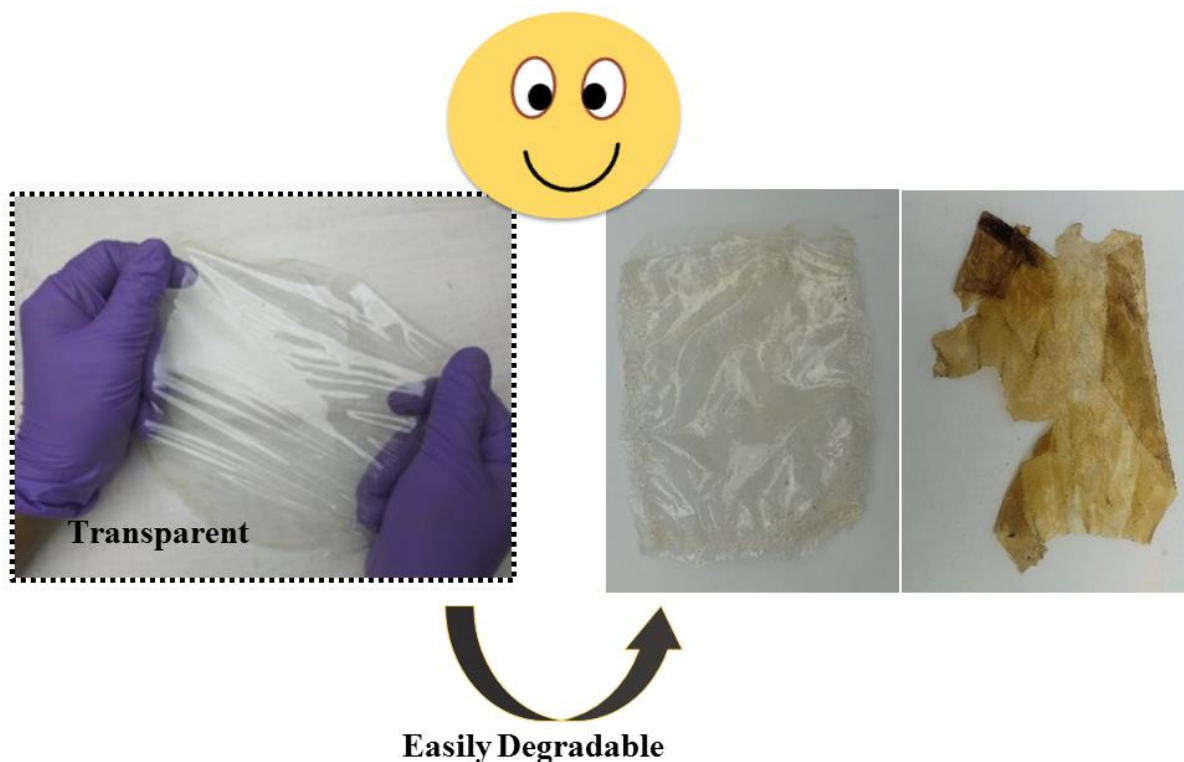


Figure 4.10: Represents the 3D presentation of film degradation. The complete film gets degraded easily without causing damage to the environment. The image of degradation of the film was taken after keeping the film buried in soil for 10 days, later the same film was further buried to continue its degradability.

4.4.9 Shelf Life study

To increase the shelf life of the product and to minimize the growth of micro-organisms, active packaging is one of the options that can be used. In case of food preservation, two types of antimicrobial films are considered. One that contains an antimicrobial agent which can migrate to the food and secondly where there is no migration of agent to the food from the film. For testing the shelf life of the food, bread was packed using 2D heterostructure based composite film (as shown in **Figure 4.11**) and stored for an adequate duration to check its efficacy and functionality. The performance of 2D heterostructure based film was analysed after two weeks and it resulted no odours, no flavours, no colour change and no microbial growth in the food items. Due to blending of 2D nanomaterials with PVA, it improves the barrier properties and decreases the water sorption ability of

the film and hence this essential daily life food was fresh till 15 days. Owing to high surface selectivity and ability to act as partial moisture barrier, this film reduces the respiration and transpiration rates of the food.

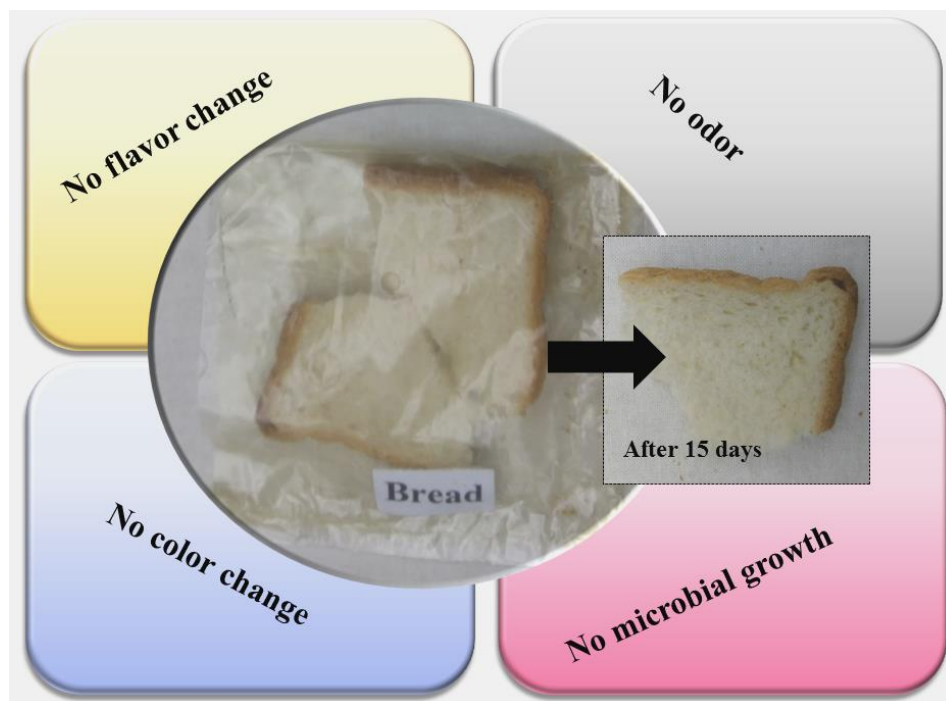


Figure 4.11: Shelf life of food stored using 2D heterostructure based composite film.

4.5 Conclusion

Promising biodegradable material is fabricated and prepared for developing a suitable substitute to plastics for food packaging. In this chapter, we have discussed about development of a composite film of 2D g-C₃N₄ nanosheets and CuSe nanoflakes with PVA for efficient food packaging. Presence of CuSe within the system facilitates the interactions with microorganisms, enhancing their antimicrobial efficacy in the final packaging material. These 2D nanoflakes with high surface areas can easily penetrate the cell membrane of bacteria. Accumulation of both g- C₃N₄ and CuSe as active packaging system has enabled

multiple active functionalities such as good thermal stability, mechanical characteristics, and antimicrobial properties and protects the food from deterioration and increases shelf-life for sustainable food packaging. Presence of 2D g-C₃N₄ not only enhances the barrier properties against gas permeations, but also increases the tensile strength up to 2.3 folds and young's modulus up to 5.7 folds than that of pristine PVA film. Presence of CuSe nanoflakes within g-C₃N₄ sheets prevents the film against microbial growth. Addition of 2D heterostructure increases the surface contact angle to 87.6° illustrating its superior hydrophobic behaviour. Further, decomposition of the film has been investigated using vermicomposting technique which confirms its easy biodegradability. From the above observations, we can conclude that this biodegradable 2D heterostructure based film can be used as a sustainable and smart packaging material.

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