WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 605-619. Conference Article ISSN 2277– 7105

PREPARATION AND CHARACTERIZATION OF CHITOSAN-POVIDONE BLEND WITH GLUTARALDEHYDE AS CROSSLINKING AGENT

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Article Received on
05 March 2018,
Revised on 25 March 2018,
Accepted on 15 April 2018
DOI: 10.20959/wjpr20188-11460

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ABSTRACT

Biocompatible Chitosan-Povidone/Poly (vinylpyrrolidone) (PVP) blends were successfully prepared by Solution Casting method. The prepared blends were characterized by using techniques such as Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction studies (XRD), Thermal studies (DSC and TGA), and SEM. FTIR was used to characterize the presence of specific chemical groups in the blends prepared. The Crystallite size and Lattice strain was calculated using Scherrer equation. The addition of PVP was beneficial for the thermal stability of chitosan, but resulted in a compatibility lower strength performance. Thermogravimetric analysis indicated that the thermal stability of the blend was increased with addition of glutaraldehyde as a crosslinking agent. DSC and XRD were used to find the thermal stability, miscibility and crystallinity of the blends. Overall, the performed studies confirmed CS–PVP blends can be a potential candidate for biomedical applications and for water treatment applications.

KEYWORDS: Chitosan, Crystallinity, Blend, Crystallite size, Povidone, Compatibility.

INTRODUCTION

The utilization of blended polymers is the most effective way to produce new multi-purpose materials. In recent years, researchers have paid particular attention to the study of the polymer mixture. Chitosan is the second largest biological polysaccharide (after cellulose) present in nature. It is present in soft-bodied insects and can be synthesized through a

deacetylase reaction using chitin as a raw material. As a water-soluble polymer, polyvinylpyrrolidone (PVP) has beneficial effects on protection, viscosity, absorbency, solubilization, and condensation, with its most significant features being excellent solubility and biological compatibility. Additionally, PVP has low toxicity and is utilized in a broad range of areas, such as medical, food, cosmetics, and health-related domains. However, issues concerned with the rigid but fragile nature of PVP and its lack of sturdiness have resulted in processing difficulties.[1] Poly (vinyl pyrolidone) (PVP) is a synthetic linear, non-toxic, biocompatible polymer, frequently used in controlled drug release, tissue engineering and wound dressings.[2,3] It is easily soluble in water and in many organic solvents. PVP is amphiphilic in nature, hence exhibiting good adhesive and cohesive properties. Due to its structure, high polarity, and ability to accept protons, PVP is cross-linkable and forms chemical complexes. Consequently, it is used in the preparation of tablets to improve the penetration of moisture and improve dispersion, which allows a better formation of the matrix. At the same time, PVP is physiologically inert and hemocompatible.[4] Glutaraldehyde (GLU) is a highly reactive dialdehydes reagent that has been widely used as a fixative and crosslinker in biological assays. Through coupling reaction of the aldehyde groups at the two separate terminals with the amino groups of proteins, the oligomeric proteins can be crosslinked via a mild aldehyde–ammonia condensation reaction.[5] Crosslinking of chitosan with the bifunctional glutaraldehyde agent.[6] The purpose of the present research was to blend the two materials, chitosan and PVP, with and without crosslinking agent with the aim of producing a new material possessing the benefits of both. The prepared blends were characterized and then the results were investigated.

2. MATERIALS AND METHODS

2.1 MATERIALS

Chitosan was kind gift from India Sea Foods, Cochin, Kerala which is 92% deacetylated. Polyvinylpyrrolidone is purchased from SD Fine Chemicals. All other chemical used were of analytical grade.

2.2 Preparation of Chitosan /PVP blends

CS/PVP (2:1) blend was prepared by about 2g of Chitosan was weighed and dissolved in 2% acetic acid. Simultaneously 1 g of (PVP) was dispersed in minimum amount of deionized water. The dispersed PVP was slowly added to the chitosan suspension and then the mixture was stirred in magnetic stirrer for 2 hours then poured into the petriplates and dried. For

CS/PVP/GLU blend the above same procedure was followed and finally 7ml of glutaradehyde was added slowly and then the mixture was stirred in magnetic stirrer for 2 hours then poured into the petriplates and dried. The digital image of prepared blends with and without crosslinker were shown in Fig.1.

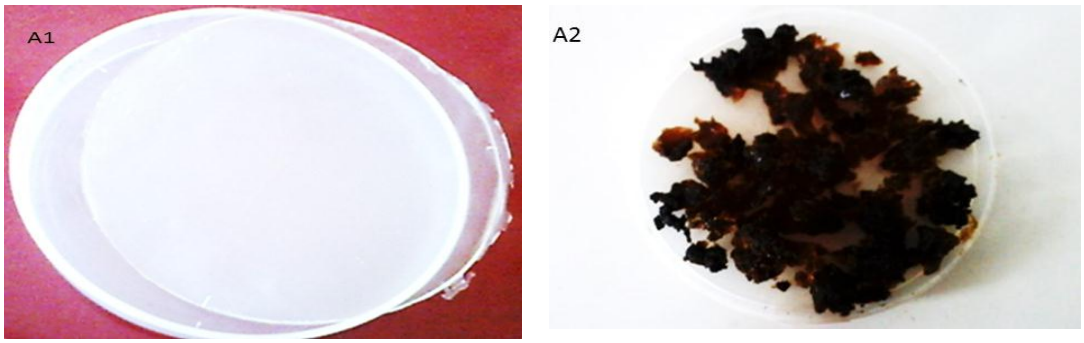


Fig.1: Prepared Chitosan /PVP blend (A1); Chitosan /PVP blend with GLU (A2).

3. CHARACTERIZATION OF POLYMER BLENDS

The FT-IR spectra of Chitosan /PVP blend of ratio (2:1) with and without crosslinker glutaraldehyde were recorded by Fourier Transform Infra-Red Spectrophotometer (FT-IR) using the Alpha Bruker FTIR Spectrophotometer. The X-ray diffraction patterns of the prepared blends were tested by an X-ray scattering D8 ADVANCE Diffractometer using Ni filter Cu Kα radiation source (λ=0.154nm), set at scan rate = 10°C/min, using a voltage of 40kV and a current of 30 mA. The TGA study of the prepared blends was carried out using SDT Q600 V8.0 Build 95 instrument at a heating rate of 10°C per minute in nitrogen atmosphere. The weight losses at different stages were analyzed. Differential Scanning Calorimeter (DSC) was used to examine the thermal property of the blends. The measurements were performed with NETZSCH DSC 200 PC in a pan Al, pierced lid in the N_2 atmosphere at a heating rate of 10° K/min. The Surface and Cross sectional morphology of the blends was observed with Scanning Electron Microscopy to confirm the compatibility of the mixtures. For the examination, the samples were cut into pieces of various sizes and wiped with a thin gold – palladium layer by a sputter coater unit (VG – microtech, UCK field, UK) and the cross section topography was analyzed with a Cambridge stereoscan 440 scanning electron microscope (SEM, Leica, Cambridge.).The particle size and Lattice strain was calculated using Scherrer equation and it can be written as: $\tau = K \lambda / \beta \cos \theta$, τ is the mean size of the ordered (crystalline) domains, K is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.94, but varies with the actual shape of the crystallite; λ is the X-ray wavelength; β is the line broadening at half the

maximum intensity (FWHM) and θ is the Bragg angle. The results were recorded and analyzed.

4. RESULTS AND DISCUSSION

4.1 FT-IR spectroscopy

FTIR spectroscopy is an appropriate technique to establish the variations introduced by different treatments on the chemical structure of the isolated samples. The literature revealed that for pure PVP, the FTIR spectrum shows a band at 2924 cm^{-1} and 2892 cm^{-1}, 1664 cm^{-1}, 1461 cm^{-1}, 962 cm^{-1} corresponds to CH_2 symmetric stretching, –C=O and C=C stretching, CH_2 bending and out-of-plane rings C–H bending [7] respectively in PVP and a band at about 1286.2 cm^{-1} was assigned to the characteristic vibration of C-N in PVP. When PVP and chitosan are mixed, physical and chemical interactions has taken place and it was reflected by change in characteristic peaks. [8] Fig. 2 show the FTIR spectrum of CS/PVP (2:1) blend. The prominent broad peak which was observed at 3400 cm^{-1} corresponds to the presence of intermolecular hydrogen bonded O-H, N-H stretching and polymeric association. The band at 3700 cm^{-1} represents the free OH bond of water molecules that straddle the interface. The peak observed at 2922 cm^{-1} and 2881.88 cm^{-1} was due to asymmetric -CH_2 stretching vibration, symmetric CH_2 stretching vibration attributed to pyranose ring respectively and the band for amide (I) and (II) were observed at 1628 cm^{-1} and 1540.38 cm^{-1}. The peaks at 1407.25 cm^{-1}, 1288.79 cm^{-1}, 1150.01 cm^{-1}, 1067.43 cm^{-1}, 1020.03 cm^{-1}, 779.38 cm^{-1} and 644.39 cm^{-1} shows the presence of CH_2 bending, O-H in plane bending, C-N stretching, C-O-C in glycosidic linkage and C-O stretching vibrations respectively. The peak at 499.19 cm^{-1} is responsible for C-C stretching vibration.

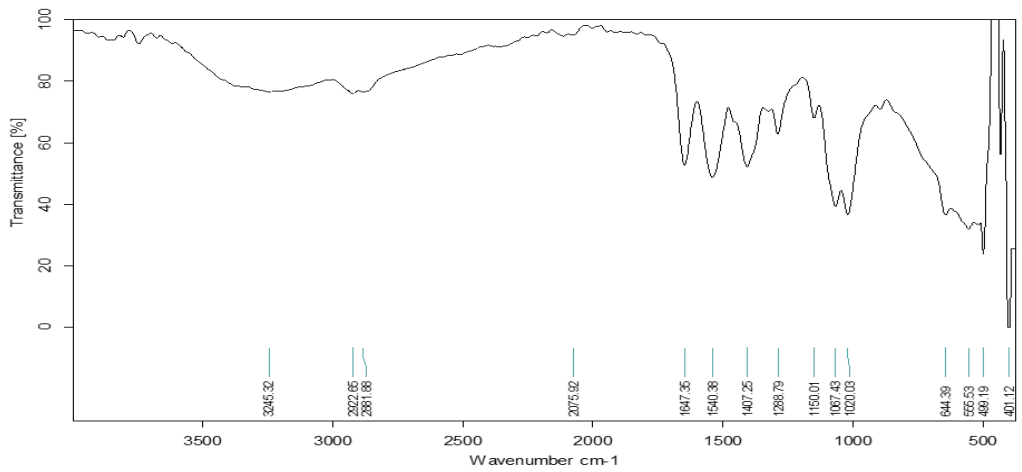


Fig. –2: FTIR spectrum of CS/PVP (2:1) blend (A1).

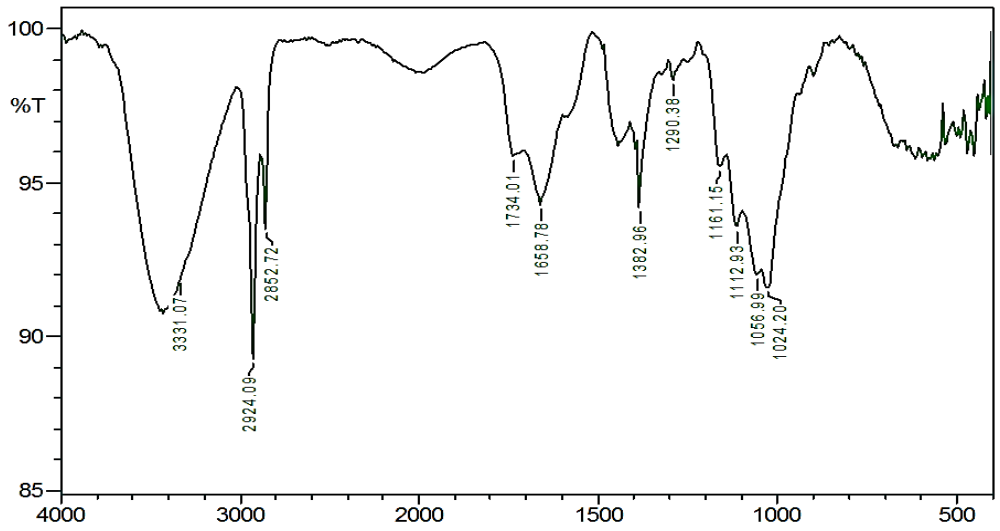


Fig. –2a: FTIR spectrum of CS/PVP/GLU (2:1) blend (A2).

In the typical spectrum of CS/PVP blend, the amide peak and amino peak of chitosan were shifted from 1628cm^{-1} to 1647 cm^{-1} and 1540 cm^{-1} to 1540.38 cm^{-1} with the addition of PVP. The formation of new band at 1647 cm^{-1} for C=N stretching vibration (Schiff base) during blending, confirms the interaction between the amine group of chitosan and carbonyl group of PVP. The FT-IR spectra of CS/PVP- GLU (2:1) blend Fig.-2a shows the prominent peaks at 3331.07 cm^{-1}, 2924.09 cm^{-1}, 1658.78 cm^{-1}. The broad band at 3331.07 cm^{-1} shows the presence of N-H stretching, O-H stretching, polymeric association and intermolecular hydrogen bonding. The peak at 2924.09 cm^{-1} and 2852.72 cm^{-1} shows the presence of aliphatic C-H stretching vibration. A peak for amide (II) band is at 1598cm^{-1}. The peaks at 1382.96 cm^{-1}, 1230.38 cm^{-1}, 1161.15 cm^{-1}, 1056.99 cm^{-1} and 1024.20 cm^{-1} shows the presence of CH_2 bending, O-H in plane, C-O-C stretching, C-O stretching vibrations respectively. Increase in the intensity of CH_2 stretching vibration confirms the presence of crosslinking agent glutaraldehyde. The peaks at 480.12 cm^{-1} is responsible for, C-C bending vibration. The band at 1658.78 cm^{-1} was ascribed to the imine bond formed during blending and crosslinking. After mixing the two polymers the broad band appeared at 3454 cm^{-1} of the –OH stretching of chitosan was shifted to 3331.07cm^{-1}, which overlaps the –NH_2 stretching of CS in the same region. Lower peak wave number indicates the stronger interactions. [9] The peak for NH_2 bending at 1540 cm^{-1} for pure Chitosan was shifted to 1598 cm^{-1} after blending. This result indicated that interactions were present between the carbonyl groups of PVP and glutaraldehyde and the amino groups of chitosan. It also shows a certain degree of miscibility between the PVP and Chitosan in the presence of crosslinker.

4.2 X-Ray Diffraction studies (XRD)

The XRD pattern of pure chitosan powder shows broad peak at 2θ values of 20^{o} (Rana et al., 2010).[10] **Fig.3** represents the XRD diffractogram of CS/PVP blend with little sharpness in peaks at various 2θ values of 11°, 15°, 18°, 19°, 20°, 22°, 24° and 30°. The peaks obtained at range of 10˚ and 20˚ were attributed to the presence of chitosan and the 2θ values at 11.5˚ and 19° shows the presence of polyvinylpyrrolidone in the blend. From the diffractogram of unmodified CS/PVP blend it can be noted that the XRD patterns of the plain blend suggest semi-crystallinity with sharp peaks at around 10˚ and 20˚ of 2θ, indicating the average intermolecular distance of the amorphous part. It can also be seen that there are two distinct bands having their maxima at 2θ = 11˚ and 2θ at 20˚, the former corresponding to the reactive functional groups present in the polymer. The presence of two broad bands with strong peaks centered and Liu et al. (2004) [11] reported that two typical peaks at 2θ =10.31° and 19.41° were observed for pure PVP. The degree of crystallinity of uncrosslinked blend was calculated and it was found to be 19.35%.

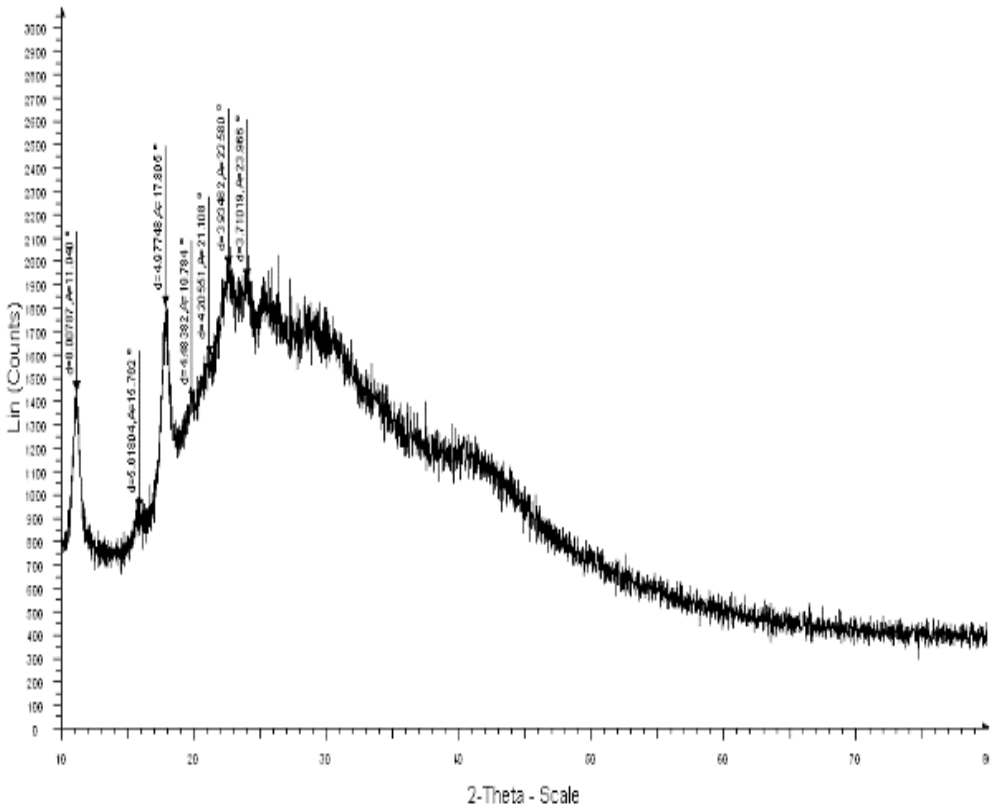


Fig.3: XRD diffractogram of CS/PVP (2:1) blend (A1).

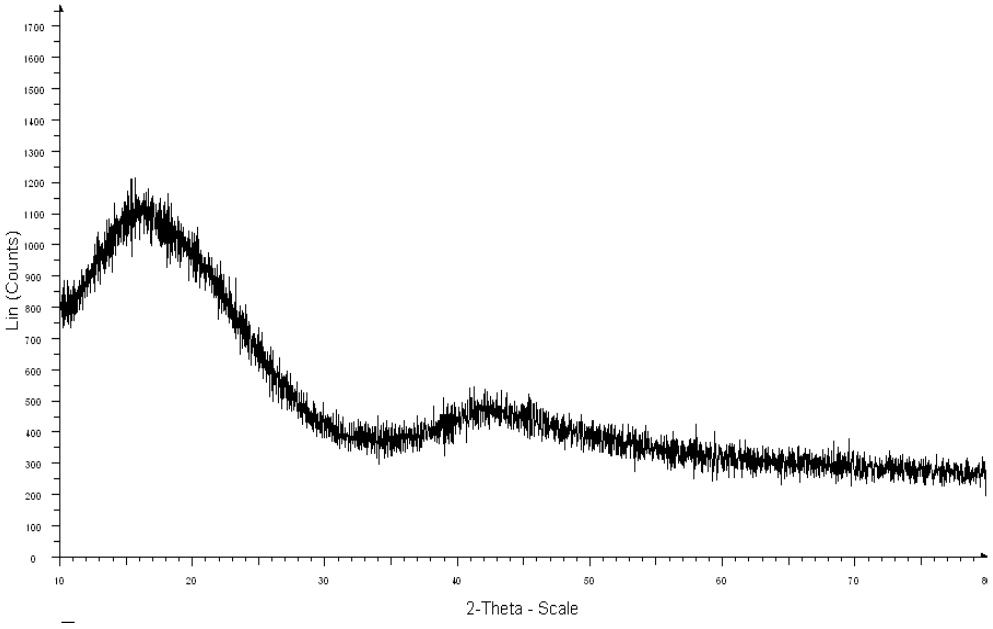


Fig.3a: XRD diffractogram of CS/PVP-GLU (2:1) (A2).

Fig.3a represents the XRD diffractogram of the CS/PVP-GLU blend which shows peaks at various 2θ values such as 16° and 42° and the degree of crystallinity was found to be 3.33%. These peaks conclude that poor crystalline state or amorphous forms were introduced when compared to the uncrosslinked CS/PVP blend. From the broad peak obtained we can conclude that the blend was found to be amorphous in nature when compared to the chitosan which could prove that samples CS/PVP-GLU have blended. In agreement with the result of previous studies (Raut and Khairkar, 2014) [12] the crystallinity of CS/PVP decreases after crosslinking with glutaraldehyde. This could be attributed to the deformation of the strong hydrogen bond in original chitosan due to the substitution of hydroxyl and amino groups, which efficiently destroyed the regularity of the packing of the original chitosan chains and resulted in the formation of amorphous Chitosan/PVP. This could suggest that there was an interaction between these two components that lead to new crystalline structures. Mohamed and Fahmy, (2012)[13] reported that the incorporation of hydrophilic cross-linker into chitosan allowed the synthesis of hydrogels with higher hydrophilicity, with greater positive charge density and with higher antimicrobial activities.

A reduction in effective d-spacing value from 0.8008 nm for uncrosslinked to 0.5536 nm for the glutaraldehyde cross-linked blend (CS/PVP-GLU) gives an indication of shrinkage in cell size or Inter-segmental spacing resulting from crosslinking. This reduction suggests an improvement in selectivity of the crosslinked blend. Further, ionic crosslinking, which increases packing of the Chitosan chains, can deform the crystalline regions. [14] Thus the

interaction detected in this work decreases crystallinity, and changes in crystallinity can be used to monitor the progress of the cross-linking reaction. By applying Scherrer equation the crystallite size were calculated and it was tabulated Table-1 along with corresponding d-spacing, full width half maximum values and percentage of crystallinity.

Table -1: Crystallite size values using Scherrer equation:

2 θ (degree)	d spacing value (nm)	FWHM Full width at half maximum (radian)	Crystallite size (nm)	Lattice strain	Degree of Crystallinity Xc%
CS/PVP (2:1) A1					
11.04	0.8008	0.0139	10.41	0.0362	19.35
15.76	0.5619	0.0098	14.91	0.0177	
17.80	0.4977	0.0086	16.88	0.0139	
19.76	0.4484	0.0078	18.79	0.0112	
21.10	0.4206	0.0073	20.08	0.0099	
22.58	0.3915	0.0068	21.61	0.0086	
23.08	0.3710	0.0065	22.84	0.0079	
CS/PVP-GLU (2:1) A2					
16	0.5536	0.00966	15.14	0.0172	3.33
41	0.2150	0.00375	39.26	0.0051	

Crystallinity depends up on the periodicity i.e) long range order of atoms or ions or molecules in a particular sample. If periodicity is more we can get high intensity of hkl planes. If the intensity of hkl planes are less indicates the periodicity is less. There is no relationship between crystallite size and crystallinity. The crystallite size value of the blends was calculated using scherrer equation and it was in the range of 10.41-22.84 nm for CS/PVP (2:1) and 15.14-39.26 for CS/PVP-GLU (2:1).

4.3 Thermogravimetric Analysis

Fig.4 shows the TGA thermogram of CS/PVP (2:1) blend. About 90% of the sample gets disintegrated with the decomposition temperature of 550°C. The maximum weight loss of the blend occurs at the temperature range of 225°C - 550°C. At the end of the experiment i.e. at 790°C only 1.337% of the sample remains as residue. Three major weight losses are observed for the blend CS/PVP (2:1). The first stage ranges between 30°C -100°C and shows about 10% loss in weight. This is due to the elimination of moisture. The second stage of weight loss begins at about 200°C-450°C corresponding to the loss of decomposition of linkages between CS and PVP.[15] The third stage weight loss begins at about 450°C-790°C is probably due to degradation decomposition of the main chain with the production of H_2O, CO and CO_2 and some other fragments from the glucosaminic ring.

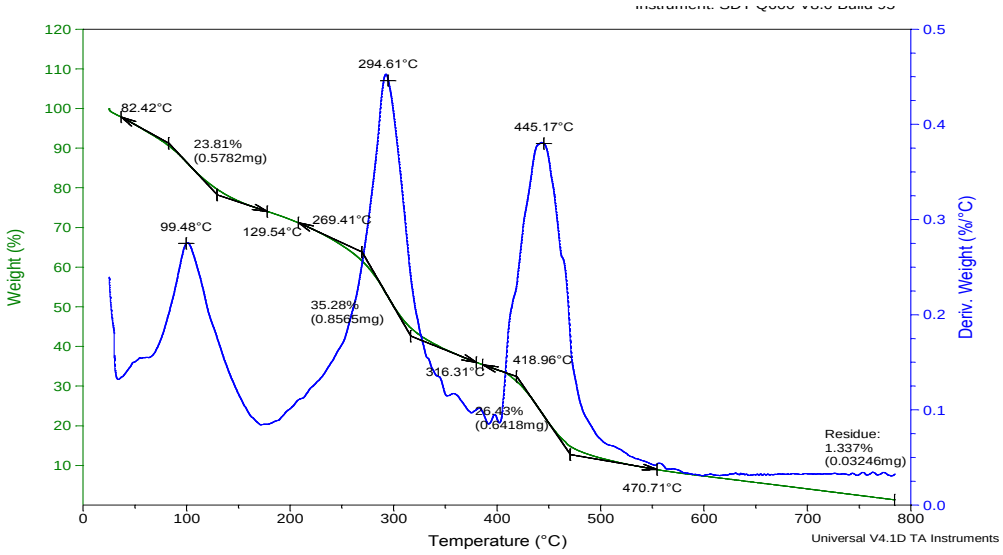


Fig.4: TGA thermogram of CS/PVP (2:1) (A1).

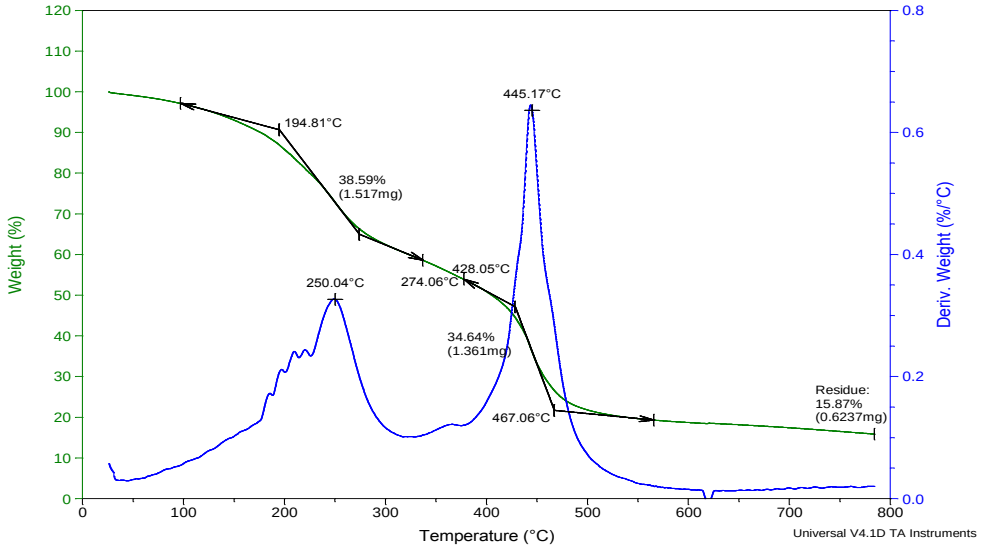


Fig.4a: TGA thermogram of CS/PVP-GLU (2:1) (A2).

Table 2: TGA thermal studies of CS/PVP (2:1) (A1) and CS/PVP-GLU (2:1) (A2).

Percentage decomposition (%)	Decomposition temperature (^{0}C)	
	CS/PVP (2:1) (A1)	CS/PVP-GLU (2:1) (A2)
10	90	200
20	140	240
30	230	260
40	290	310
50	310	400
60	340	440
70	430	460
80	460	560
90	550	>560

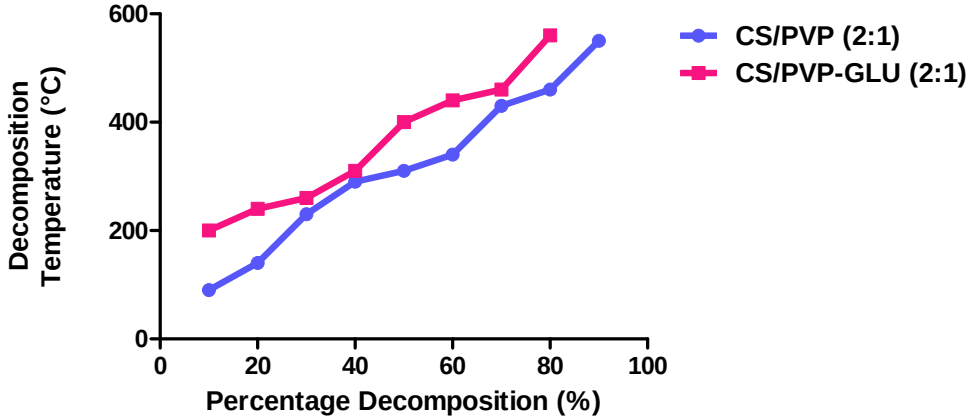


Fig.-4a: TGA thermogram details of CS/PVP-GLU (2:1) (A4).

Fig.-4a shows the TGA thermogram of CS/PVP-GLU (2:1) blend. The initial decomposition of the sample took place at about 200°C. The maximum weight loss occurs at the temperature range of 250°C to 500°C. Here also three stages of weight loss observed on compared with CS/PVP blend. The first stage ranges between 30°C - 250°C which was due to the elimination of moisture and the vaporization of small moieties. The second weight loss was at 250°C - 450°C attributed to the decomposition of linkages between the polymers. The third stage weight loss was in the range of 470°C - 790°C is probably due to degradation of chitosan backbone. At about 560°C, 80% of the sample was disintegrated. At the end of the experiment at 790°C, about 15.87% of the crosslinked sample remains as residue. From the above results it is evident that cross linked sample had more thermal stability when compared with uncrosslinked sample. The cross linked chitosan improved the strength and stability of polymer blends. [16] On comparing the CS/PVP (2:1) blend prepared in the absence and the presence of glutaraldehyde, the crosslinked blend was found to be thermally more stable. The total weight loss at about 500°C was found to be 73% for crosslinked and 86% for uncrosslinked. At the end of the experiment (i.e.) at 790°C only 1.337% of uncrosslinked and 15.87% of crosslinked blend remained as the residue. Also the initial decomposition was found to be more for the crosslinked blend which clearly indicates the addition of glutaraldehyde improves the thermal stability.

4.4 Differential Scanning Calorimetry (DSC)

Fig.5 represents the DSC curve of CS/PVP blend. The glass transition temperature of the blend was observed at 190°C (Tg) whereas for pure chitosan it was found to be 203°C (Tg). [17] The DSC curve showed an exothermic peak at 290.63°C (Tm) showing the melting nature

of the blend and an endothermic peak observed 94.63°C (Tc) shows the crystallization of the blend at a lower temperature. This peak is attributed to the loss of water associated with the hydrophilic groups of the polymer (Gonzalez et al., 2000).[18]

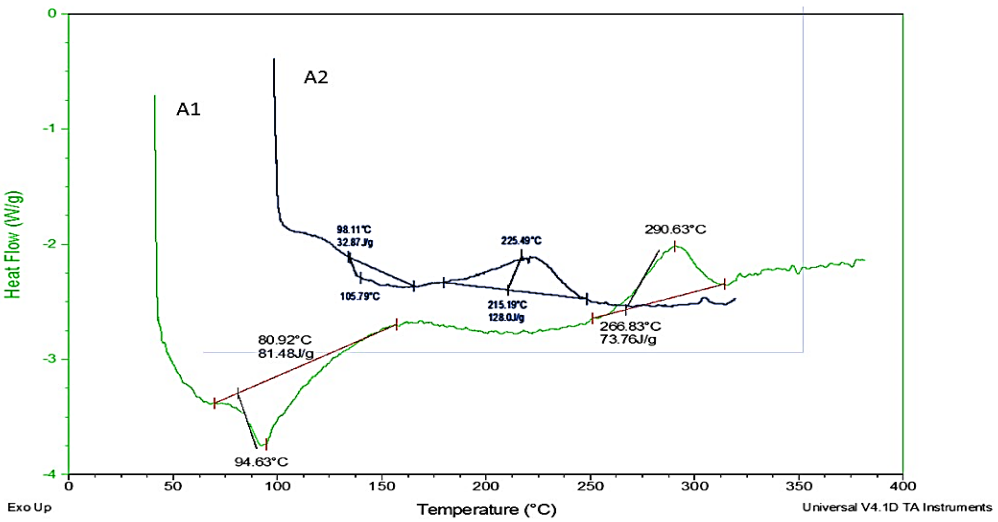


Fig.5: DSC thermogram of CS/PVP (2:1) (A1), CS/PVP-GLU (2:1) (A2).

On analyzing the DSC thermograms of CS, PVP and their blends in the literature several conflicting values of a glass transition temperature (Tg) of PVP were found ranging from 54°C to 175°C.[19] These may be attributed to the large influence of sorbed moisture due to the hydrophilic nature of the material, as will be shown by present measurements. Therefore, for hydrophilic polymers such as PVP, an accurate drying at the maximum possible temperature, preferably in a nitrogen atmosphere, is necessary to obtain the correct Tg values.

In Fig.5 A2 represents the DSC curve of CS/PVP-GLU blend. The glass transition temperature of the blend is 160°C (Tg) which shows the blend were amorphous Table-3. The DSC curve showed an exothermic peak at 225.49°C (Tm) showing the melting of the sample at this temperature and an endothermic peak at 105.79°C (Tc) shows the crystallization of the sample at a lower temperature. This peak is attributed to the loss of water associated with the hydrophilic groups of the polymer.

Table 3: DSC thermogram details of CS/PVP blend with and without crosslinking agent.

Code	Samples	Tg °C	Tc °C	Tm °C
A1	**CS/PVP**	190	94.63	290.63
A2	**CS/PVP-GLU**	160	105.79	225.49

The DSC thermogram of the CS/PVP blends shows single glass transition temperature values showing good miscibility of the materials used.

4.5 SEM analysis

Fig.6 shows the surface structure of prepared CS/PVP (2:1) blend. From SEM micrograph it was observed that due to the chitosan the surface were uniform and firm binding formed on the surface of the blend was flat and smooth, which is beneficial to resist bacteria adhesion.[20] Chitosan and PVP could form a homogeneous phase construction through strong inter and intra-molecular hydrogen bonding. PVP as a hydrophilic polymer molecular added could enhance the hydrophilicity and swelling properties of the sample. From the SEM micrograph of CS/PVP the surface becomes smooth and glazes after the addition of PVP. SEM micrographs also revealed that the surface structure of prepared CS/PVP blend exhibited little amorphous feature.

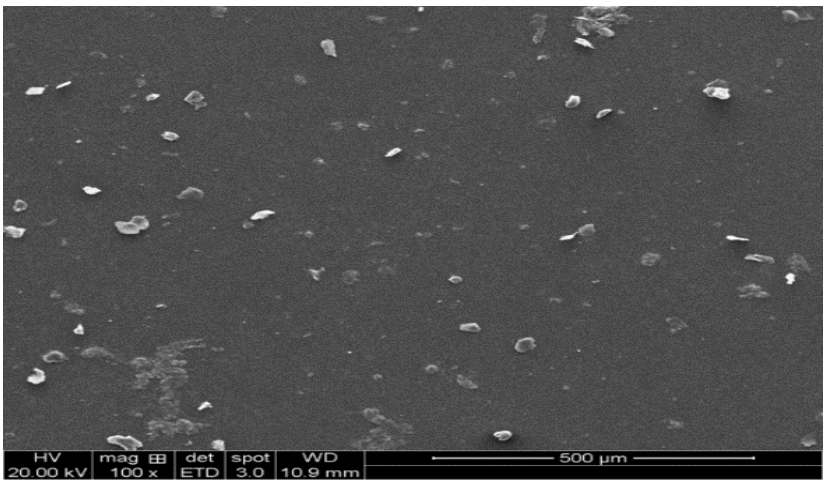


Fig.6: SEM micrograph of CS/PVP (2:1) (A1).

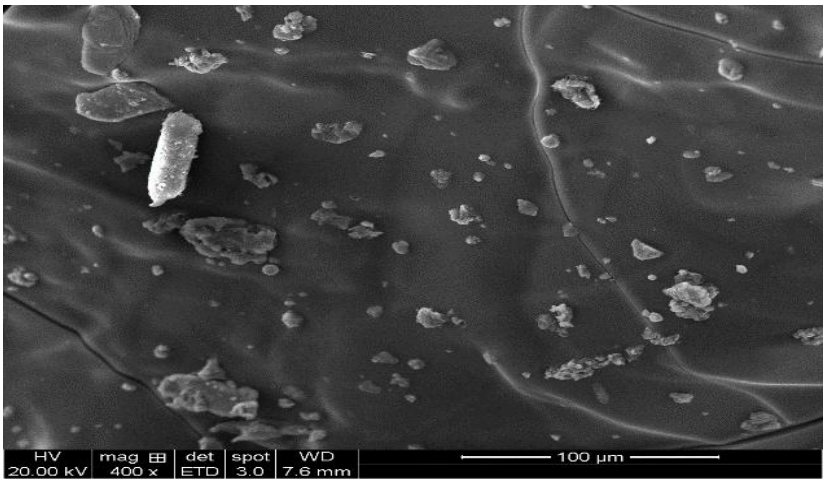


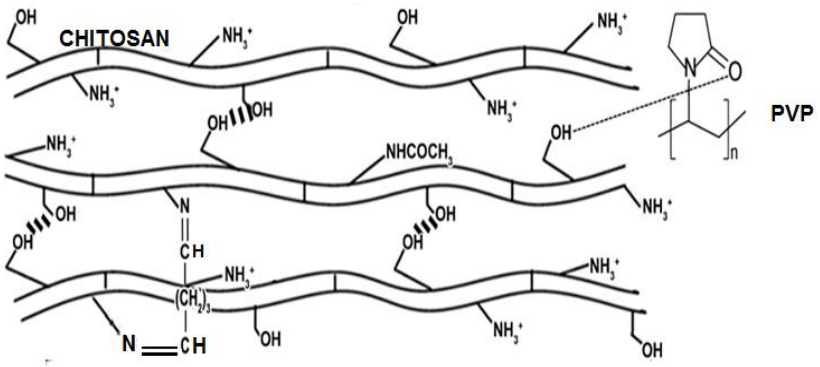
Fig.6a: SEM micrograph of CS/PVP- GLU (2:1) (A2).

Fig.6a shows the surface structure of prepared CS/PVP-GLU (2:1) blend. From SEM micrograph it was observed that the blend has a rough, porous and uneven agglomerates were found. This rough surface allows more water molecules to be adsorbed and it can also hold and allows the cells to grow on it. SEM micrographs also revealed that the surface structure of prepared CS/PVP-GLU blend exhibited little amorphous feature.

The rough surface morphology may be due to the insufficient cross-linking of Chitosan or the glutaraldehyde groups partly grafted on chitosan, indicating that the reaction has taken place on the surface. Furthermore, the porous structure of crosslinked chitosan may offer multiple adsorption sites for the adsorbate,which generally supported the fact that glutaraldehyde-crosslinked chitosan has been widely applied in the uptake of heavy metals[21] and drug delivery.[22]

4.6 MECHANISM

Glutaraldehyde is mainly used as a cross linker in nanoemulsion method. It is an aldehyde and the most commonly used cross linker with chitosan. Glutaraldehyde is a dipolar anionic linear molecule and reacts with free amine groups present in chitosan.



Mechanism -A: Possible interactions of CS/PVP-GLU.

CONCLUSION

The present study was aimed to prepare a novel blend and to carry out a comparative study on effects of crosslinker on the blend. The FT-IR analysis manifested the specific interaction in the polymer blends, therefore, it provides insight in to the possible interactions between the Chitosan/PVP and glutaraldehyde. The results of TGA and DSC of blends with and without cross-linking agent high thermal stability and miscibility were achieved after crosslinked. SEM micrographs shows a high specific surface and a porous structure of the chitosan matrix was achieved during blending and the uncrosslinked blend has a smooth, wide surface with

many pores, while the cross-linked sample has a rough and bent surface suitable for cell growth. From the above analysis, it has been found that mixing of polymers through the blending has been well formed with modified thermal and crystalline properties compared to the individual homopolymers. The addition of PVP to chitosan was useful for the thermoresistance of the blended polymer. The dimensions of the crystallite were calculated using Scherrer method and the results admit that a reduction in crystalline size was achieved in the blends and could be a potential candidate for exploring applications in the biomedical field.

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