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SYNTHESIS, CHARACTERIZATION AND IN VITRO DRUG RELEASE OF CURCUMIN LOADED CASSAVA STARCH ACETATE–POLY VINYL ALCOHOL/ CLOSITE 30B NANOCOMPOSITES

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ABSTRACT

The aim of the present study is to examine the feasibility of *Manihot esculenta* (cassava) starch acetate (CSA)–polyvinyl alcohol (PVA)–Closite30B nanocomposites as controlled drug delivery systems. It is one of the novel drug vehicles which can be used for the controlled release of an anticancer drug curcumin (CUM). Simple nano-precipitation method was used to prepare the carriers CSA–PVA-C30B nanocomposites and they were used for entrapping CUM. The physico chemical properties are studied using FTIR, SEM, TGA and XRD. Also we have to study drug encapsulation efficiency, drug loading capacity and in vitro release of CUM. In finally it was proved that the cross linked CSA-PVA-C30B nanocomposites can be a potential polymeric carrier for cancer treatment.

KEYWORD: ***Starch, PVA. Curcumin, Drug delivery***

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INTRODUCTION

Sustained drug delivery technology represents one of the frontier areas of science, which involves a multidisciplinary scientific approach, contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity and improved patient compliance and convenience. It involves releasing the drug into the blood stream at predetermined rates over a period of time.[1-3] Drug delivery systems that can precisely control the release rates or target drugs to a Specific body site have had an enormous impact on the healthcare system. The last two decades in the pharmaceutical industry have witnessed an avant-garde interaction among the fields of polymer and material science, resulting in the development of novel drug delivery systems. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc. which modulates the release and absorption characteristics of the drug.[4,5] Starch, a biodegradable polymer is a promising carrier for drug delivery. It has been used in various fields like biomedical, agriculture and food etc. However, native starch cannot fit into some parental controlled drug delivery systems, as many drugs are released quickly from such unmodified starch-based systems due to considerable swelling and quick enzymatic degradation of native starch in biological systems.[6-11] The key objective of the current study is to encapsulate the anticancer drug CUM into Cassava starch acetate/PVA/Closite30B (CSA/PVA/C30B) nanocomposites through the interaction between CUM and CSA/PVA/C30B nanocomposites. The viability of CUM-loaded polymeric nanocomposites as a drug delivery system was verified by evaluating its in vitro studies and instrumental characteristics.

MATERIALS AND METHODS

Materials

Native Cassava starch powder (CS) was obtained from Sago Serve Industries (Salem, India). Acetic acid (P99%) and acetic anhydride (98%) were of analytical grade procured from Sigma–Aldrich. PEG 10000, gelatin Type- B, Sodium hydroxide (NaOH) and absolute ethanol were purchased from Merck. (Vapour density 13 (vs air), form: powder; solubility: DMSO>11mg/ml and ethanol 1mg/ml mp: 175^{0}C, storage temp :-20^{0}C) Curcumin was obtained from sigma Aldrich. All chemicals were used without additional purification.

Preparation of cassava starch acetate (CSA)

Cassava starch was dried in an oven for 20 h at 45–60^{0}C. Dried starch was mixed with acetic anhydride (1:4) through the medium of pyridine (200 g). The reaction was carried out at 120 ^{0}C for a period of 3 h. The final product was precipitated with ethanol, filtered and dried in vacuum oven. Lastly, the modified starch was milled and sifted to homogeneous particle size and stored in desiccators until further study.[12]

Preparation- Starch-PVA nanocomposite

Cassava starch acetate -PVA were taken in water in different composition, like 30:70, 50:50, 70:30.The reaction mixture was heated at 70ºC until uniformity appears. After cooling solution at 35ºC, all the solutions are mixed and modified with 40% acetyl chloride. Then the solutions are poured onto casting mold and dried under oven at 75ºC to remove water contents. After complete drying, the films are stored in moisture free environment. A specified amount of closite 30B (1%, 2%, 3%, 4% by wt. of starch-PVA used) was dissolved in the starch-PVA solution. The starch solution containing the clay was heated to 70°C, held at that temperature for 20 min, then cooled to 50°C and poured onto Petri dish and kept in an oven at 75°C for drying and peeled off.

Preparation of CUM– Starch-PVA nanocomposite

CUM of different loadings, i.e., 10 wt%, 20 wt%, 30 wt%, 40 wt% and 50 wt% were then added to the Starch-PVA nanocomposite (70:30) solution having 2 % of Cloisite 30B and stirred for 1 hr and then the composites were kept at room temperature for drying.

Characterization

FT-IR Spectral Analysis

The Fourier Transmission Infrared Spectra (FT–IR) were obtained through a Perkin Elmer Spectrum RX1 FT–IR spectrometer at Hanyang University, South Korea.

Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis has been done using a Universal V4.5A TA Instruments, TGA Q500 V20.10 Build 36 instrument. The Experiments have been carried out from room temperature to 800°C at heating rate 10°s per minute in air.

X-ray Diffraction (XRD)

The change in gallery height of the blend was investigated by WAXD experiments, which were carried out using an X-ray diffractometer (BEDE D-3 system) with Cu Ka radiation at a generator voltage of 40 kV and a generator current of 100 mA. Samples were scanned from 2θ = 1–10^o at a scanning rate of 2^o/min.

Scanning electron microscopy (SEM)

The Starch-PVA nanocomposites were sprinkled onto double-sided tape, sputter-coated with gold to about 500! 10K8 cm thickness using an Hitachi coating unit IB-2 coater under a high vaccum, 0.1 Torr, high voltage, 1.2 kV and 50 mA. Coated samples were examined using Hitachi S-2300 scanning electron microscopy.

Dissolution experiments

Dissolution experiments were performed at 37^o C using the dissolution tester (Disso test, Lab India, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 900 ml of phosphate buffer solution (pH 3.4 and 7.4) was used as the dissolution media to stimulate gastrointestinal tract (GIT) conditions. A 5 ml aliquot was used each time for analyzing the CUM content at a fixed time interval. The dissolution media was replenished with a fresh stock solution. The amount of CUM released was analyzed using a UV

spectrophotometer (Systronics, India) at the λ_{max} value of 421 nm.

Swelling Studies

Water absorption of the polymer-drug conjugates was measured following ASTM D 570-81. The samples were preconditioned at 50° C for 24h and then cooled in desiccators before being weighed. The preconditioned samples were submerged in distilled water at 25° C for 24h.The samples were removed and dried with a paper towel before weighing. Water absorption was calculated as a percentage of initial weight. The soluble material loss was checked by weighting the specimens after drying them in an oven at 50° C for another 24h. The total water absorption for 24h was calculated including the soluble material loss

$$\% \text{ Swelling} = \frac{W_1 - W_2}{W_2} \times 100$$

Where, W_1=Weight of Swollen composite after 24 hr., W_2= Weight of Dry Composite

RESULTS

FTIR

The FT-IR spectra of native and acetylated cassava starch are given in Fig. 1. In the spectrum of native starch, there are some discernible absorbances at 1159, 1014 cm $^{-1}$, which are attributed to C–O bond stretching[13]. Other characteristic absorption bands at 926, 858, 765, and 574 cm $^{-1}$ are due to the whole anhydroglucose ring stretching vibrations[14]. The very broad band between 3000–3600 cm^{-1} and 2928 cm $^{-1}$ corresponds to OH and CH stretching respectively [15] whiles the peaks at 1643 cm $^{-1}$ and 1426 cm $^{-1}$ correspond to (OH) and (CH) bendings . Compared to native starch, starch acetates had a strong absorption band at 1732 cm^{-1} that is attributed to the stretching vibration of the ester carbonyl C,O and indicated the acetylation of starch. The stretching and bending vibration of the hydrogen bonding -OH group of PVA and PVA/CSA blends occurred at 3500-3200 cm^{-1} and 1653 cm^{-1}, respectively. The stretching vibration of C-O bond in C-O-C group in the anhydrous glucose ring appeared at 790-775 cm^{-1}. The characteristic peaks at 1745 and 1713 cm^{-1} in PVA are attributed to the residual acetate groups due to the manufacture of PVA from hydrolysis of polyvinyl acetate. All spectra exhibit the characteristic absorption bands of pure PVA which are 3500-3200, 2955, 1745, 1456 and 1430-1275cm^{-1}. The vibrational peaks are assigned to O–H stretching, C–H stretching, C=O stretching, C–H bend of CH_2, and C-H wagging of PVA and they existed in the FTIR spectra of PVA/CSA blends, indicating the success of blending of PVA with starch. The C-H rocking mode of PVA was appeared at 918 cm^{-1} and is shifted to 916, 926, 916 and 943 cm^{-1} [16].The IR spectra of CSA/PVA/Closite30B, the Carbonyl group is the most prominent band due to the stretching of the –C=O at 1761 cm^{-1} and carbonyl bending at 1382 cm^{-1}. The –OH stretching band at 3504 cm^{-1} and 2995 and 2945 cm^{-1} correspond to $–CH_2$ and $–CH_3$. The hydroxyl bending is in 1185 cm^{-1}.

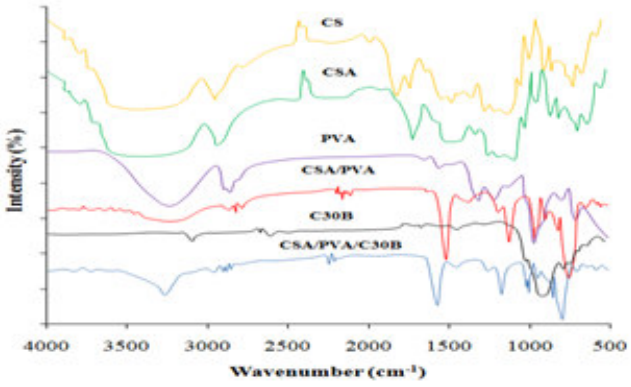

Figure1
FTIR of CS, CSA, PVA, CSA/PVA, C30B and CSA/PVA/C30B

TGA

TGA has been done for all the samples with and without closite 30B to understand the thermal stability and its behavior at high temperature. The TGA data of the PVA without any starch has also been done for reference. The PVA and all the PVA starch blends and their nano composites undergo three step degradation and the results are tabulated in the Table 1.

Table 1
TGA results of CSA/PVA/Closite 30B based samples

Sample	1st peak	2nd peak	3rd peak	Residue Left (%)
PVA	150	350	435	
CSA/PVA (30:70)	60.21	289.32	410.21	10.21
CSA/PVA (50:50)	67.64	302.31	421.89	12.32
CSA/PVA (70:30)	70.65	303.45	437.23	14.89
CSA/PVA (80:20)	69.54	302.12	433.23	13.39
CSA/PVA (70:30) 1% Closite 30B	94.32	284.65	432.34	23.12
CSA/PVA (70:30) 2% Closite 30B	96.56	289.45	438.32	23.21
CSA/PVA (70:30) 3%Closite 30B	109.34	301.67	443.38	23.78
CSA/PVA (70:30) 4% Closite 30B	100.32	278.32	435.38	23.56

From the above result, we have concluded that CSA was added to the PVA at different ratio, the stability of the blend lowered down compared to pure PVA. The TGA studies of the unfilled and filled CSA-PVA blend and pure PVA shows that addition of CSA to PVA matrix has reduced the thermal stability of PVA. But addition of closite 30B at 1%, 2%, 3% and 4% loading has increased the stability. The best stability among all the clay filled samples is shown by 3% clay loaded CSA-PVA (70:30) sample again confirming the optimum dispersion of the closite 30B in the blend matrix[17].

XRD

The X-ray Diffraction studies have been conducted on the CSA-PVA (70:30) /C30B blend nanocomposite samples where the Cloisite 30 B clay loading has been varied in figure. 2. In figure 2a, the variations in the XRD peak intensities are observed was indicated towards the interaction of PVA phase in preference to the starch phase. The XRD spectra of the CSA-PVA blend with 1%, 2% and 3% nanoclay loading have been shown in the Figures 2b respectively. The variations of intensities of the above mentioned three peaks in the three compositions refer to the interference of nano clay at 1% and 2% loading with the crystalline phases of both PVA and starch. At 3% clay loading, probably the nanoclay starts to form agglomerates and hence does not interfere with the crystalline structure of the constituent polymers leading to sharper peaks.[18]

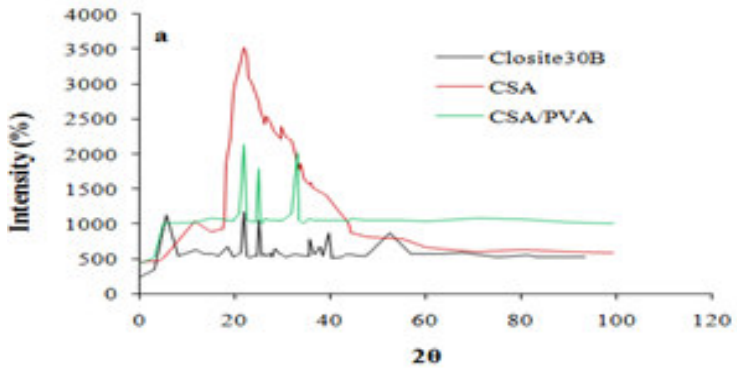

Figure 2a
XRD spectra of CSA, PVA, Pure closite30B and CSA/PVA blend

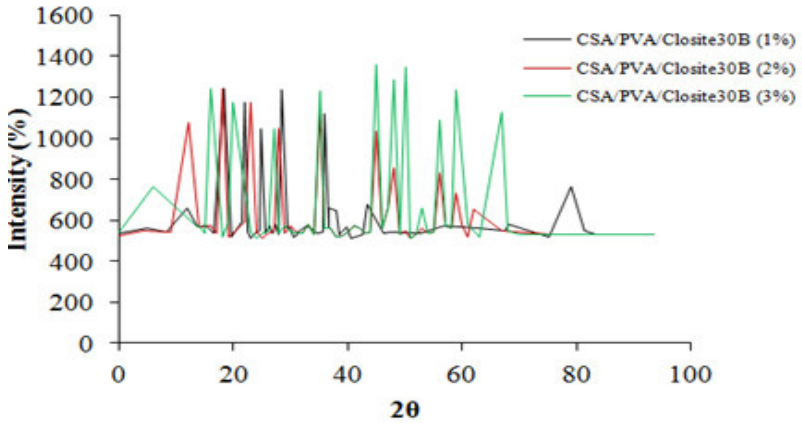

Figure 2b
XRD spectra of CSA-PVA blend with 1%, 2% and 3% closite 30B loading

SEM

SEM has been employed for the observation of the surface morphology of the different CSA-PVA (70:30)/closite 30B nanocomposites. The microstructure obtained by SEM for the CSA-PVA/closite30B nanocomposites prepared by mixing, showed that PVA are relatively well dispersed in the CSA matrix. In Fig.3 shows that CSA-PVA/closite30B nanocomposites is homogenous at low concentration 1% C30B , 2% C30B .As the concentration of the closite 30B increases from 1% to 3% the homogeneity of the surfaces increases because of the intercalation of the nanocomposite along the polymer matrix This might enhance the surface modification.[19]

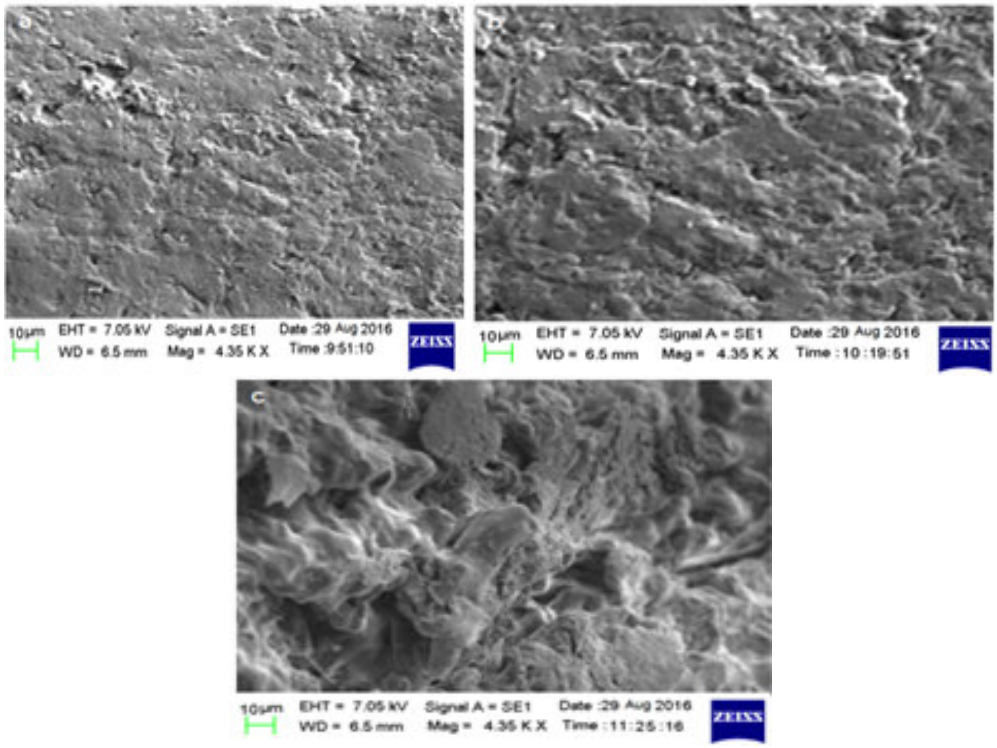

Figure 3

SEM of CSA/PVA/Closite 30B nanocomposites (A) CSA/PVA/Closite 1% (B) CSA/PVA/Closite 2% (C) CSA/PVA/Closite 3%

Swelling Studies

The swelling behavior of CSA/PVA/Closite 30B (70:30: 3%) depends upon the nature of the polymer, polymer solvent compatibility and degree of cross-linking. However, in the case of ionic networks, swelling behavior depends upon mass transfer limitations, ion exchange and ionic interaction. Swelling studies are important to understand the drug release characteristics of the polymer drug conjugate. It depends upon the nature and extent of interaction between solvent molecules and polymer chains in addition to porosity of the polymer and the nature of hydrophilic groups present on the polymer. Here the percentage of swelling increases with increase in the percentage of drug loading in CSA/PVA/Closite 30B nanocomposites.

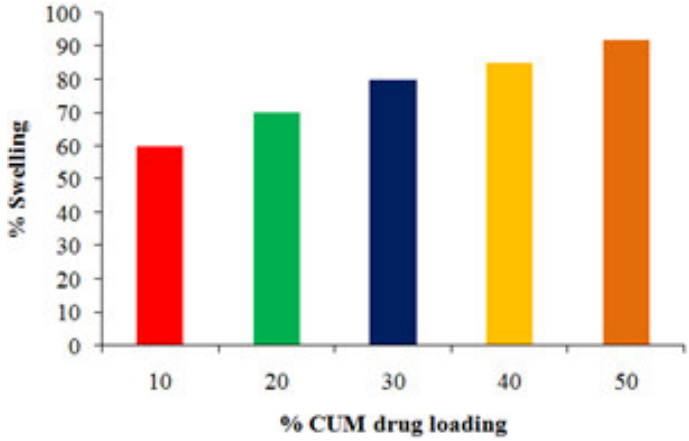

Figure 4

Water Absorption of the CSA/PVA/Closite 30B (70:30: 3%) nanocomposites with different % drug loadings

In-vitro drug release

Effect of pH

In order to investigate the effect of pH on the swelling of composite CSA/PVA/Closite 30B (70:30 /3%), the % cumulative release in both pH 3.4 and 7.4 media was measured. The cumulative release data presented in Figure 5 indicates that by increasing the pH from 3.4 to 7.4, a considerable increase in the cumulative release was observed for all composites. From Figure 5a and b, it can be seen that the 50 % drug- polymer composites have shown longer drug release rates than the other composites. Thus, drug release depends upon the nature of the polymer matrix as well as pH of the media. This suggests that the drugs in the blend can be used to be suitable for the basic environment of the large intestine, colon, and rectal mucosa for which there are different emptying times[19]

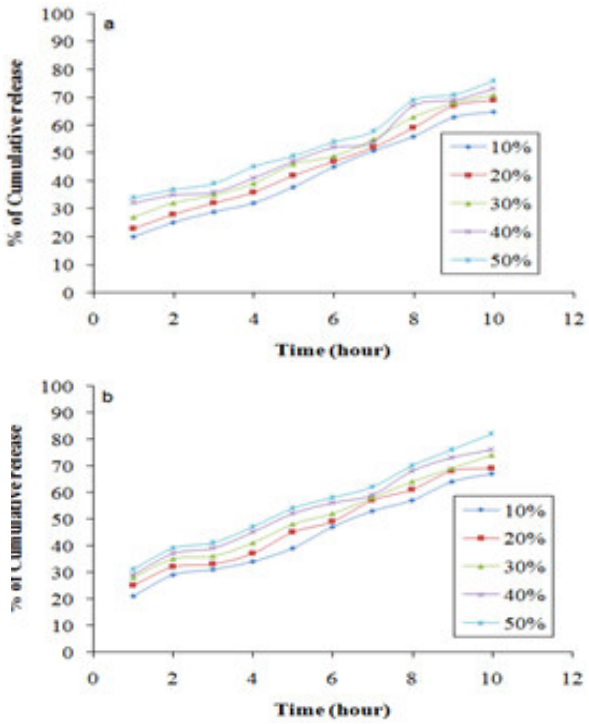

Figure 5
Percentage of Cumulative release Vs time for different CUM formulation loaded with CSA/PVA/Closite 30B (70:30 /3%), at (a) pH 7.4 and (b) pH 3.4.

Effect of Time

Interestingly, CUM is being released more rapidly at pH 7.4 than at pH 3.4, the release half times t_{50} (time required for releasing 50 wt% of drug) for 10, 20, 30, 40, 50 % drug loading are 2.05, 2.08, 3.0, 3.01 and 4.0 h at pH 7.4, and 3.0, 3.05, 6.0, 7.0 and 8.0 h at pH 3.4, respectively are shown in Figure 6. More than 80 wt% CUM is released from composites at pH 7.4 within 8 h, whereas less than 44 wt% of the drug is released at pH 3.4 within 4 h. This suggests that the drugs in the composites can be used to be suitable for the basic environment. Further the electrostatic interaction of composites is more easily broken at pH 7.4 than at pH 3.4, leading to CUM being released more rapidly at pH 7.4 than 3.4.

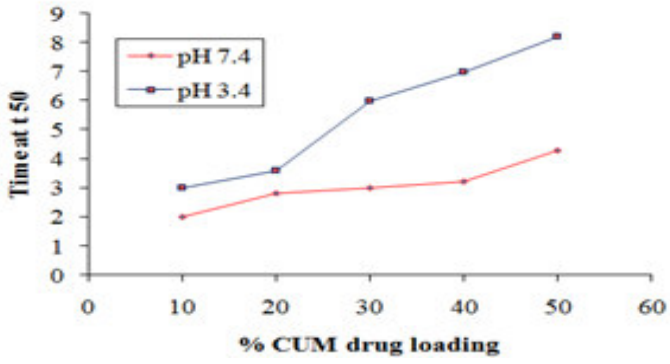

Figure 6
Drug release at time t50 vs. Drug loading in Composite CSA/PVA/Closite 30B (70:30 /3%) at (A) pH 7.4 and (B) pH 3.4

CONCLUSIONS

In this study, a novel formulation of CUM loaded CSA, CSA/PVA, CSA/PVA/Closite30B nanocomposites was successfully developed and characterized. Size and shape of the prepared nanocomposites were examined using SEM. Also, the various suspended groups present in the composites have been determined through the FT-IR studies. The nanocomposites showed pH and time dependent drug release as confirmed by the in vitro drug dissolution profiles. Drug penetration and in vitro tests suggest that further study is required to develop an in vivo drug delivery system. These results suggest that the CUM coated CSA, CSA/PVA and CSA/PVA/Closite30B nanocomposites might be used as great potential carriers for controlled drug delivery system.

CONFLICT OF INTEREST

Conflict of interest declared none.

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