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Controlled Drug Delivery Studies of Biological Macromolecules: Sodium Alginate and Lignosulphonic Acid Films

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ABSTRACT: The ciprofloxacin (CPX)-loaded blends made of sodium alginate and lignosulfonic acid (LS) were prepared by solution casting method in the ratio of 80/20. The blends were crosslinked for different intervals of time to control the drug release. The drug release was investigated for 24 hours in different pH medium (1, 4, 7, and 9). It was confirmed that drug release is controlled by diffusion through the polymer matrix followed by the erosion of the polymer. The pH of the surrounding medium influences the drug solubility, swelling, and degradation rate of the polymer and therefore the overall drug release process. The blend shows minimal drug release at pH 1 and 9, whereas moderate release at pH 4, but rapid release at pH 7. Further FTIR, XRD, and SEM characterization are carried, to confirm the chemical-interaction, crystallization effects, and compatibility between the blend matrixes. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40442.

KEYWORDS: biodegradable; biocompatibility; blends; drug delivery systems

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INTRODUCTION

There are steady efforts from decades to release the drug in a controlled manner from biological macromolecules. In most cases, the purpose is to make a product that maintains a prolonged therapeutic effect at a reduced dosing frequency. It is important to supply drugs to the patient in a controlled manner enabling the optimum concentration of drugs and prolonged effectiveness. Crosslinked blend films have been investigated as controlled drug delivery systems, taking advantage of their function to release drugs.[1–3] The drugs confined in a polymer network are released in a controlled rate by the swelling behavior of blend film, the pore size of a polymer network, the affinity between drugs and polymer and their degradation with various pH medium *in vivo*.[4–11] In the last decade, medical and pharmaceutical industries are showing an increased interest, especially in biological macromolecules and alginates in particular. These materials have found numerous applications in pharmaceutical sciences due to their usefulness in specific applications as it enhances efficient treatment of esophageal reflux, creates multiquality calcium fibers for dermatology and wound healing.[12–17] It is also used for high and low-gel strength dental impression materials. Besides this, it naturally degradable, tablet binder and offers an attractive alternative for sustained-release systems. Sodium alginate (SA) is a freely natural available Biodegradable polymer; it is non-toxic, biocompatible and offers advantages over synthetic polymers as it form hydrogels in aqueous medium.[18–24] All these advantages make alginates very useful material for biomedical applications. It was reported that, the drugs can be incorporated either as dissolved or dispersed phase into the polymeric matrix, which degrades in contact with the biological fluids, which allows a progressive release of the drug content.[25–27] But water solubility and mechanical weakness of SA membrane has been a drawback in its possible use for drug delivery applications. To improve the mechanical stability of membrane, SA needs to be blended with other polymer such as lignosulfonic acid (LS). LS is a natural biodegradable polymer, a plant byproduct formed from sulfite cooking of wood and is large tonnage wastes from pulp and paper industry which is well known, as super plasticizer.

In this study, we prepared SA/LS blend films. To investigate these films in several controlled release applications, it was necessary to have an overall understanding of their properties. We used ciprofloxacin (CPX) as a model drug, to study the influence of drug release from SA/LS films as function of pH of the release medium and the crosslinking time with calcium chloride solution etc. We wish this film can lead to a successful application for localized drug delivery *in vivo* or *in vitro* environment. Further, the drug loaded blends are subjected for FTIR characterization, XRD, and SEM to test the compatibility between the polymer matrixes and drug.

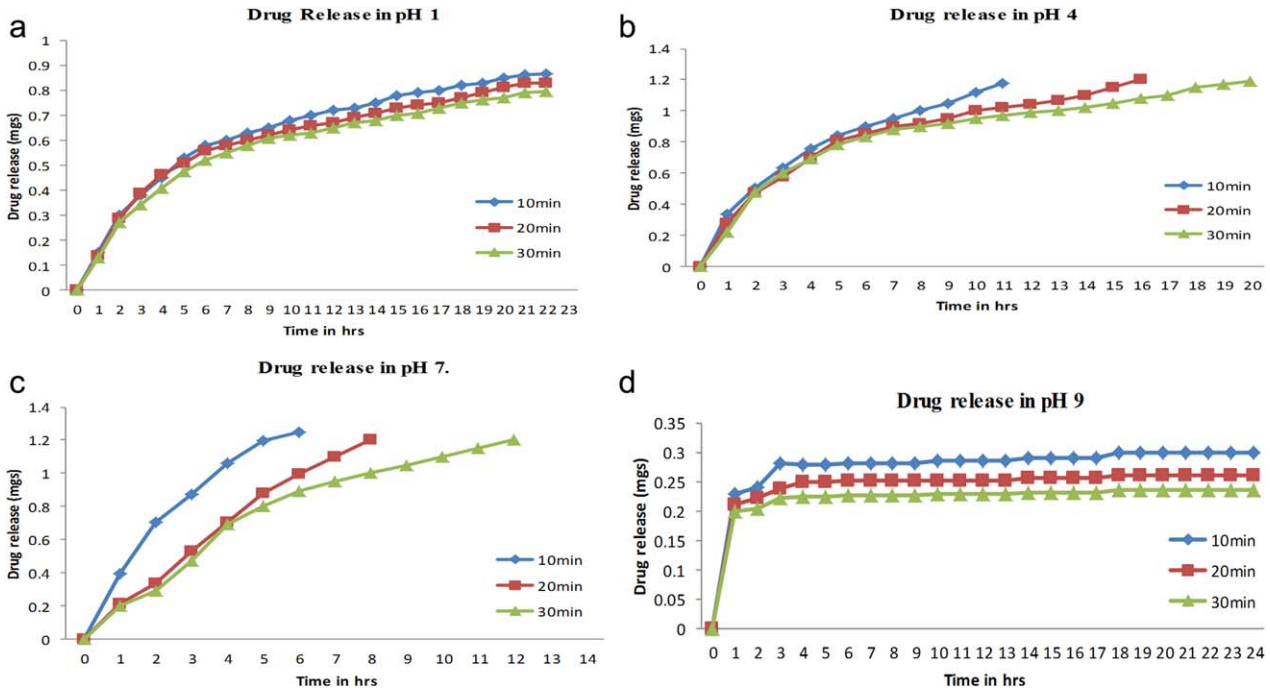

Figure 1. Controlled release of drug from SA/LS (80/20) blends crosslinked for different intervals of time (10, 20, and 30 min) in the medium of a) pH 1 b) pH 4 c) pH 7 & d) pH 9. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

EXPERIMENTAL

Chemicals

SA (MW = 300,000 gm/mol) and LS (MW = 50,000 gm/mol) were used as received from Sigma Aldrich, Bangalore, India. Calcium chloride, 0.1*M* HCl as pH 1, buffer capsules of pH 4, pH 7, and pH 9 were used as received from SD Fine Chem. Ciprofloxacin (CPX) a biochemical reagent (used as model drug) was received as gift sample from Dr.Reddy's Laboratory, Hyderabad.

Preparation of Drug Loaded Blend Films

The aqueous solution of SA (preliminary swollen in distilled water for 24 h) and LS solutions with a concentration of 2 wt % was prepared in distilled water in composition 80/20 by weight. To this composition, 50 mg of CPX (dissolved in 50 mL of distilled water) was added and stirred for 30 min with the help of magnetic stirrer, to make solution completely homogeneous. The SA/LS drug loaded films were produced by solution casting technique on glass substrates, dried at room temperature for 72 h, and then dried at 60°C to constant weight in a vacuum oven. The dried films of thickness 0.2 mm were obtained and the blends were cut in to circular shape of diameter 1.9 cm as reported.[28]

Curing Drug Loaded SA/LS Blends Using Calcium Chloride as Crosslinking Agent

The drug loaded blends were crosslinked by dipping in 2% $CaCl_2$ solution for various intervals of time (10, 20, and 30 min), then allowed to dry at 30°C in a dust free chamber till they attained constant weight. The blends are dried at moderate temperature; otherwise, it may result in surface cracking, which can facilitate the surface erosion upon rehydration. This will ultimately affect the swelling/degradation behavior. Experimental conditions were maintained uniform throughout the investigations.

CHARACTERIZATION

Release Studies

The drug loaded blend film was placed in a beaker containing 50 mL buffer solution at room temperature. The solutions were withdrawn in to a 5 mL cuvette and the amount of CPX drug released from the drug loaded films were evaluated at an interval of every 1 h by Shimadzu scanning UV spectrophotometer (UV-3101 PC) at 320 nm. Then, an equal volume of the same dissolution medium was added back to maintain a constant volume. The medium conditions for the controlled release studies were four typical solutions: pH 1 (0.1*N* HCl solution, acts as simulated gastric fluid), pH 4 (HAc–NaAc buffered solution), pH 7, and 9 (NaH_2PO_4–Na_2HPO_4 buffered solution) solutions (acts as simulated intestinal fluid). All the experiments were done in triplicates.

Fourier Transform Infrared Spectroscopy (FTIR) Studies

FTIR spectra of the blends were measured on a BRUKER Optik GmbH, Model No. TENSOR 27, Software–OPUS version 6.5. The samples were prepared by making KBr pellets containing 3 wt % of materials. Samples were scanned for characteristic functional group absorption in the range 4000–400 cm^{-1}. The instrument employed a pyroelectric detector, which scanned the samples in the form of KBr pellets or as a smear on NaCl plate. Each interferogram was generated by signal averaging 32 scans at a resolution of 4 cm^{-1} and the spectra were obtained as percentage transmittance versus per centimeter.

X-ray Diffraction Studies

A Brucker D8 advanced powder X-ray diffractometer was used to study the solid state morphology of SA, LS, and SA/LS

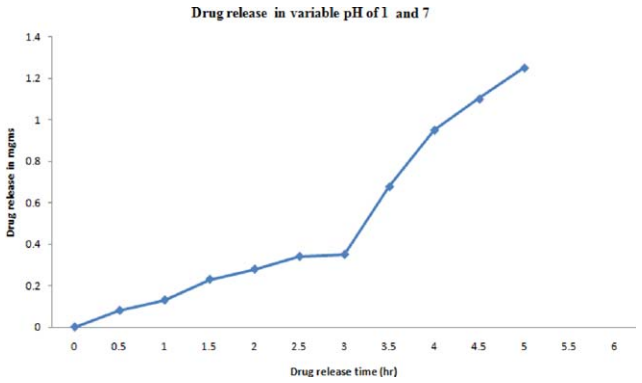

Figure 2. Controlled drug release in a variable pH medium of 1 and 7 of 30 min crosslinked SA/LS blend. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Blends in powdered form. X-rays of 1.548 A° wavelengths were generated by a Cu Kα source. The angle of diffraction was varied from 5° to 60° to identify the change in the crystal structure and intermolecular distances between the intersegmental chains in blends.

Scanning Electron Microscope (SEM)

Scanning electron images of all films were obtained using a VEGA3 TESCAN SEM. Samples were fractured after immersing in liquid nitrogen for few minutes. The fractured edge was then imaged using SEM after coating in gold for few minutes. The surface morphology of each film was examined for signs of separation of the polymer blends and drug into separate domains.

RESULTS AND DISCUSSIONS

Control Release Studies of CPX from SA/LS Blends

Controlled Drug Release Studies in pH 1. The drug loaded crosslinked SA/LS (80/20) blends are used in order to investigate the control release of drug in an aqueous medium of pH 1. It was observed from Figure 1(a), that the release of drug is very minimal in 10 min crosslinked blend, that is, about 0.5 mg

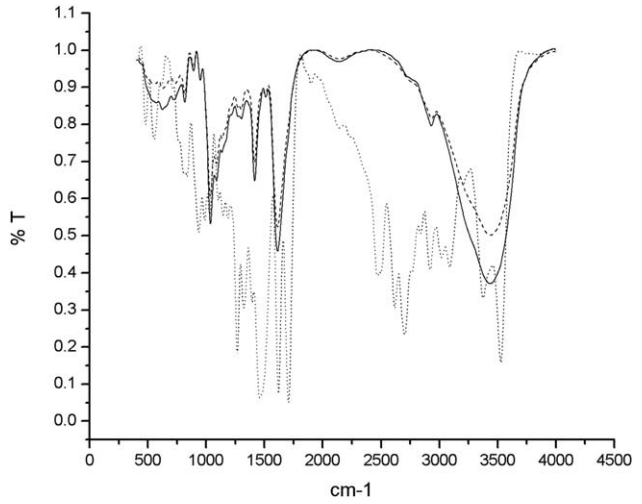

Figure 3. FTIR spectra of (a) SA/LS : 80/20 (—); (b) CPX loaded (SA/LS) blend (-----); (c) pure CPX (. . .).

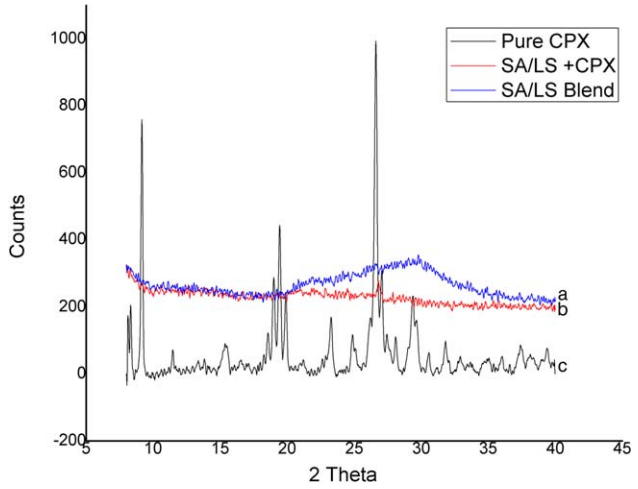

Figure 4. XRD spectra of (a) SA/LS (80/20); (b) CPX loaded SA/LS blend; (c) pure CPX. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in 6 h (out of the total 1.2 mg drug loaded in blend). The drug release gradually slows down and releases only 0.8 mg (about 66% of drug) in 24 h. This can be explained that, in acidic pH medium, the protonation of carboxylate groups attached at polyguluronate blocks of alginate results in decrease in swelling, thereby affecting the drug release.[29] Similar trends were observed in 20 and 30 min crosslinked blends, releasing about 55 and 60% of the drug in 24 h.

Controlled Drug Delivery in pH 4. The drug release is moderate in an aqueous medium of pH 4 as depicted in Figure 1(b). The 10 min crosslinked blend releases about 0.8 mg of drug in 6 h and complete release in 11 h. Whereas 20 and 30 min crosslinked blend releases complete drug in 16 and 20 h.

Controlled Drug Delivery in pH 7. The drug release was rapid in short period of time in an aqueous medium of pH 7, which is assumed to be simulated intestinal fluid. From Figure 1(c), it was observed that, the drug release is fast in 10 min crosslinked blend and releases complete drug in 6 h. Whereas 20 and 30 min crosslinked blend, drug release is moderate with time and total release takes place in 8 and 12 h. The high amount of drug release is due to the ion-exchange between Ca^{+2} ions of blend and Na^{+} ions of phosphate buffer (pH 7) makes the structure loose, thus enhancing the uptake of water resulting in maximum swelling of low molecular weight segments of SA and linear chains of LS. This swelled film was unable to retain the hydrated structure and hence the blend begins to disintegrate, thereby release the drug rapidly.

Controlled Drug Delivery in pH 9. It has been observed from Figure 1(d) that the drug release is not favorable in pH 9. Because, pH 9 medium is highly alkaline, blend cannot swell much in higher pH medium. Since the drug release is swelling dependant resulting in to a very low release of drug even in 24 h.

Control Release Studies in Variable pH Medium of 1 and 7. To test the suitability of the blends for gastrointestinal drug delivery, the drug release behavior of SA/LS blend in ratio of 80/20,

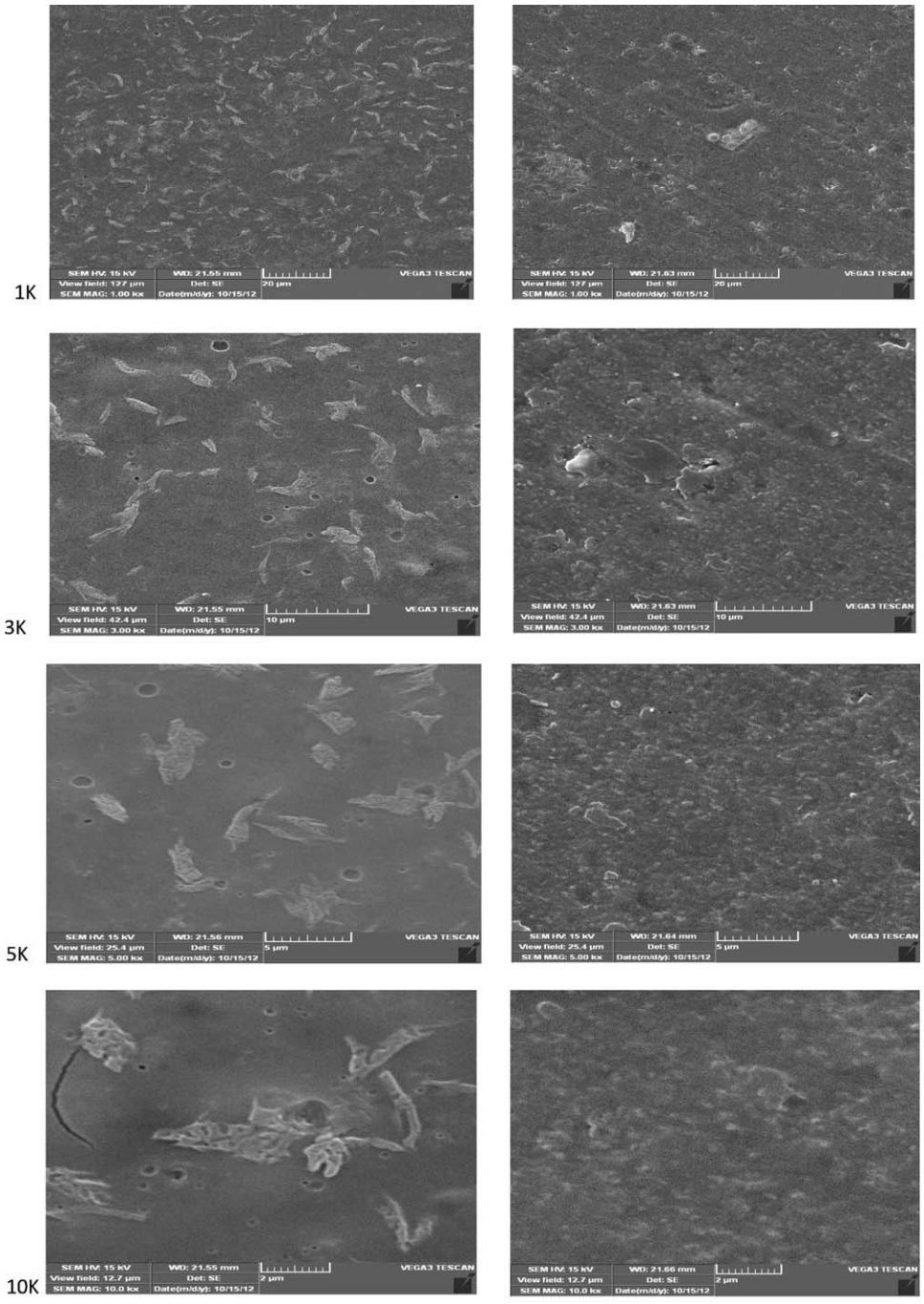

Figure 5. SEM images of (a) blend SA/LS : 80/20; (b) CPX loaded blend (SA/LS : 80/20).

crosslinked for 30 min using calcium chloride solution as crosslinking agent was studied. The SA/LS (80/20) blend loaded with drug (CPX) was subjected for control release in variable pH medium of 1 and 7. From Figure 2, it is found that drug release was very low in pH 1 medium in 3 h, because alginates do not swell significantly at low pH,[30] which in turn affecting drug release. It is well known, when drug is consumed orally, goes to the stomach, resides there for some time, then passes on to small intestine, finally passes to colon. In this way, the polymer loaded with drug has to get exposed to a medium in the pH range 1–2 (gastric fluid) to pH range 7–8 (intestinal fluid). Therefore, to mimic the transition of proposed drug loaded blend from mouth to colon, they should be exposed to the media of varying pH medium. It was reported by a several pharmaceutical researchers regarding the transit time of a dosage form along GI tract,[31] we exposed the drug loaded blend to the medium of pH 1 for a period of 3 h and then same blend is transferred to the phosphate buffer of pH 7. The drug release was nearly 0.3 mg (out of 1.2 mg) in the pH 1 medium in first 3 h and when it is transferred into medium of pH 7, a drastic

release of drug was observed. The SA/LS (80/20) blend releases complete drug in next 2 h, and then starts disintegrating. This can be explained as follows; when the blend is placed in the medium of pH 1, they exhibit almost minimum swelling. This is due to the fact that their stay in the medium of pH 1 results in acid–catalyzed hydrolysis of alginates into low molecular alginic acid.[32] Moreover, in acidic pH medium, the protonation of carboxylate groups, attached at polyguluronate blocks of alginate results in decrease in ionic crosslinking of the blend, thereby affecting the drug release. Later, when this blend is transferred into the phosphate buffer medium of pH 7, the blend begins to take up water. The ion-exchange between Ca^{+2} ions of blend and Na^{+} ions of phosphate buffer also makes the structure loose as explained earlier. Finally, when the blend attains maximum swelling low molecular weight segments of alginic acid and linear chains of LS become unable to retain the hydrated structure and hence the blend begins to disintegrate, thereby releasing the complete drug.

FTIR Spectroscopic Analysis

In the FTIR spectra "c" of CPX as shown in Figure 3 shows characteristic peak between 3510 and 3450 cm^{-1} was due to OH stretching vibration and band at 3000–2950 cm^{-1} represented aromatic C—H stretching. Another bands at 1750–1700 cm^{-1} represented carbonyl C=O stretching, while the peak at 1650–1600 cm^{-1} is seems to, the framework of quinolones. The bands at the 1450–1400 cm^{-1} represented —C—O and the ones at 1300–1250 cm^{-1} suggested bending vibration of O—H group, which indicated the presence of carboxylic acid. It was reported that, a strong absorption peak between 1050 and 1000 cm^{-1} was assigned to C—F group.[33] It was observed from Figure 3 that spectra "a" of the SA/LS blend band shows characteristic peaks at 1600 and 3500 cm^{-1} due to the aromatic structure and hydroxyl groups due to LS present in the blend. However, the characteristic bands of SA appeared at 1611 and 3500 cm^{-1} were observed due to presence of —C=O and —OH groups of SA in the blend. Whereas the FTIR spectra "b" of drug loaded blend film, we can see that the characteristic absorption bands of SA/LS blends are not shifted. At the same time, there were no new characteristic absorption bands of drug loaded films, that is, the conclusion has been drawn that, there were no chemical reaction taking place between the drug and the matrix of the blend.

X-ray Diffraction Studies

It was observed from X-ray diffraction studies shown in Figure 4 spectra "a" reveals that the diffraction peaks of SA/LS blend intensified 2θ value around 29° and CPX shows sharp peaks at 8°, 18°, and 27°, indicating highly crystalline substance. In spectra "b," it may be seen that CPX changed the diffraction patterns of the blank matrix SA/LS. Comparing the X-ray diffraction patterns of blend and drug loaded film (i.e., spectra "a" and "b") it is possible to verify that after the addition of the drug to the blend, the diffraction intensities of blend has disappeared the peak at 29°. These results have indicated that the addition of CPX destroyed the ordered packing of the molecules of film to form the regular crystallites. The mixing of blend and drug reinforces the existence of good compatibility, due to both kind of strong interactions like hydrogen bonds and ionic interactions.[34]

Morphological Studies by SEM

The cross-sectional images of SA/LS blend and drug loaded samples were examined by scanning electron microscopy at different resolution power as depicted in Figure 5. For SA/LS(80/20) blend a multiphase morphology was observed, while samples with drug (SA/LS(80/20)+ CPX) showed a more homogeneous structure and uniform phase indicated good compatibility between the matrix and the CPX.

CONCLUSIONS

CPX loaded biodegradable blends of SA and LS (80/20) are prepared by solution casting method. These blends were crosslinked with calcium chloride solution at different intervals of time, that is, 10, 20, and 30 min, to investigate the control drug release. The drug release was studied in different pH medium of 1, 4, 7, and 9. It was concluded that the release is very low in acidic pH of 1 and moderate in pH 4 and very high release in neutral medium of pH 7. Whereas very low drug release was observed in pH 9, for the reason that swelling of SA is very poor in alkaline medium. The FTIR spectrum of blends and drug loaded blends confirms that, there was no chemical interaction between blend and drug, thereby retaining the drugs originality. XRD images further justifies that inclusion of CPX strengthens the compatibility between the blend and drug. SEM images of SA/LS(80/20) blend shows multiphase morphology, leading to the characteristics of immiscible blends. However, single phase morphology was observed in CPX loaded SA/LS (80/20) blend, providing the good compatibility between drug and blend. Based on the above observations, the SA/LS blends are appear to be suitable for controlled drug delivery for gastrointestinal applications.

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