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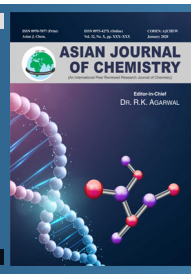

Cassia alata Seed Extract Loaded Chitosan: Synthesis and Release Study

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In present study, a herbal extract, *Cassia alata* seed extract has been loaded into chitosan matrix as drug delivery systems. The major components of *Cassia alata* seed extract are flavonoids and anthraquinone. *Cassia alata* seed extract loaded chitosan has been characterized by FTIR, SEM, XRD, thermal and NMR analysis. The characterization has shown successful loading of *Cassia alata* seed extract into chitosan. Further, swelling properties and drug release profiles of *Cassia alata* seed extract loaded chitosan has been carried out at two different pH 2 and 7.4. It is observed that the swelling behaviour and drug release profile was much better at pH 2 than pH 7.4. Thus, the loaded chitosan can be used as a biomedicine.

Keywords: Chitosan, *Cassia alata*, Flavonoids, Swelling study, Drug release study.

INTRODUCTION

Deacetylation of Chitin, a naturally occurring substance mainly seen in aquatic marine crustaceans, produces chitosan, a copolymer formed between glucosamine and N-acetyl glucosamine joined by a β-1,4 glycosidic linkage [1]. Due to its abundance, biodegradability, better biocompatibility it is used in biomedical applications, food processing, pharmaceutical fields, *etc*. The presence of -OH and -NH_2 groups in chitosan increases its applicability like surface modification as well as tissue engineering [2,3]. Chitosan shows microbicidal and non-toxic property which contributes to its use in medicine, food packaging, preservation. *etc*. [4]. The chitosan hydrogels are also used in drug delivery, wound dressing, biosensors, *etc*. [5]. Chitosan hydrogels can be prepared by adjusting the pH and by freezing the solution. The presence of hydrophilic groups like hydroxyl, carboxyl and amide groups in chitosan helps it in swelling in water as well as biological fluids [6,7]. All these properties enable it to have wide applications in biomedical and pharmaceutical areas. In the present study, by making use of above properties of chitosan, it has been used in the drug delivery.

In the present study, a herbal drug derived from *Cassia alata*, also known as candle bush, which belongs to the Fabaceae family, has been chosen. It is 1-4 m tall shrub usually seen in tropical areas [8]. The different parts of this plant have been used in traditional medicine because of the presence of anthraquinones, polyphenols, tannins, saponins, alkaloids, steroids, flavonoids, *etc*. In present study, *Cassia alata* seed extract has been used as the drug. The seed extract of *Cassia alata* contains flavonoids [9]. The presence of these flavonoids enhances its medicinal value. *Cassia* seeds have many medicinal benefits like it can be used for correcting bowel movements, provides relief from cold and flu and also restores the functions of the liver. Other benefits include laxative property, expectorant, anticancer, antioxidant, anti-inflammatory and anthelmintic [10-14]. However, in general the herbal drugs have astringent taste, unpleasant flavour, brownish color, not very stable and are sensitive to heat and light [15]. Thus, to overcome such drawbacks, *Cassia alata* seed extract is encapsulated in chitosan and the drug delivery studies are carried out.

EXPERIMENTAL

Chitosan, (deacetylation degree > 85 %) was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India. Sodium hydroxide, isopropyl alcohol, ethyl alcohol, chloroform, KOH, *etc*. are obtained from Merck Chem. Co. and used as received. pH meter (PHS-25) is used to measure the pH of the solution. Millipore water has been used throughout the experiment.

Plant: *Cassia alata* seeds were collected from different parts of India like rural areas of Chhattisgarh (India) and Kerala (India) during early winter (October-November, 2018). The collected seeds were identified by comparison with the herbariums present in Botanical Department of Saurashtra University, Rajkot India. These seeds were washed, air dried and also dried in a hot air oven at 40 ºC for 4-5 h prior to use. The dried seeds were powdered in an electronic mill and passed through a sieve (20 mesh).

Preparation of *Cassia alata* seed extract: The most convenient and effective method for extraction of *Cassia alata* seed employed here was maceration. Powdered *Cassia alata* seeds are macerated with 80 % ethanol. The extraction was repeated until the extract is free from anthraquinone and flavonoids. The maceration extracts were combined, filtered using muslin cloth and evaporated to dryness by using rotary evaporator to yield a macerated crude extract powder.

Identification of major constituents in the seed extract: The extract on treatment with few drops of NaOH solution gives an intense yellow colour which turns colourless on further addition of dil. HCl indicates the presence of flavonoids. Further, the extract is subjected to Borntrager's reaction to detect the presence of anthraquinone. For that, first HCl (2 M) is added to the extract and then heated on a hot water bath for 10 min. This is then cooled, filtered and then chloroform was added to the extract filtrate. Now added 10 % KOH to the chloroform layer and shake. Appearance of pink-red layer shows the presence of anthraquinone.

Encapsulation of *Cassia alata* seed extract with chitosan: Encapsulation of *Cassia alata* seed extract with chitosan has been done by using the modified reported procedure [16]. Blank chitosan microspheres were prepared first. Dissolved chitosan in 2 % acetic acid solution by stirring it for 5 h. The resultant solution is dropped into a mixture containing H_2O:MeOH: NaOH (4:5:1) to form chitosan microspheres and then let it stand for 1 h. Thus obtained chitosan microspheres were washed with distilled water until the pH was less than 7 and are dried 37 ºC [17].

Further, to prepare *Cassia alata* seed extract encapsulated with chitosan, 0.05 g of chitosan was dissolved in 20 mL acetic acid and stirred (100 rpm) overnight at 37 ºC. *Cassia alata* seed extract was weighed and dissolved in millipore water. This drug solution was dripped into chitosan solution. The loading efficiency (LE %) was calculated using following equation:

$$\text{LE (\%)} = \frac{\text{Amount of drug in chitosan}}{\text{Total weight}} \times 100$$

Swelling studies: Water absorption of chitosan-drug hydrogels was measured at different pH for different time intervals from 1 day to 8 days [8]. A known amount of sample (0.5 g) was immersed in 10 mL of different buffer solutions (pH 2.0 and 7.4). The weight of swollen sample was determined at different intervals by removing excess of solvent by gently absorbing by using filter paper. Once again it was immersed in the solvent to ensure equilibrium. The percentage of swelling for each sample at different time interval was calculated using the following formula:

$$\text{Swelling (\%)} = \frac{W_t - W_o}{W_o} \times 100$$

where W_t = weight of the samples at time t after immersion in solution; W_o = weight of dried samples.

Release study of *Cassia alata* seed extract: Drug release studies are carried out at two different pH 2 and 7.4 (Table-1) for 24 h. *Cassia alata* seed extract loaded chitosan is weighed (0.05 g) and was kept in buffer solution (either pH 2.0 and 7.4) with constant stirring (100 rpm) at 37 ºC for 24 h. To find the release of the drug, 1 mL of solution was withdrawn at different intervals and fresh buffer (1 mL) was added to replace it [14,15]. The amount of drug release in particular time was then measured by using UV-visible spectrophotometer at 285 nm.

TABLE-1
COMPOSITION OF *Cassia alata* SEED EXTRACT LOADED CHITOSAN

Sample code	Chitosan (g)	Acetic acid (2 %)	Drug (g)	LE (%)	Yield (%)
S1	1	40 mL	0.75	54.4	79.4
S2	1	40 mL	0.50	50.6	72.3
S3	1	40 mL	0.25	43.3	66.6
S4	1	40 mL	0.10	40.2	61.1

A Bruker Alpha Model FTIR Spectrophotometer made in Germany is used to record FTIR data by using KBr pellet method in the range of 4000-400 cm^{-1}. The ^1H NMR spectra of raw chitosan and *Cassia alata* seed extract loaded chitosan were determined using a Bruker AVANCE III 500, 11.75 Tesla, spectrometer operating at 500.13 MHz for ^1H. The chitosan and seed extract loaded chitosan were dissolved in CD_3COOD 1 % (v/v) and DMSO 1 % (v/v) respectively. Thermogravimetric analysis was used to study the thermal behaviour of the samples. The instrument was Shimadzu TGA 50. About 8 mg of sample was used in each measurement. The study was carried out under nitrogen atmosphere with a gas flow of 50 mL min^{-1} by heating the material from 40 ºC to 750 ºC at a heating rate of 20°C min^{-1}. The X-ray diffraction pattern of samples was obtained by using an X-Ray Diffractometer (General Instrument Co. Ltd., Beijing XD-3) at 40 kV with the scan range from (2θ) of 5º to 60º and a scan rate of 2º/min. The XRD method helps to study the crystallographic nature of a material. Scanning electron microscopy (SEM) was performed with a Cold Field Emission S-4800 Scanning Electron Microscope (Hitachi, Tokyo, Japan) operated under an acceleration voltage of 5 kV. The samples were mounted on a double sided carbon tape and then coated with a thin layer of platinum.

RESULTS AND DISCUSSION

FTIR analysis: FTIR spectra of chitosan, *Cassia alata* seed extract and *Cassia alata* seed extract loaded chitosan are shown in Fig. 1. FTIR spectrum of chitosan (Fig. 1a) showed all the characteristic absorption bands of chitosan. The absorption at 3429 cm^{-1} is due to -NH_2 group stretching vibration intermolecularly H-bonded with -OH group; the peak at 2924 cm^{-1} is due to aliphatic C-H stretching vibration, while the peak at 1653 cm^{-1} is due to N-H bending at primary amine and the band at 1373 cm^{-1} can be attributed to carboxylic group and 1073 cm^{-1} shows the skeletal vibration of C-O-C stretching. FTIR spectrum of *Cassia alata* seed extract (Fig 1b) shows a

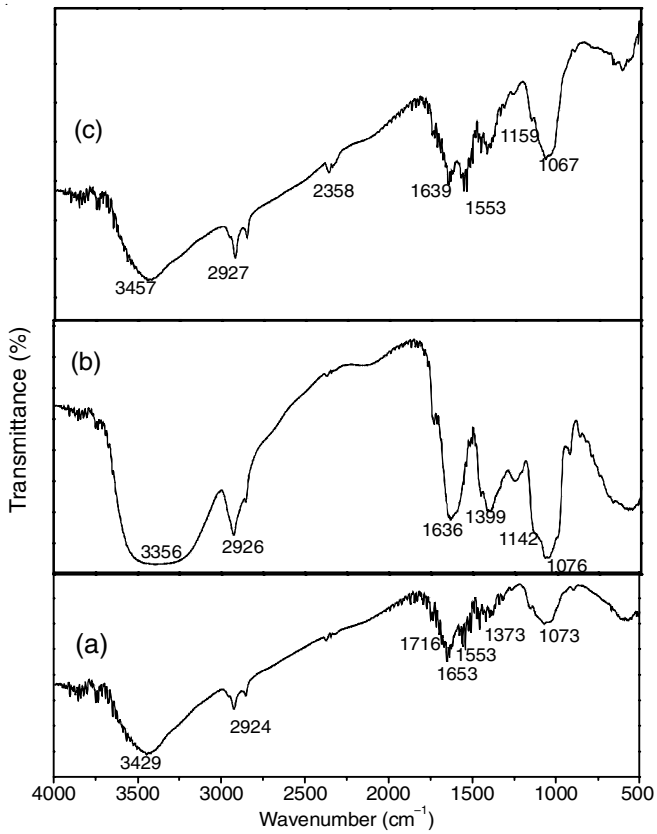

Fig. 1. FTIR spectrum of (a) chitosan and (b) *Cassia alata* seed extract (c) loaded chitosan

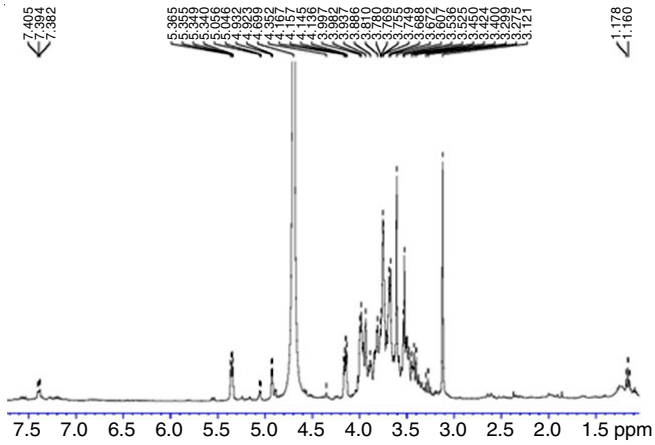

Fig. 2. NMR of *Cassia alata* seed extract loaded chitosan

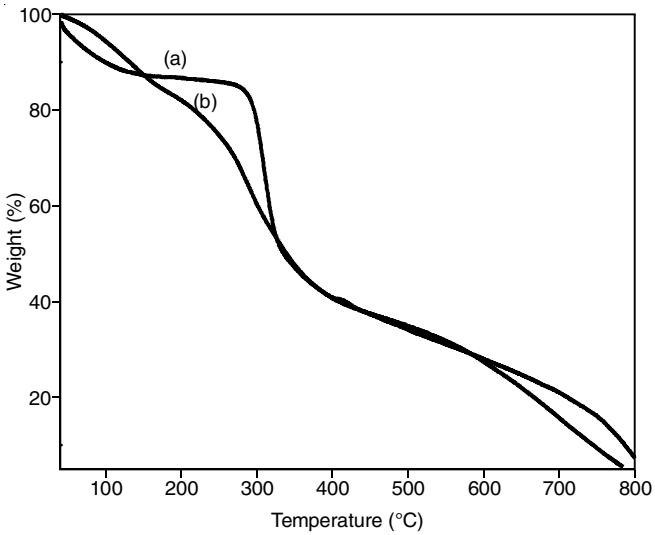

Fig. 3. TG curves of (a) chitosan and (b) *Cassia alata* seed extract loaded chitosan

broad peak at 3356 cm^{-1} due to OH groups; at 2926 cm^{-1} due to C-H stretching, moreover the peak at 1636 cm^{-1} due to ketonic groups of anthraquinones and flavonoids present in the seed extract. Further, FTIR spectrum of *Cassia alata* seed extract loaded chitosan shows most of the peaks present in chitosan and the seed extract and thus confirms the loading of seed extract on chitosan matrix [17-22]. All these informations are pointing towards the successful loading of seed extract on to chitosan.

^{1}H NMR analysis: ^{1}H NMR spectrum of chitosan shows resonance of acetyl-protons (δ 2 ppm); peak at δ 3.6-3.9 ppm and 3.3 ppm are found to be the basic peaks of chitosan resonance. NMR spectrum of *Cassia alata* seed extract loaded chitosan (Fig. 2) shows the resonance peaks due to chitosan as well as flavonoids and anthraquinone. NMR peaks of flavonoids such as 7-hydroxy flavone and 5,7-dihydroxy- 3′, 4′,5′-trimethoxy-flavone are generally seen at around 5 ppm [21]. The peaks at around 5.03-5.04 ppm attributes to the presence of -OH group of 7-hydroxyflavone as well as 5,7-dihydroxy-3′,4′,5′-trimethoxy-flavones. The peaks at 7.3-7.4 ppm is due to the protons on C-2 and C-6 protons of 7-hydroxyflavone. Also the peaks at around 3.74 ppm shows the presence methoxy groups of 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone. Thus, NMR clearly explains the encapsulation of flavonoids from the seed extract of *Cassia alata* on chitosan. A clear peak at 4.7 ppm is due to the solvent DMSO.

Thermogravimetric analysis: From the thermogram of chitosan (Fig. 3a), it is observed that it has three stages of weight loss [23]. The thermal event starts at about 50 ºC and extends to 290 ºC for chitosan. The first stage of weight loss is due to loss of adsorbed water. The second stage mass loss up to 290-410 ºC was due to the decomposition and depolymerization of acetylated and deacetylated chitosan unit. The third stage mass loss at 410-650 ºC was due to the decomposition of the remaining glucosamine. In contrast, the thermogram of *Cassia alata* seed extract loaded chitosan (Fig. 3b) shows a steady loss in mass as temperature increases especially from 200 ºC to 300 ºC. This shows the loss of thermal stability for the drug loaded chitosan than the pure chitosan.

X-Ray diffraction analysis: XRD of chitosan (Fig. 4a) shows peaks at 10.78º and 19.8º and also shows the semi-crystalline nature of chitosan [24,25]. However, XRD of *Cassia alata* seed extract loaded chitosan (Fig. 4 b) shows a broad peak at around 20º and also shows more amorphous nature than chitosan. Incorporation of seed extract decreased the crystallinity of chitosan and increased porosity which in turn increased the absorption/adsorption of seed extract in the chitosan matrix.

SEM analysis: Comparison of morphological behaviour of both chitosan and seed extract loaded chitosan (Fig. 5) shows the adsorption of seed extract powdered particles on the surface of chitosan matrix.

Swelling studies: The nature of polymer, polymer solvent compatibility and degree of cross-linking are the main factors on which swelling depends [23]. But in case of ionic networks, swelling behaviour depends upon mass transfer limitations,

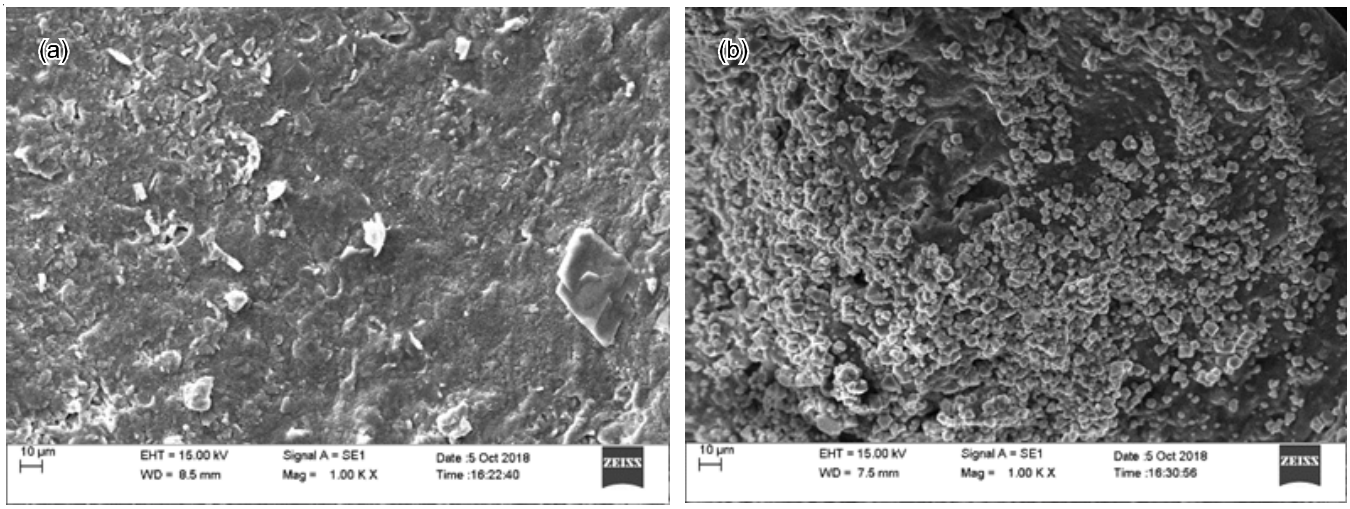

Fig. 5. SEM images of (a) chitosan and (b) *Cassia alata* seed extract loaded chitosan

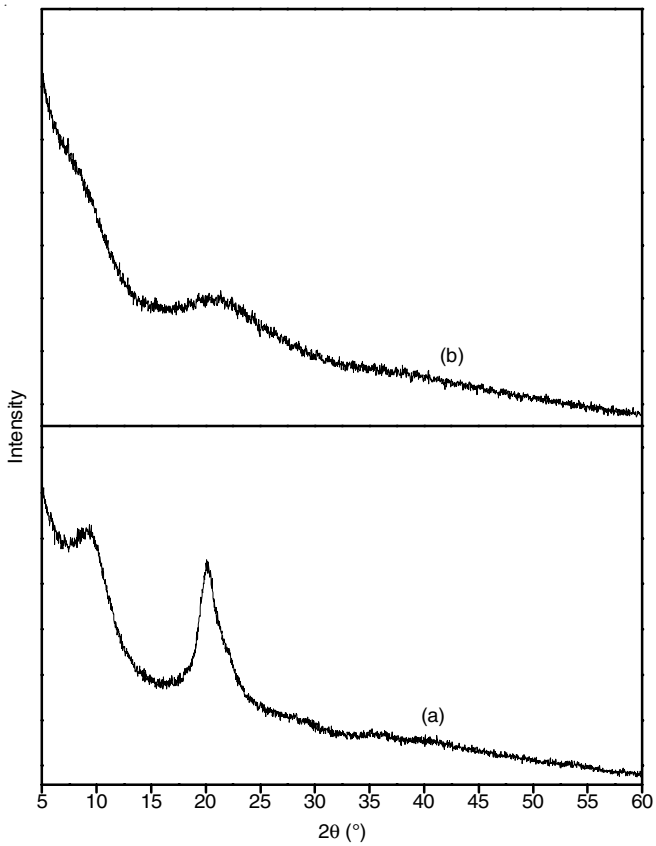

Fig. 4. XRD pattern of chitosan (a) and *Cassia alata* loaded Chitosan (b)

ion exchange and ionic interaction. Chitosan based hydrogels cross-linked by itself will have a thick scale-mesh like network which only allow for passive diffusion of nutrients. When the hydrogel-seed extract mixture is placed in the solution, the solution enters into the hydrogel and hydrogel-seed extract mixture swells.

The swelling behaviour of hydrogel (Fig. 6) with different drug loadings at 37 ºC at both pH = 2.0 and 7.4, it is observed that percentage of swelling is more in acidic media (pH = 2.0) than in basic media (pH = 7.4). This is because at acidic pH the amount of H^+ ions in the medium is higher. Thus, the protonation of amino groups in the polymer is more prominent in

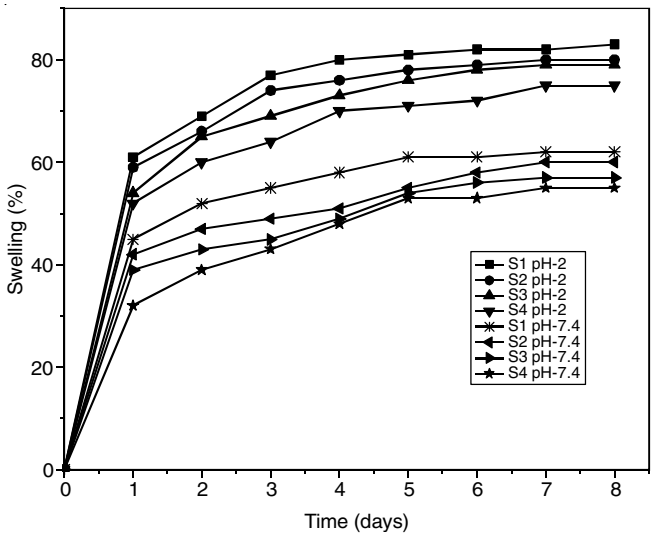

Fig. 6. Swelling behaviour of samples (**S1-S4**) as a function of time in pH 2.0 and pH 7.4 at 37 °C

acidic medium. The porous nature of chitosan is also found to be less in lower pH. In basic media the degree of ionization of charged species decreases which in turn decreases the protonation of amino group, and thus water absorption decreases. *i.e.* the hydrophobicity nature of chitosan dominates at higher pH and this prevents it from swelling in basic media. Similarly, in lower pH the interaction of amino and hydroxyl groups with proton gets reduced due to its unavailability and thus decreases swelling. Further, it is observed that the percentage of swelling increased with the amount of *Cassia alata* seed extract.

Drug release studies: *Cassia alata* seed extract was selected as the herbal drug. This selection is made mainly based on three factors of the drug like ease of availability, herbal nature and non toxicity. The presence of anthraquinones and flavonoid components of *Cassia alata* makes it a better medicine. Moreover, seed extract of *Cassia alata* has several medicinal functions such as antimicrobial, antifungal and are found to be antioxidants due to the presence of flavonoids in it. By varrying the amount of extract to be loaded, drug release studies are carried out (Table-1). The results of release studies are shown in Fig. 7. *Cassia alata* seed extract loaded chitosan (0.05 g) is weighed and was

kept in phosphate buffer saline (both pH = 2.0 and 7.4) under constant stirring (100 rpm) [14]. The amount of drug release in particular time was then measured using UV-visible spectrophotometer at 285 nm. The drug absorption on chitosan hydrogel takes place through the H-bonds formed between the polar group of drug molecule and hydroxyl and amino groups of chitosan [23].

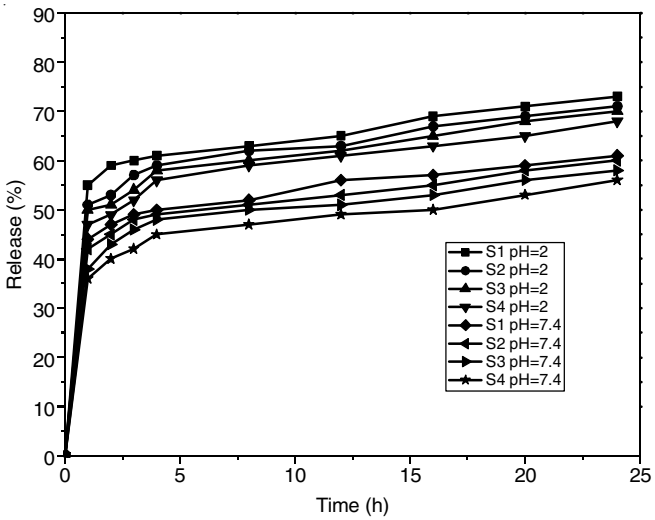

Fig. 7. Release behaviour of *Cassia alata* seed extract loaded chitosan at pH = 2 and pH = 7.4

Quick release of drug is observed in the first 5 h in both pH = 2.0 and 7.4 (*i.e.* S1 > S2 > S3 > S4). The experiments were repeated by keeping chitosan to drug concentration same while changing the pH. It is clearly showing the significance of pH in drug release. Acidic pH = 2.0 shows better release than the alkaline pH = 7.4 and by 24 h almost 73 % release is observed. The sudden release of major portion of drug in the initial stage may due to the release of drug molecules present on the surface of hydrogel. At pH = 2.0, $-NH_2$ group gets protonated and exists as $-NH_3^+$. Thus, there will be repulsion among similar charges. This force helps hydrogel to get expanded and to have more space among polymer chain, which helps it to release the drug molecules [26].

Conclusion

In the present study, a novel method for the loading of *Cassia alata* seed extract into chitosan matrix has been carried out successfully. The incorporation of *Cassia alata* seed extract into chitosan is confirmed by FTIR, SEM and NMR studies. The NMR studies explained incorporation of flavonoid and anthraquinone components of the seed extract into chitosan. The incorporation of *Cassia alata* seed extract into chitosan destroyed the semi-crystalline nature of chitosan which was proved through XRD studies. The drug release studies showed its pH dependency and clearly indicated that in acidic pH, the release of drug as well as the swelling of the sample is found to be high in comparison to alkaline pH.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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