

Preparation and in vitro controlled release behavior of a novel pH-sensitive drug carrier for colon delivery

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Abstract The synthesis of a novel complex system designed for colon-targeting drug delivery was reported. The complex was prepared by dialdehyde konjac glucomannan and adipic dihydrazides to form steady Schiff base, and crosslinking with 5-aminosalicylic acid (5-ASA) through glutaraldehyde as a cross-linking agent. The structure was characterized by Fourier transform infrared (FTIR) spectroscopy, ^{13}C NMR, wide angle X-ray diffraction (WAXRD) and thermogravimetric analysis. In vitro release of 5-ASA from the complex showed that the total released 5-ASA after 24 h in buffer solution at pH 1.2, 6.8, and 7.4 were 4, 59, and 21%, respectively. The release rate of 5-ASA can be controlled by tuning the pH value more effectively. The results indicated that the novel pH-sensitive complex could be potentially useful for colon-targeting drug delivery system.

Keywords Konjac glucomannan · pH-sensitivity · Colon-targeting · Drug delivery

Introduction

Oral colon-targeting drug delivery systems (OCTDDS) have been paid much attention during the past two decades. Drugs in OCTDDS can offer several advantages over the conventional drugs delivery systems (CDDS), including longer retention time in the body [1], lower noxious side effects, enhancing curative effect,

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and avoiding drugs degradation by the stomach and small intestine enzyme to improve the bioavailability of drugs such as protein-, peptide- based drugs, bacterin and chronic enteritis [2]. There are several types of controlled drug delivery systems, such as prodrugs [3], pH-sensitive [4], time-dependent [5], and microflora-activated [6] systems, based on cellulose, impulse stuff-bursa, methylcellulose acetate phthalate, azo-polymer, and polysaccharides. Although prodrugs can provide site-specific drug delivery, they are new chemical entities and their detailed toxicological studies need to be more investigated [7].

Polysaccharides playing an important role in OCTDDs due to their abundant, biocompatibility, biodegradation, nontoxicity and easily modified ability are suggested their applications in OCTDDs [8]. Chitosan [9], cyclodextrins [10], guar gum [11], alginate [12], and cellulose derivatives [13] have been studied for their potential as colon-specific drug carrier systems [8]. Recently, it was reported that KGM could hold potentialities in drug carrier delivery for its nonimmunogenicity and bioadhesive properties [14, 15]. However, all of above polysaccharides are only used as coating materials, by which drug is inevitable dissolved partially in stomach by the high pH value, when passing through stomach, and would reduce curative effect [16].

In this paper, a novel pH-sensitive complex was designed based on dialdehyde konjac glucomannan (DAKGM) prepared by konjac glucomannan using sodium periodate as oxidant. The structure and properties of the resulted products were characterized by FTIR, ^{13}C NMR, thermogravimetric analysis (TGA), wide angle X-ray diffraction (WAXRD), and pH dependent evaluation. The release behavior of the system was investigated by using 5-aminosalicylic acid (5-ASA) as a model drug.

Experimental

Materials

Konjac glucomannan powder (KGM, $M_w = 980,000$, $M_w/M_n = 1.7$, a donation from Multi-Ring Health Products, Ltd, Beihai, China). Adipic dihydrazide (ADH) and 5-ASA were used without any further purification. All other chemicals used in this work were from commercial sources and of analytical grade.

Instruments

FTIR spectra were recorded using a Vector 33 spectrometer (Germany). KGM samples were mixed with KBr and pressed to a plate for measurement. ^{13}C NMR measurement was conducted on Avance AV 400 spectrometer (Switzerland), at room temperature, using CF_3COOD as solvent. TGA analysis was conducted with Netzsch 209F1 instrument (Germany) under a nitrogen atmosphere with the sample weight was about 8–10 mg. The scan of TG was carried out at a heating rate of $20\text{ }^\circ\text{C min}^{-1}$ from 30 to $800\text{ }^\circ\text{C}$. X-ray diffraction of the films was analyzed using D-MAX 2200 VPC (Japan) diffractometer equipped with a scan rate of 4° min^{-1} . The diffraction angle was ranging from $2\theta = 3^\circ$ to $2\theta = 60^\circ$.

Preparation of DAKGM-ADH-ASA complex

Dialdehyde konjac glucomannan (DAKGM) was prepared as described in brief: 2 g (0.012 mol) of KGM was dissolved in 0.018 M aqueous sodium periodate (100 ml). The mixture was reacted for 9 h at room temperature in the dark. The reaction mixture was dialyzed in distilled water for 3 days till the dialysate was free from iodate (checked with silver nitrate). The dried samples were stored in a desiccator over silica gel for further use.

Preparation of DAKGM-ADH: DAKGM and Adipic dihydrazides (ADH) (mole rate 1:1 mol) were mixed with appropriate distilled water. The reactants were stirring for 2 h at 40 °C. The white precipitate was washed with distilled water, and then vacuum evaporation at 50 °C.

Preparation of DAKGM-ADH-ASA: DAKGM-ADH and 5-ASA were dispersed with ethanol in a three-necked round-bottomed flask and heated to 50 °C. Glutaraldehyde (glutaraldehyde:ethanol, 1:2 v/v) was added dropwise to the ethanol solution, while stirring with a magnetic stirrer. After 4 h, the yellow powder was gained and rinsed with distilled water and acetone alternatively. The resulting yellow powder was dispersed in ethanol and deoxidized by sodium borohydride. The crude sample was purified by repeated washing with distilled water until neutral, and then was extracted with acetone for 48 h to remove unreacted components and soluble fragments or byproducts. Finally, the ultimate product (K1) was dried at 60 °C for 24 h and stored in an airtight desiccator for further use.

Preparation of KGM–ASA blend

KGM (0.012 mol) was dissolved in distilled water under stirring for 8 h to form 1% (w/v) homogeneous solution at room temperature. Amount of ASA (0.006 mol) was added to the solution and continuous stirred for 8 h. The resulting mixture was high-speed centrifugal at 10,000 rpm for the removal of the residual water. The sedimentation (K2) was freeze-dried at –60 °C for 24 h.

In vitro drug release study

5-Aminosalicylic acid release from the complexes in a 100-mL flask at different pH (pH = 1.0, 6.8, 7.4, respectively), in vitro was performed in a thermostatic rotary shaker at shaking speed of 100 rpm and temperature at 37 °C. Samples at appropriate intervals, 4 mL were withdrawn and equal volume of same dissolution medium was added to maintain a constant volume. The amount of 5-ASA cumulative released from the matrix was determined with UV–visible spectrophotometer at 299 nm (Hitachi UV-3010, Japan) and calculated the drug release in complex. The percentage of cumulative drug release efficiency is determined from the following equation:

$$\text{Cumulative drug release efficiency (\%)} = \frac{L}{L_0} \times 100$$

where L and L_0 are the content of 5-ASA released within a known content of complexes base on experiment data and calculation of concentration, respectively.

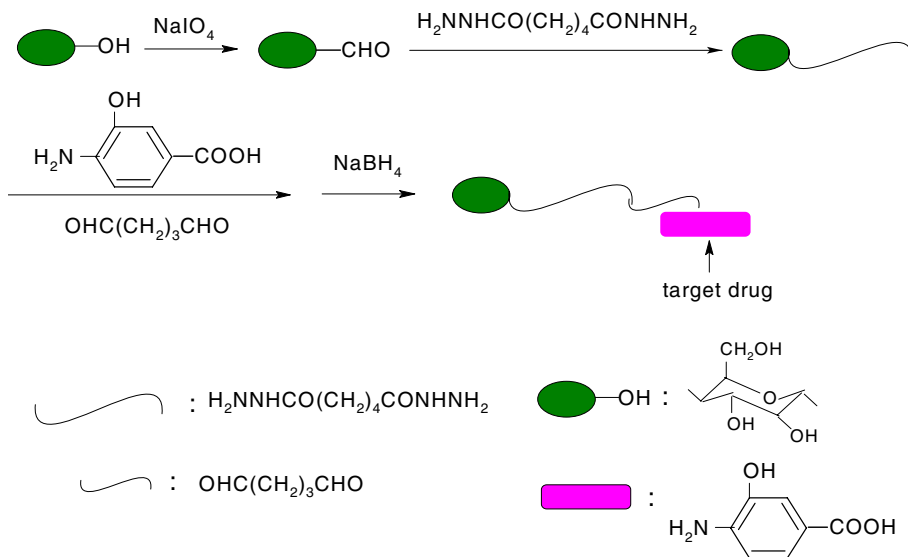
Results and discussion

Synthesis of the DAKGM-ADH-ASA complex

DAKGM-ADH-ASA complex was designed by KGM oxidated to DAKGM via sodium periodate as oxidant, then DAKGM with ADH formed to konjac glucomannan Schiff base, finally crosslinked with 5-ASA by glutaraldehyde crosslinker. The complex formation was illustrated in Scheme 1.

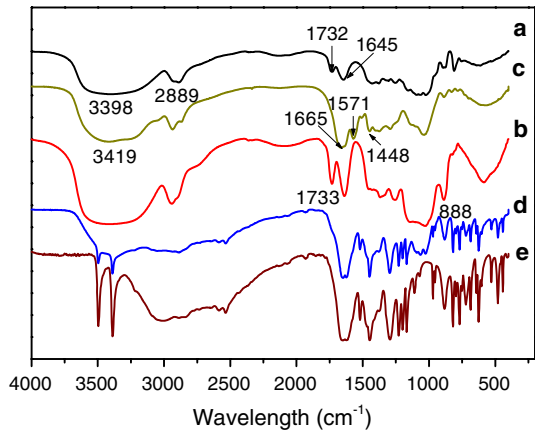
FTIR characterization of KGM samples

Figure 1 show FTIR spectra of KGM (a), DAKGM (b), DAKGM-ADH-ASA complex (c, K1), KGM-ASA blend (d, K2) and pure 5-ASA (e). In the IR spectrum of KGM (curve a), the broad peak at $3,398\text{ cm}^{-1}$ is attributed to the stretching of $-\text{OH}$ groups. The peak at $2,889\text{ cm}^{-1}$ indicated $-\text{C}-\text{H}$ stretching vibration. The absorption band of the carbonyl of acetyl groups is at $1,732\text{ cm}^{-1}$. The intense peak at $1,645\text{ cm}^{-1}$ is due to the in-plane deformation of the water molecule [17]. However, the oxidation leads to the appearance of two characteristic bands of DAKGM (curve b) around $1,733$ and 888 cm^{-1} region. The former is also ascribed to the aldehyde symmetric vibrational band (carbonyl), which changed from a small shoulder in pure KGM to a distinct peak in DAKGM. The latter can be assigned to



Scheme 1 Routine of synthesis of DAKGM-ADH-ASA complex

Fig. 1 FTIR spectra of KGM (a), DAKGM (b), DAKGM-ADH-ASA complex (c, K1), KGM-ASA blend (d, K2) and 5-ASA (e)



the hemiacetal structure between the aldehyde groups and neighboring hydroxyl groups [18]. The chemical interaction is reflected by changes in characteristic spectra peaks as follow in curve c (K1 complex). The peaks at $3,419\text{ cm}^{-1}$ is owed to symmetrical stretching of -N-H groups. This coupling reaction is followed by the disappearance of the aldehyde symmetric vibrational band ($1,733\text{ cm}^{-1}$) and the appearance of a carbonyl band for the hydrazide at $1,665\text{ cm}^{-1}$, which was the absorption of carboxyl stretching of benzene [19]. The peaks near $1,571$, $1,448\text{ cm}^{-1}$ were attributed to the symmetrical stretching of -C=C- and stretching vibrations absorption of -CH groups of benzene, respectively. Compared with the K1 complex, the results from the FTIR spectra of pure 5-ASA (curve e) is similar to that of the K2 blend (d), which was showed that it was physical blend, low compatibility between KGM and 5-ASA.

^{13}C NMR spectrum of DAKGM-ADH-ASA complex

Figure 2 shows the ^{13}C NMR spectrum of DAKGM-ADH-ASA complex in CF_3COOD . The chemical shift of C atom in the copolymer was as follows: (a) 60–80 ppm (CH_2 group of KGM), (b) 160 ppm (NH-CO), (c) 24–38 ppm ($(\text{CH}_2)_4$), (d) 168 ppm (CO-NH), (e) 24–38 ppm ($(\text{CH}_2)_2$), (f) 14 ppm (CH_2), (e) 24–38 ppm ($(\text{CH}_2)_2$), and (g) 184 ppm (COOH). The FTIR and ^{13}C NMR spectra confirmed that DAKGM was successfully crosslinked with ADH and 5-ASA.

Thermogravimetric analysis and X-ray diffraction analysis

Thermogravimetric analysis method is used to thermally characterize the complex in comparison with the pure polysaccharide. The thermograms recorded for KGM, DAKGM, DAKGM-ADH-ASA complex, KGM-ASA blend and 5-ASA are shown in Fig. 3. In the thermogram of KGM (curve a), two decomposition steps could be observed, the first occurs in the range of $50\text{--}120\text{ }^\circ\text{C}$, which is due to the loss of water. The second occurs in the range of $225\text{--}350\text{ }^\circ\text{C}$ showing 65% weight loss could be attributed to the degradation of the saccharide structure of the molecule,

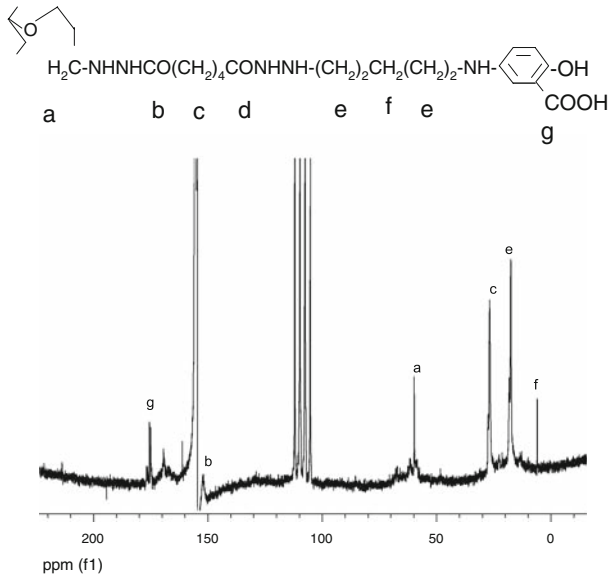
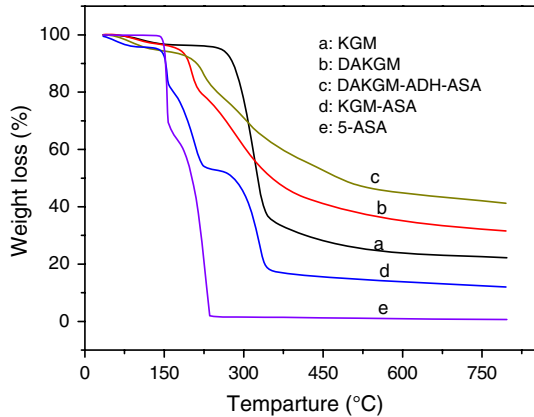


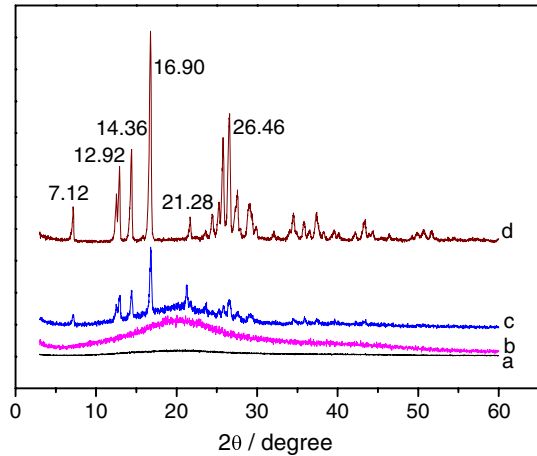
Fig. 2 ^{13}C NMR spectrum of DAKGM-ADH-ASA complex

Fig. 3 TGA curves of samples



including the dehydration of saccharide rings and decomposition of the acetylated units of KGM. In the dialdehyde konjac glucomannan (DAKGM) sample, the TGA curve of DAKGM (curve b) has three distinct stages, and shows three maximum peaks at 91, 204 and 290 °C. Similarly, the slight weight loss of DAKGM in the first stage is due to the loss of water. In the second stage, the weight loss starts at 162 °C and continues to 228 °C with a 21% weight loss due to the degradation of dialdehyde bond of DAKGM. The third stage of weight loss starts at 232 °C and it continues up to 369 °C indicating 52% weight loss because crosslinking of KGM exists in these remnants. In the thermogram of pure 5-ASA (curve e), it is showed that about 35% weight loss takes place at 170 °C for 5-ASA, and it can degrade

Fig. 4 WAXRD patterns of KGM (a), K1complex (b), K2 (c) blend and 5-ASA (d)



completely at 235°C . However, in the DAKGM-ADH-ASA complex sample, a residual weight of 40% was observed at 369°C . The sample cannot degrade completely at 600°C . Therefore, it was indicated that the chemical crosslinking of DAKGM and 5-ASA may provide feasible route for the improvement of the thermal stability. KGM–ASA blend shows a two step mechanism for thermal degradation. The first step weight loss is related to the decomposition of 5-ASA, occurs at about 170°C . The second occurs within the range of $225\text{--}350^\circ\text{C}$ about 65% weight loss due to the degradation of the saccharide structure of the KGM molecule. The results in this work are somewhat similar to the previous reports of pure 5-ASA and pure KGM, respectively. It is because the two components (5-ASA, KGM) in blends have weak intermolecular interaction, namely, degrade independently, and then one has expected residual weight percent based on the additive rule.

To investigate the physical state of the drug within KGM-based complex, WAXRD patterns on the samples are carried out as shown in Fig. 4. Amorphous KGM does not show any diffraction, but exhibits a weak broad peak at 2θ of around 20° . Similarly, K1 complex exhibit a broad peak at 2θ of about 20° . These results showed that K1 complex is not exhibited any characteristic crystalline peak, indicating that they are amorphous. This could be a consequence of strong covalent bond interaction in the K1 complex. However, in the WAXRD of pure 5-ASA, some typical peaks at $2\theta = 7.12, 12.92, 14.36, 16.90, 21.28,$ and 26.46° are observed. Upon mixing with KGM, these peaks intensity of K2 blend become weaker, so it is generalized that the interactions between KGM and 5-ASA make the blend partially miscible and decrease the crystallinity. These results of the WAXRD analysis are in agreement with results of IR and TG analysis.

In vitro release study

In vitro release studies were performed under experimental conditions that simulate the gastrointestinal fluids. Figures 5–7 show the drug-release behavior of 5-ASA in the HCl solution of pH 1.0 (simulated gastric fluid) and phosphate buffer solutions

of pH 6.8 (simulated intestinal fluid) and 7.4 (simulated colon fluid), respectively. From Fig. 5, it can be seen that an initial rapid release of about 93% in the first 1 h of K2 blend. However, the release amount is quite low and remains essentially constant (3%) of the K1 complex. Under both conditions at pH 6.8 and pH 7.4, the amount of 5-ASA release is similar for the K2 blend, where sustained about 55–60% of the drug was released after 24 h (Figs. 6, 7). A large burst of drug release is mainly attributed to the presence of the drug at the surface of the blend.

Figure 8 shows the drug-release behavior of 5-ASA loaded K1 complex in different pH (1.0, 6.8 and 7.4). The maximum content of 5-ASA released from K1 complex reached 17% at 12 h in buffer solution of pH 7.4 and 56% in buffer solution of pH 6.8. On the contrary, at pH 1.0, the release amount is quite low and remains essentially constant of about 4%. It is very interesting to note that there is a steady release with stepwise increasing from 14 to 56 wt% during 12 h when at pH 6.8. This result indicates that drug delivery in our system at a mildly acidic pH value such as pH 6.8 may be long term and continuous, which is helpful for maintaining the concentration of drugs in the certain sites of the body within the optimum range.

Fig. 5 In vitro release profile of 5-ASA from K1 complex and K2 blend in phosphate buffer (pH 1.0) at 37 °C

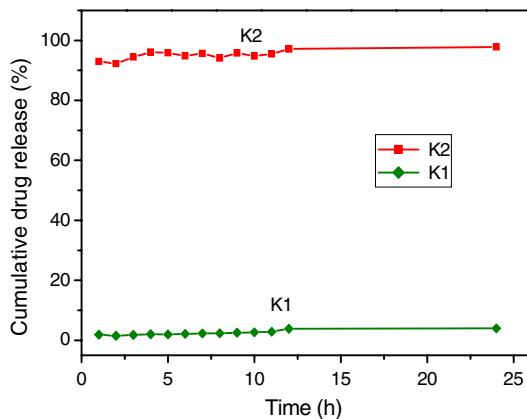


Fig. 6 In vitro release profile of 5-ASA from K1 complex and K2 blend in phosphate buffer (pH 6.8) at 37 °C

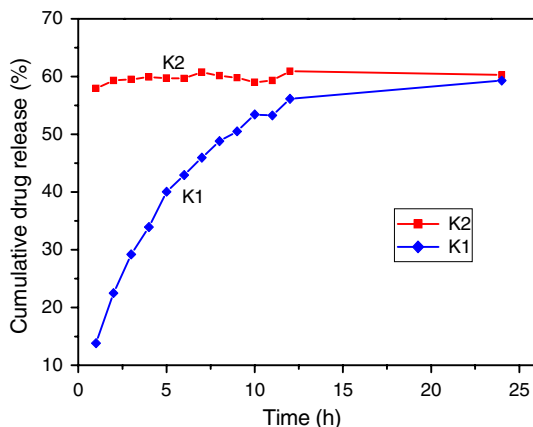


Fig. 7 In vitro release profile of 5-ASA from K1 complex and K2 blend in phosphate buffer (pH 7.4) at 37 °C

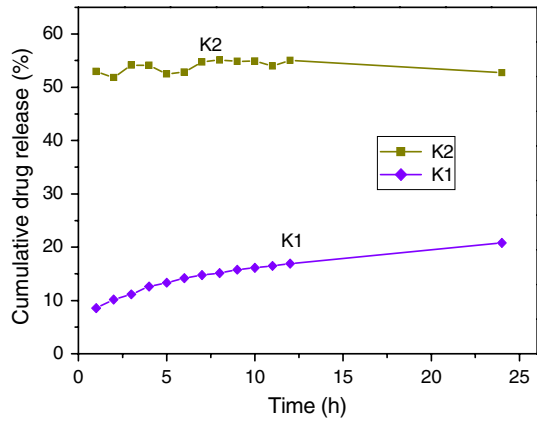
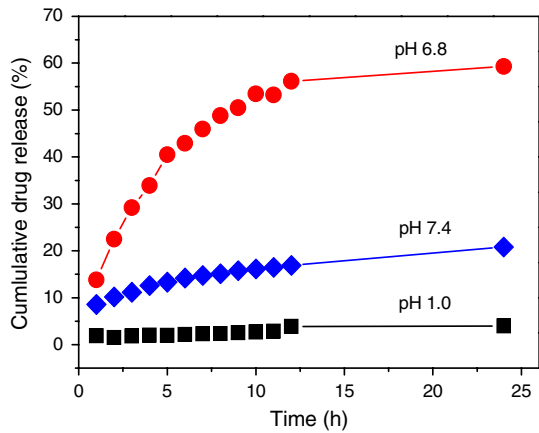


Fig. 8 In vitro release profile of 5-ASA from K1 in phosphate buffer with various pH values at 37 °C



Further, the release mechanism of 5-ASA from K1 and K2 blends was investigated by the Peppas equation [20, 21]:

$$M_t/M_\infty = Kt^n$$

where M_t/M_∞ is the fraction of drug released, K is a constant depended on the system, t is the release time and n is the diffusion exponent, indicating the type of drug release mechanism. If n approaches to 0.45, the release mechanism could be Fickian diffusion. If n approaches to 0.89, the release mechanism could be zero order, and on the other hand if $0.45 < n < 0.89$ non-Fickian transport (a synergistic effect of drug diffusion and matrices erosion) could be attained.

Table 1 shows the diffusion exponent n for K1 complex and K2 blend at different pH values. The results showed that the release of 5-ASA from K2 blend could be a Fickian (diffusion) mechanism being observed for the release at pH1.0, 6.8 and 7.4, respectively. It is correlative to several systems containing polysaccharides show release by diffusion mechanism [22, 23]. However, the drug release mechanism of 5-ASA from K1 complex could be independent on the pH value. Release of 5-ASA from the K1 complex shows release mechanisms via Fickian diffusion phenomena

Table 1 Kinetic data for blends at different pH values

pH	K1		K2	
	<i>K</i> values	<i>n</i> values	<i>K</i> values	<i>n</i> values
1.0	0.4365	0.2889	86.956	0.0153
6.8	2.2480	0.4877	56.299	0.0100
7.4	2.6620	0.4821	50.6874	0.0102

at pH 1.0. While the release from K1 complex is mainly occurred by non-Fickian mechanism and the diffusion is relaxation controlled at 6.8 and 7.4, respectively.

The mentioned results indicate that covalent bond link is an important factor within molecular interaction and the level of 5-ASA release from this system is much stronger dependent on the pH value than of K2 blend.

Conclusion

A novel pH-sensitivity complex was synthesized by DAKGM crosslinking with 5-ASA by the crosslinker of glutaraldehyde, and the structure characteristics and thermal characteristics were investigated. 5-ASA in vitro release behavior was also studied. The results showed that the DAKGM-ADH-ASA complex was sensitive to pH. 5-ASA was released from the complex at pH 6.8 and 7.4 much higher than at pH 1.0. This investigation of konjac glucomannan-based, pH-responsive complex indicated that the rate of drug release can be modulated by the appropriate chemical modification of the crosslinking densities. The DAKGM-ADH-ASA complex could be a promising oral drug delivery to the intestinal environment.

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