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Research paper

Synthesis, characterization and in vitro release of 5-aminosalicylic acid and 5-acetyl aminosalicylic acid of polyanhydride – P(CBFAS)

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Abstract

A novel polyanhydride, poly[(5-carboxybutyl formamide)-2-acetyl salicylic anhydride] (P(CBFAS)), with 5-aminosalicylic acid (5-ASA) incorporated into the polymer backbone was synthesized and characterized by infrared, ^{1}H-nuclear magnetic resonance, differential scanning calorimetry, vapor pressure osmometry, etc. The polyanhydride was subjected to degradation and simultaneously released 5-ASA and its derivative 5-acetyl aminosalicylic acid (5-acetyl ASA) in vitro under various conditions. The factors influencing the release profiles of 5-ASA and 5-acetyl ASA, including polymer molecular weights, pH value, enzyme and rat gastrointestinal contents, were examined. The results showed that the release rate of 5-ASA and 5-acetyl ASA increases with increasing pH value and with decreasing molecular weights. In PBS (pH 8.0, 37 °C) total ASA released was 8.0% for P(CBFAS)$_1$ (Mn 10770) in 13 h, but only 1.1 and 2.6% at pH 2.0 and 6.5, respectively. Enzymes including pepsin and trypsin, as well as rat gastric and jejunum contents had little effect on the release rate of 5-ASA and 5-acetyl ASA at pH 2.0 and 6.5 (less than 4% in 13 h). However, the release rate of 5-ASA and 5-acetyl ASA was much fast in PBS(pH 8.0) containing 5% of cecal contents, the total ASA released was 13.6% for the polymer in 13 h.. Considering the high drug loading of the polymer (50.2% of 5-ASA moieties in the backbones) and the degradation characters, it is possible to reach high local concentration of 5-ASA in the colon site via oral administration. Therefore, P(CBFAS) may be potentially useful in the colon specific delivery of 5-ASA.

Keywords: 5-Aminosalicylic acid; 5-Acetyl-aminosalicylic acid; Polyanhydride; Colon-specific drug delivery; Polymer prodrug

1. Introduction

5-Aminosalicylic acid (5-ASA) is widely accepted in the treatment of Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease. When orally administered, 5-ASA is unstable in the gastric conditions and prone to be absorbed in the upper intestine, which causes low drug bioavailability and low efficiency for inflammatory colon disease. Therefore, the colon-specific delivery of 5-ASA is an important issue.

Generally, the colon-specific delivery of 5-ASA can be achieved by several routes, these being: (1) coating with pH-sensitive polymer [1,2], (2) time-controlled formulation and device [3,4], (3) coating with polymer which can be degraded by intestinal microflora [5–7], (4) pressure controlled devices [8–10], and (5) polymeric prodrug approaches [11].

Of the colon-specific prodrugs of 5-ASA, the earliest-accepted formulation is sulfasalazine, an azo-conjugate of 5-ASA with sulfapyridine (SP) [12].With the knowledge that the adverse effects associated with sulfasalazine are due to SP [13], an investigation was started for the choice of a suitable carrier for 5-ASA with minimum adverse effects. Therefore, SP was replaced by 4-aminobenzoyl-β-alanine in balsalazide and by another 5-ASA molecule in olsalazine. Non azo-conjugates of 5-ASA, including 5-aminosalicyl-glycine [14] and dextran-5-ASA ester [15], were also developed.

Polymeric prodrugs with 5-ASA linked to the polymer backbones via spacers can successfully delivery 5-ASA to the colon [16–19].The linkages between 5-ASA and the polymer backbone, including azo, ester and amide bonds, are susceptible to enzymatic attack in the large intestine and 5-ASA is released at this site. However, the drug loading in

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these systems usually is less than 20%, and could not fit the requirements of large doses by patients.

Polyanhydride is a biocompatible and degradable material and widely applied in the drug controlled release system [20–24]. Recently we synthesized a novel polyanhydride prodrug, poly[(5-carboxybutyl formamido)-2-acetyl salicylic anhydride] (P(CBFAS)), in which the 5-ASA moieties were incorporated into the polymer backbone with high loading percentage (50.2%). Here we report some preliminary results on the polymer synthesis, characterization, and in vitro release characteristics of 5-ASA and its derivative 5-acetyl ASA under various conditions.

2. Experiments

2.1. Materials and instruments

Adipic acid was purchased from Shanghai Chemical Co. (Shanghai, China) and recrystallized with acetone. 5-Aminosalicylic acid (5-ASA) was a gift from Genglou Chemical Co. (Hangzhou, China), and recrystallized with distilled water. Acetonitrile supplied by Linhai Chemical Co.(Linhai, Zhejiang, China) was chromatographic grade. Dimethylformamide (DMF) was dried with CaH_2 and distilled under reduced pressure. All other chemicals were analytical grade and used as received.

Infrared (IR) spectra were recorded on a Bruker Vector 22 spectrometer. Samples were film cast onto NaCl plates or pressed into discs with KBr powder. ^{1}H-nuclear magnetic resonance (NMR) spectra were obtained on a Bruker DMX500 NMR spectrometer operating at 500 MHz, with d_6-DMSO as solvent and tetramethylsilane (TMS) as internal standard. Thermal analysis was performed on a Perkin Elmer DSC-7 thermal analysis system at a heating rate of 10 °C/min. Molecular weights of polymeric prodrugs were determined on a vapor pressure osmometer (VPO) apparatus (Knauer) with DMF as solvent at 90 °C. The static contact angles of distilled water on the polymer surface were used to evaluate polymer hydrophobicity by a contact angle meter (Krüss DSA-10). The polymer was dissolved in DMF and the solution was cast on silanized glass microscopy slides and dried at 40 °C under vacuum. Contact angles were measured on six different regions of each polymer surface and an average value was taken.

High-performance liquid chromatography (HPLC) detection was performed on a system consisting of double pumps (Waters, model 1525), autosampler (Waters,717), a dual λ UV detector (Waters, model 2487), with a 5-μm Diamonsil C_{18} reverse phase column (150 × 4.6 mm, Dikma), and a workstation (Breeze V3.2). The mobile phase consisting of 0.1 M acetic acid, acetonitrile and triethylamine (920:80:2, by volume) was applied and operated in 1.0 ml/min of flow rate at 25 °C.

According to the literature [14], Sprague–Dawley rats (Supplied by Experimental Animal Center, Medical School, Zhejiang University) weighing 350–450 g were used as the source of gastrointestinal (GI) contents. The animals were anesthetized by ethyl ether and killed, and the stomach, small intestine and cecum were excised. The contents in the lumens were taken out and diluted to the concentration of 5% (w/v) with buffer solution. The suspensions were stored in refrigerator at 4 °C before use.

2.2. Synthesis of polymeric prodrugs

2.2.1. Synthesis of 5-(carboxybutylformamido)salicylic acid (CBFS)

7.65 g (0.05 mol) of 5-ASA and 6.4 g (0.05 mol) of adipic anhydride prepared according to the literature [25] were dissolved in 50 ml anhydrous DMF, stirred overnight, the reaction mixture was poured into 500 ml acidic water (pH 2.0) under stirring. After filtration the collection was washed with distilled water, dried and extracted with warm ethyl acetate, 12.3 g of CBFS was obtained with yield 87.5%, m.p. 216–217 °C. IR spectrum (KBr, cm^{-1}): 3284 (amide N–H), 1695 (aliphatic acid C═O), 1673 (aromatic acid C═O), 1650 (amide C═O), 1540 (amide C–N stretching and N–H vibration), ^{1}H-NMR spectrum (DMSO-d_6, δ ppm): 6.9–8.0 (Aromatic, 3H), 2.3 (-$\underline{CH_2}CH_2CH_2\underline{CH_2}$-, 4H), and 1.6 (-$\underline{CH_2CH_2}$-, 4H).

2.2.2. Polymerization

5.62 g (0.02 mol) of CBFS was refluxed in 50 ml of acetic anhydride at 140 °C under dried nitrogen atmosphere for 3 h. After excess acetic anhydride was removed under reduced pressure, a dark oily prepolymer was obtained. Then it was polymerized under high vacuum (< 2 mmHg) for 2 h at high temperature. The products were dissolved in anhydrous DMF and precipitated from ethyl ether.

2.3. Hydrolysis and 5-ASA and 5-acetyl-ASA releasing from polymeric prodrugs

2.3.1. Degradation of P(CBFAS)

One hundred milligrams of polymers were compressed into discs at the temperature 20 °C higher than their T_gs and under the compression force of 500 kg/cm^2 for 5 min in a home-made mould. The discs were 11 mm in diameter and ca. 0.64 mm in thickness. The discs were placed into vessels with 30 ml solution with different pH, enzyme or rat gastrointestinal contents. At the selected time interval, 3 ml dissolution solution was removed for determining degradation products and replaced by fresh buffer liquor with the same volume.

2.3.2. Measurements of 5-ASA and 5-acetyl-ASA in the P(CBFAS) degradation solution

Pure 5-ASA and 5-acetyl-ASA were used as the external standards. The amount of 5-ASA and 5-acetyl-ASA in the degradation solution was analyzed by HPLC. The degradation solution was filtrated by a membrane with pore

diameter in 0.45 μm (Xingya Purification Co., Shanghai, China) before elution.

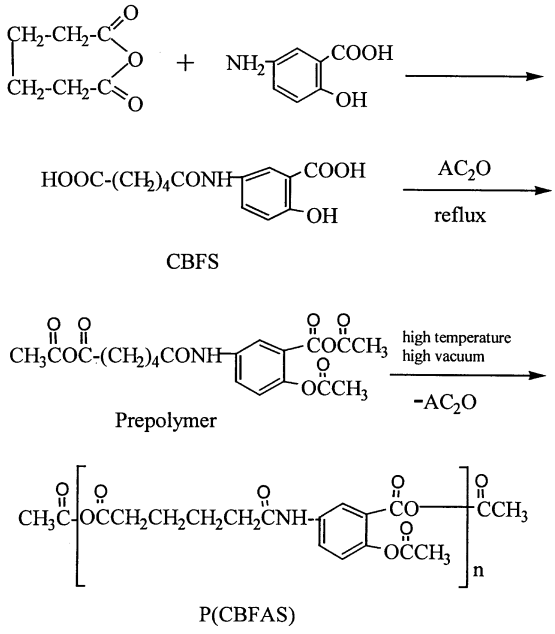
3. Results and discussion

3.1. Synthesis of P(CBFAS)

Adipic anhydride (AA) is an extremely active monomer, which can easily react with proton-donor groups. Although there are two possible reaction sites in the molecular structure of 5-ASA, i.e. the amino group and hydroxyl group, the former is more active than the latter in reacting with AA at the ambient temperature. From the IR spectrum of CBFS, we can find the information of amide bond at 3284,1650 and 1540 cm^{-1}. Together with the ^{1}HNMR spectrum, the structure of CBFS can be confirmed as that illustrated in Scheme 1.

The IR and ^{1}H NMR spectra of P(CBFAS)$_3$ are shown in Figs. 1 and 2, respectively. IR spectrum (KBr, cm^{-1}): 3350(N–H), 3070 (Ar–H), 1814, 1719 (anhydride, C=O), and 1757 (acetyl ester, C=O). ^{1}H NMR spectrum δ (ppm): 7.2–8.5 (e, g, f, 3H), 2.3–2.6 (a, c, 4H), 2.2 (h, 3H), 1.4–1.6 (b, 4H), 2.1 (i, 0.41H), and 2.5 (d_6-DMSO). From the confirmed structure of P(CBFAS) the ASA moieties in the polymer units were calculated to be 50.2%.

Table 1 summarizes some characteristics of P(CBFAS). It can be seen that as the polymerization temperature raising from 150 to 180 °C, the molecular weights increased from 1320 to 10 770, and the solubility of the polymer in DMF became more difficult. The thermal properties of P(CBFAS) were determined by differential scanning calorimetry (DSC). The samples were scanned from -50 to 200 °C,



Scheme 1.

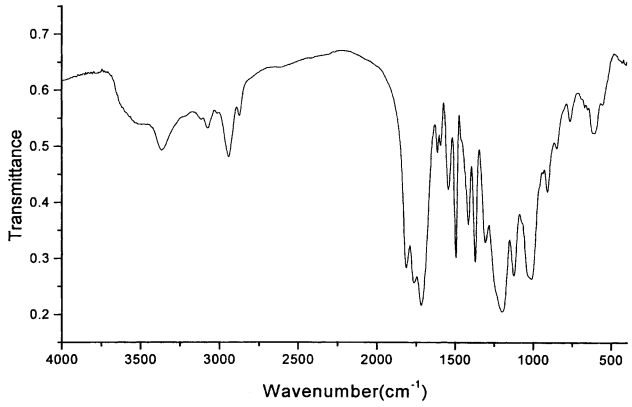


Fig. 1. Infrared spectrum of P(CBFAS)$_3$ (KBr method).

only T_g can be observed and the T_g's value increased with increasing the polymer molecular weight. The hydrophobicity of the polymers reflected by static contact angle also shows an increase as the polymer molecular weight increases.

While the polymerization was carried out below 150 °C, the polymers obtained were sticky state with poor mechanical properties at room temperature If the reaction temperature was over 180 °C, the polymers became very difficult to dissolve in DMF even in heating to 90 °C.

3.2. In vitro degradation and 5-ASA/5-acetyl-ASA release

3.2.1. Calibration curves of 5-ASA and 5-acetyl-ASA

Since 5-acetyl-ASA is the main metabolite of 5-ASA, they both need to be determined in degradation process of the polymers. The calibration curves of 5-ASA and 5-acetyl-ASA for HPLC determination were plotted referring to Yu's work [26], the linear regression equations are as follows: $A = -25760 + 22633.6C$ ($r = 0.99965$) for 5-ASA; $A = -1160 + 11838.8C$ ($r = 0.99987$) for 5-acetyl-ASA.

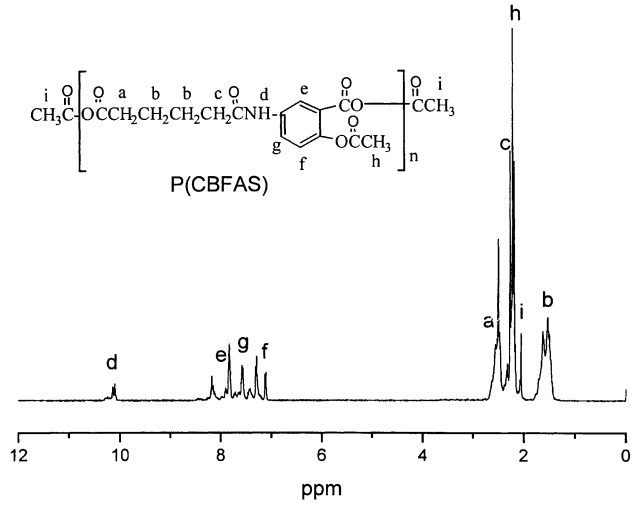


Fig. 2. ^{1}H-NMR spectrum of P(CBFAS)$_3$ in d_6-DMSO with TMS as internal standard at 500 MHz.

Table 1
Characteristics of P(CBFAS)

Polymer	Polymerization temperature (°C)	Mn[a] (g/mol)	T_g[b] (°C)	Static contact angle (°)[c]	Solubility in DMF[d]
$P(CBFAS)_1$	180	10770	114	89	90 °C in 2 h
$P(CBFAS)_2$	165	4870	97	73	90 °C in 10 min
$P(CBFAS)_3$	150	1320	80	55	Room temperature in 10 min

[a] Determined by VPO in DMF at 90 °C.
[b] Determined by DSC at rate of 10 °C/min.
[c] Determined by Krüss DSA10.
[d] 100 mg in 10 ml DMF.

In the concentration range of 2–100 μg/ml (for 5-ASA) and 1-200 μg/ml (for 5-acetyl-ASA), there is a perfect linear relationship between concentration and peak area. At a flow rate of 1.0 ml/min, the retention time of 5-ASA and 5-acetyl-ASA is 1.85 and 9.6 min, respectively.

3.2.2. Detection of 5-ASA and 5-acetyl-ASA released from P(CBFAS)

At the specific time interval, a sample of the degradation solution of P(CBFAS) was analyzed by HPLC. In Fig. 3, peaks other than those at 1.85 min (5-ASA) and 9.6 min (5-acetyl-ASA) can also be observed in the chromatogram. Using CBFAS and CBFS as references, it can be confirmed that the peak at 16 min corresponds to CBFAS, while that at 37 min corresponds to CBFS.

3.2.3. Molecular weight effect on 5-ASA and 5-acetyl-ASA release

Fig. 4 shows the release of 5-ASA and 5-acetyl-ASA from P(CBFAS) with variable molecular weights in PBS at pH 6.5. From the curves with solid symbols, we can see that the release rate of 5-ASA increases with time at the first stage (initial 13 h), then decreases. The release rate of 5-acetyl-ASA increases more slowly than that of 5-ASA at the initial stage, but becomes fast at the latter stage. However, the total ASA released increases with time (see the inset in Fig. 4).

No apparent burst release was observed during the early stage of degradation. As molecular weight increases, the polymer has higher hydrophobicity, which is reflected by the static contact angle data (Table 1). The higher hydrophobicity hinders water accessing to the anhydride bond, thus polymer degradation is retarded and the drug release rate reduced. We also examined the sticky polymer with low molecular weight, it was totally disintegrated in 4 h and has rapid drug release (data not shown).

3.2.4. pH effect on 5-ASA and 5-acetyl-ASA release

Fig. 5 shows the pH value has a critical effect on the release of 5-ASA and 5-acetyl-ASA from $P(CBFAS)_1$. Total ASA released from the polymer at acidic condition (pH 2.0) in 35 h was only 1.4%, while that at basic condition (pH 8.0) was 9%.

It was noticed that at the early stage of degradation (in 3.5 h), the release curves of 5-ASA or 5-acetyl-ASA are close with low release percentage (totally less than 1%). This lag time may be due to the high T_g and hydrophobic character of P(CBFAS) [27].

3.2.5. Enzymes and GI contents effect on 5-ASA and 5-acetyl-ASA release

Fig. 6 shows the effect of enzymes and rat cecal contents on the release of 5-ASA and 5-acetyl-ASA (Fig. 6a) and total ASA release (Fig. 6b) from $P(CBFAS)_1$ at pH 8.0. It can be seen that, the polymer in 0.1 M PBS at pH 8.0 containing 0.1% trypsin has no obvious change in the release rate of 5-ASA and 5-acetyl-ASA (ca. 5.2%), compared to that without any enzyme and GI contents in the same buffer solution. By adding 5% cecal contents, the release percentage of total ASA apparently rose to 13.6% at pH 8.0 PBS in 13 h. This may be attributed to the degrading oligomers; e.g. CBFAS and CBFS, etc., can be partially transformed into 5-ASA or 5-acetyl-ASA by the action of enzymes produced by the microflora in the cecum.

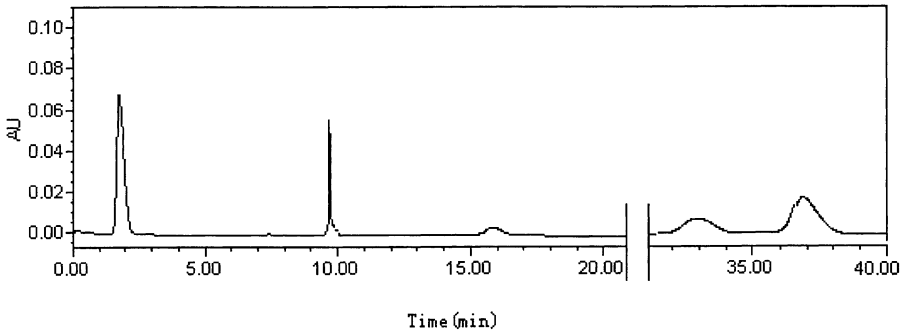


Fig. 3. Chromatogram of the degradation solution of $P(CBFAS)_1$ in 0.1 M PBS at pH 8.0, 37 °C with 5% of rat cecal contents at 13 h.

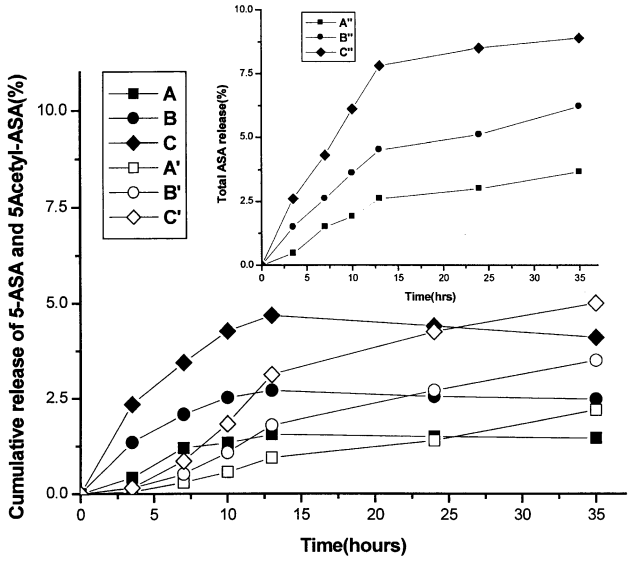


Fig. 4. 5-ASA and 5-acetyl-ASA released from P(CBFAS) with different molecular weights in 0.1 M PBS at pH 6.5, 37 °C. The lines marked with A, B, C represent releasing profiles of 5-ASA from $P(CBFAS)_1$, $P(CBFAS)_2$ and $P(CBFAS)_3$, respectively. The lines marked with A′, B′, C′ represent releasing profiles of 5-acetyl-ASA from $P(CBFAS)_1$, $P(CBFAS)_2$ and $P(CBFAS)_3$, respectively. In the inserted figure, lines marked with A″, B″, C″ represent releasing profiles of total ASA from $P(CBFAS)_1$, $P(CBFAS)_2$ and $P(CBFAS)_3$, respectively ($n = 3$).

We also examined the release behavior of 5-ASA and 5-acetyl-ASA in PBS at pH 2.0 and 6.5 with enzymes and upper GI contents; total ASA released is less than 4% in both cases in 13 h.

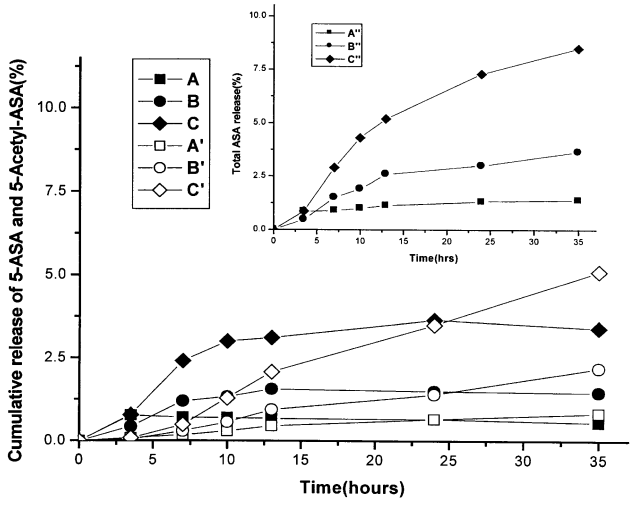


Fig. 5. 5-ASA and 5-acetyl-ASA released from $P(CBFAS)_1$ in 0.1 M PBS at 37 °C with variable pH values: The lines marked with A,A′ for 5-ASA and 5-acetyl-ASA released at pH 2.0; B,B′ for 5-ASA and 5-acetyl-ASA at pH 6.5; and C,C′ for 5-ASA and 5-acetyl-ASA at pH 8.0. In the inserted figure, A″, B″, C″ represent the total ASA released from $P(CBFAS)_1$ at pH 2.0, 6.5, and 8.0, respectively ($n = 3$).

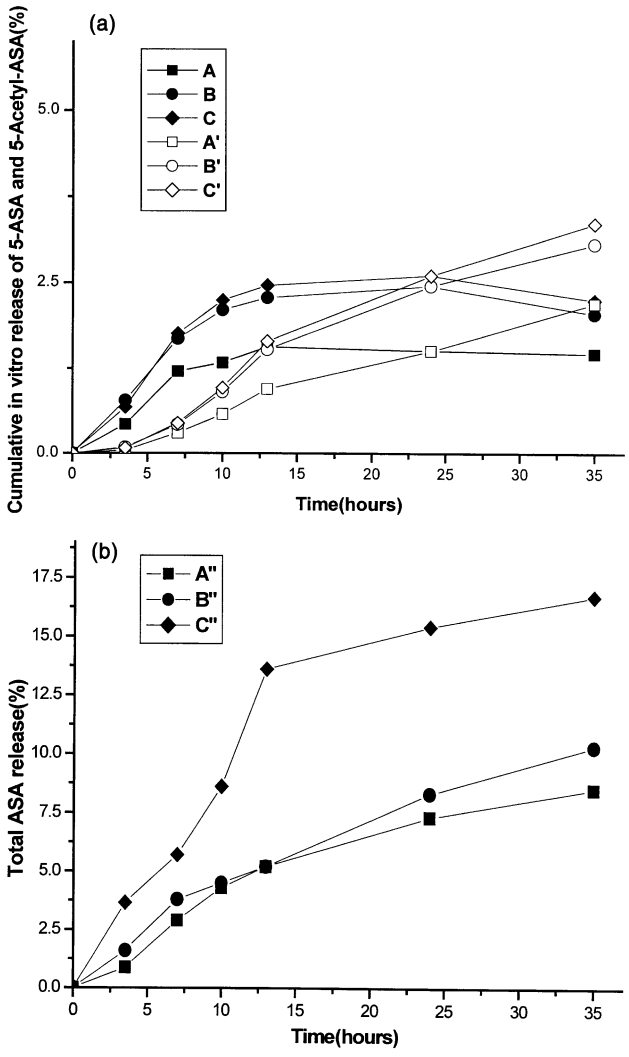


Fig. 6. 5-ASA and 5-acetyl-ASA released from $P(CBFAS)_1$ in 0.1 M PBS at pH 8.0, 37 °C. (a) The lines marked with A,A′ represent the releasing profiles of 5-ASA and 5-acetyl-ASA in PBS; B,B′ in PBS containing 0.1% trypsin; and C,C′ in PBS containing 5% rat cecal contents. (b) The lines marked with A″, B″, C′ represent the total ASA released in PBS, PBS containing 0.1% trypsin, and PBS containing 5% rat cecal contents, respectively ($n = 3$)

4. Conclusions

The polyanhydride with a bioactive agent, 5-aminosalicylic acid, incorporated into the polymer backbone was synthesized and characterized. The release of 5-ASA and 5-acetyl-ASA from P(CBFAS) in vitro is mainly influenced by the pH value and distal GI contents, as well as molecular weights of the polymers.

It was found that the release rate of 5-ASA and 5-acetyl-ASA is much lower in PBS at pH 2.0 and 6.5 than that at pH 8.0, and enzymes and upper GI contents are not apparent in promotion of the release ($< 4\%$ of total ASA released in 13 h). When the degradation was performed in PBS at pH 8.0 and the solution containing 5% cecal contents, the total ASA release rose to 13.6%. Considering P(CBFAS) has high drug

loading percentage (50.2%) and pH-sensitive degradation character, it is possible to create a high local concentration of 5-ASA and 5-acetyl-ASA in the colon sites via oral administration. Therefore, P(CBFAS) may be potentially useful for the colon-specific delivery of 5-ASA.

Acknowledgements

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