

## Controlled Release of Sulfasalazine Release from “Smart” Pectin Gel Microspheres under Physiological Simulated Fluids

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**Abstract** Sulfasalazine (SLZ) is a synthetic nonsteroidal anti-inflammatory drug used mainly for the treatment of an inflammatory bowel and other diseases. Two pectins with different methylation degrees were blended to synthesized gel microspheres by ionotropic gelation for SLZ encapsulation. The encapsulation efficiency was found to be around of 99% in all formulations tested. However, different SLZ release profiles related to the methylation degrees of pectin were observed. Mixture of low methylated (LM) and high methylated (HM) pectins in the presence of calcium(II) displayed the best microsphere morphologies among the formulations tested determined by optical and electronic microscopies. The percentage of drug release using a mixture of LM and HM pectins after 255 min in simulated gastric fluid (pH=1.2), simulated intestinal fluid (pH=6.8), and phosphate buffer (pH=7.4) were 15.0%, 47.0%, and 52.2%, respectively.

**Keywords** Pectin · Hydrogel · Drug delivery · Sulfasalazine · Biopolymers · Rheological analysis · Physiological simulated fluids

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## Introduction

Oral delivery of drugs is still the most popular among physicians and patients. The major site for drug absorption by this route is the small intestine which offers 100 m<sup>2</sup> of surface epithelia across which transfer can at least in principle take place. If the drug is poorly soluble under physiological conditions, significant absorption of the drug may occur in the large intestine [1]. Interest in polymeric matrices for pharmaceutical formulation continues to grow, especially those that react to external stimuli, allowing to target the release of drugs in specific conditions or sites. Pectins, an edible plant polysaccharide, have been shown to be useful for the construction of drug delivery systems for specific drug delivery. They are naturally occurring biodegradable polysaccharides consisting of linear chain of 1–4-linked  $\alpha$ -D-galacturonic acid residues with varying degrees of methyl ester substituents. Pectins can be classified according to the methyl esterification degree (DE) into low methylated (LM) when the methylation is below 40%, medium methylated when the DE is in between 40% and 60%, and high methylated (HM) pectin when the DE is higher than 60%. LM pectins are of particular interest in drug delivery as they can form gels with multivalent cations like calcium ion (Ca<sup>2+</sup>) which has potential applications. HM pectins are more hydrophobic than other pectins and were able to sustain the release of incorporated fragrances for long time. Less esterified pectin derivatives are able to penetrate deeper into the skin and may be useful in aromatherapy formulations [2]. Also, degradation of pectins in humans occurs by the available microflora in the colon. These properties could provide a basis for preparation of controlled release formulations, particularly for colon-specific drug delivery. Indeed, colon-specific delivery with a controlled release pattern provides more effective therapy for such chronic diseases as irritable bowel syndrome and inflammatory bowel disease, including Crohn's disease and ulcerative colitis. Sulfasalazine (SLZ) is a compound composed of 5-aminosalicylic acid and sulfapyridine and used as a prodrug for the treatment of ulcerative colitis [3–5].

In the present study, the effects of different formulation parameters upon the characteristics of pectinate beads prepared by ionotropic gelation technique for gastrointestinal delivery of SLZ were investigated. Optical and electron microscopies were used to characterize bead morphologies, rheological analysis was used to determine the performance and behavior of the formulation, and SLZ kinetic release was analyzed under different simulated physiological conditions.

## Materials and Methods

### Materials

Low methylated pectin (LM DE, 33.0%) and high methylated pectin (HM DE, 71.7%) dry powders were kindly provided by CPKelco (Buenos Aires, Argentina). SLZ Fluka was purchased from Sigma-Aldrich (Buenos Aires, Argentina) and used without further purification. All the other used chemicals and reagents were of analytical grade.

### Preparation of Drug-Loaded Calcium–Pectinate Beads

Aqueous solutions of 4% LM and HM pectins were prepared in respective buffers. SLZ with a final concentration of 1.0 mg/ml was added to the initial aqueous pectin solution (LM, HM, or a mixture of both of them) with continuous stirring and kept at 4 °C for 5 h to remove bubbles. The resulting solution was used to make the SLZ gel microspheres. Ten

milliliters of this solution was dropped into a 40-ml of gently stirred 300 mM  $\text{CaCl}_2$ –1.0% glutaraldehyde solution, through a syringe needle (0.3 mm diameter) using a peristaltic pump (LKB 2132 Microperpex peristaltic pump). The beads were allowed to harden in the calcium chloride solution for 30 min at room temperature. The beads were filtered and washed three times with distilled water and then dried at 37 °C for 96 h.

## Characterization of Pectin-SLZ Beads

### *Rheological Measurements*

A controlled-stress rheometer (Anton Paar MCR 301) with a 43-mm parallel plate (PP43/GL-SN16497 measurements system) and a P-PTD120-SN80470786 accessory system was used to characterize the rheological properties of pectin solutions. The gap used was 0.7 mm. For steady shear measurements, shear rates ranging from 1 to 1,000  $\text{s}^{-1}$  were used, and the resulting stress was recorded. All rheological measurements were carried out at 25 °C, and the reported curves were mean values of five measurements. Shear stress, shear rate, and apparent viscosity data are obtained directly from the instrument.

### *Pectin-SLZ Bead Diameter Analysis*

Pectin-SLZ bead diameter was determined using stereomicroscope and image analysis program (Image Pro<sup>®</sup> Plus, version 6.1). The average values of diameters (major and minor) was measured on 25 beads randomly taken and using the following equation [6]:

$$\text{Diameter relation} = \frac{A}{a}$$

where  $A$  is major diameter of the pectin-SLZ bead and  $a$  is the minor diameter of the pectin-SLZ bead. If the value of  $A/a$  is near to 1, the bead can be associated to a sphere, otherwise to an ellipse.

### *Scanning Electron Microscopy*

The surface characteristics of air-dried pectin-SLZ beads were determined by scanning electronic microscopy (SEM) at 22 Kv. The pectin-SLZ beads were sputter-coated with Au using a vacuum evaporator and examined using a scanning electron microscope (Philips SEM 505, Rochester, NY, USA).

### *Determination of Pectin Bead Water Content*

Each pectin bead batch was weighed (Mettler H35AR microbalance) before and after drying (37 °C for 96 h) and the mean water loss ( $W_L$ ) calculated according to the following equation [7]:

$$W_L\% = \left( \frac{W_o - W_d}{W_o} \right) \times 100$$

where  $W_o$  is the initial weight of the batch measured just after filtration and  $W_d$  is the weight after drying.

### Swelling Studies

The swelling characteristics of beads (40 mg) were determined by immersing them at dry state into conical flask containing 20 ml of each release medium and incubated at 37 °C under shaking at 90 rpm. Dry beads were swollen in simulated gastric fluid (SGF) at pH 1.2 containing 100 mM HCl and 0.2% (w/v) NaCl for 2 h, in simulated intestinal fluid (SIF) at pH 6.8 containing 5 mM KH<sub>2</sub>PO<sub>4</sub> and 3 mM NaOH, and finally in 5 mM of phosphate buffer solution (pH 7.4) [8]. At specific time intervals, samples were taken out from the different swelling medium and blotted with a piece of paper towel to absorb excess water on surface. The percentage of swelling ( $S(t)\%$ ), at each time was calculated using the following expression [9]:

$$S(t)\% = \frac{W_t - W_d}{W_t} \times 100$$

where  $W_t$  and  $W_d$  are the sample weights at time  $t$  and in the dry state, respectively. Each experiment was repeated twice times.

### Encapsulation Efficiency Determination (EE%)

Percent of entrapment efficiency (EE%) of SLZ was quantified spectrophotometrically at 360 nm (SP 2000 UV Spectrum, UV–Vis spectrophotometer). Residual SLZ concentration in the aqueous solution was determined after separating and washing pectin beads, according to the following equation [7]:

$$EE (\%) = \left( \frac{Q_t - Q_r}{Q_t} \right) \times 100$$

where  $Q_t$  is the drug content initially added during the batch preparation and  $Q_r$  is the sum of the drug content recovered in the aqueous solutions after separating and washing pectin beads.

### Drug Release Studies

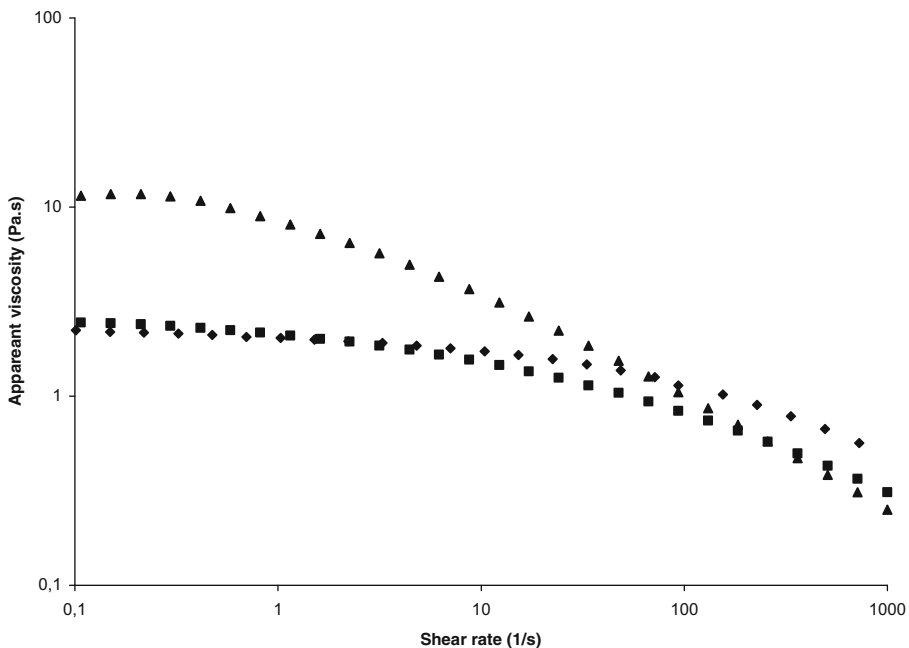
The SLZ release from loaded beads was studied at conditions described in swelling studies. At predetermined time intervals, 2.0-ml sample was withdrawn from the dissolution medium and immediately replaced by the same volume of fresh medium. The amount of SLZ released from beads was determined spectrophotometrically at 360 nm using previously calibrated standard curves at different pHs. To determine the total release in SGF solution, the pH of the release medium was adjusted to 6.8, by adding NaOH, and the concentration of SLZ was determined from calibration curve at this pH [10]. Each experiment was repeated twice times.

## Results and Discussion

### Rheological Properties of Polymers–Drug Solutions

The apparent viscosity (pascal second), shear stress (pascal), and shear rate (per second) at different revolutions per minute were recorded. The shear rate was increased between 0 and 1,000 s<sup>-1</sup> within a period of 175 s for recording the “up” curve; then it was reduced from

1,000 to  $0 \text{ s}^{-1}$  for recording the “down curve” in the same period of time to study the time-dependent nature of the polymers–drug solutions. A slight thixotropy (the area between the curves was negligible) was detected only for LM 4%-SLZ. For these samples, the down curve was used. In Fig. 1, steady-state shear viscosity is shown as a function of shear rate in log–log coordinates. At first, two different viscosity regions are observed: the Newtonian flow region showing the constant zero-shear viscosity ( $\eta_0$ ) at low shear rate and the power-law flow region showing the shear rate dependent apparent viscosity ( $\eta$ ) at relatively higher shear rate. The zero-shear viscosity is important in studying the structure–function relation of biopolymeric systems, but apparent viscosity measured in the power-law region gives practical information for the process optimization and sensory characterization of fluids. Apparent viscosity was found to decrease with an increase in shear rate indicating a shear-thinning behavior. The initial apparent viscosity for the low-grade pectin LM-SLZ was approximately four times greater the viscosity of HM-SLZ and HM–LM-SLZ. For the last two, less resistance to shear flow is observed, which means that the increase pectin degree of esterification is concomitantly with lower viscosity. Similar observations in our laboratory for pectin/doxorubicin solutions were found (data not shown). These results were in agreement with previous studies reported by several authors [11, 12]. The viscosity curves of HM–LM-SLZ and HM-SLZ were approximately equals up to  $5 \text{ s}^{-1}$ ; however, for increasing shear rate, the curve for HM–LM-SLZ presents lower viscosity values compared to the other curves except a very high shear rates where the curve now is very much closer to the LM-SLZ sample. These observations could indicate that HM–LM-SLZ presents best features to be used as a potential polymeric carrier.



**Fig. 1** Flow behavior of different pectin-SLZ solutions at 25 °C. Apparent viscosity as a function of shear rate. Symbols: SLZ in 4% HM pectin (diamond), 2% HM+2% LM pectins (square), and 4% LM pectin (triangle)

## Rheology Model

The shear-thinning region of apparent viscosity vs the shear rate can be expressed by the power-law model. The constitutive equation of this model is

$$h = K(\dot{\gamma})^{n-1}$$

where  $\eta$  is the apparent viscosity (pascal second),  $\dot{\gamma}$  the rate of shear (per second),  $K$  the consistency index (pascal (second) <sup>$n$</sup> ), and  $n$  the flow behavior index which is a measure of the degree of the non-Newtonian behavior.

For shear-thinning fluids, the flow index  $n$  lies between zero and unity with values further removed from unity indicating a more pronounced non-Newtonian behavior. As is observed in Fig. 1, there exist a transition flow region between the initial Newtonian plateau at the low shear rates and the final power-law region at the higher shear rates. The characterization of the transition region is of rheological importance because in this region, polymers can provide the mechanisms in the early disentanglement stage, depending on their molecular structure and interaction properties. The consistency index, the flow behavior index, and the correlation coefficient ( $R$ ) of pectin solutions are displayed in Tables 1 and 2. As it can be seen, the value of  $n$  is less than 1. Thus, the studied substances are exhibiting shear-thinning behavior, and the LM-SLZ solution showed the high one. The results indicate that the transition flow region can be expressed in terms of the power-law equation, and two distinct power flow regions, including the transition region, were determined for HM-SLZ, LM-SLZ, and HM-LM-SLZ pectin solutions. Similar observations in citrus pectin solutions were previously reported [13].

## Morphological Bead Characterization

When the aqueous solutions of different pectins containing SLZ were dropped into counter-ions solutions (calcium), gelled beads were produced instantaneously by ionotropic gelation. In this process, intermolecular cross-linking between the negatively charged carboxyl groups of pectin and the positively charged counter-ions generated the gel network, which is similar as previously described in the “egg-box model”-like type [14].

Two pectin solutions (4%) with different esterification degree as well as a mixture of them (2.0% of each one) were used to obtain three polymeric networks containing SLZ in

**Table 1** Rheological characteristics of pectin-SLZ solutions

Samples	Range	$\eta_0$ (Pa s)	Flow behavior index $n$	Consistency index $K$ (Pa s <sup><math>n</math></sup> )	$R^2$
HM-SLZ	0.1–0.323	2.45	0.88	2.026	0.998
	0.323–10.4		0.726	2.440	0.998
	10.4–1000				
HM-LM-SLZ	0.1–0.295	2.23	0.766	2.104	0.997
	0.295–8.73		0.730	2.101	0.998
	8.73–1000				
LM-SLZ	0.1–0.415	11.7	0.635	8.317	0.997
	0.415–12.3		0.425	13.887	0.999
	12.3–1,000				

**Table 2** Percentage of water loss in pectin-SLZ beads after drying

Beads	$W_o$ (g)	$W_d$ (g)	$W_L$ (%)
LM-SLZ	8.622	0.973	88.7
HM-LM-SLZ	9.703	1.072	89.0
HM-SLZ	14.378	1.510	89.5

the presence of Ca(II). As shown in Figs. 2a, b, the resulting beads loaded with SLZ prepared from both HM and LM pectins were irregular-shaped displaying a roundness value of  $1.14 \pm 0.11$  (collapsed pectin bead) and  $1.77 \pm 0.13$  (drop-like pectin bead), respectively. Interestingly, Fig. 2c shows that wet beads obtained with a mixture of both pectin solutions were almost spherical ( $A/a = 1.09 \pm 0.07$ ). A defined spherical shape is the desired form for controlled release system because of the high surface/area ratio and also because it is easy for the development of control release kinetic models (data not shown).

### Scanning Electron Microscopy

Scanning electron micrographs of drug-loaded beads prepared with LM–HM pectins or LM pectin are shown in Fig. 3a, b, respectively. The smooth surface of both dry formulations beads displayed numerous discrete homogeneous pores dispersed all over the bead and some surface-associated structures. Under our experimental conditions, it was not possible to evaluate scanning electron micrographs of HM-SLZ pectin beads because they were unstable. This could be attributed to the soft nature of the HM pectin hydrogel network.

Pectin, a hydrophilic macromolecule, is the “first-generation” mucoadhesive biopolymer, whose mucoadhesive properties depend upon the presence of hydrogen bond forming groups. First-generation mucoadhesives are activated by moistening, and they adhere non-specifically to many surfaces. However, their overhydration results in the formation of loosely bound slippery mucilage leading to their removal from the surfaces [9].

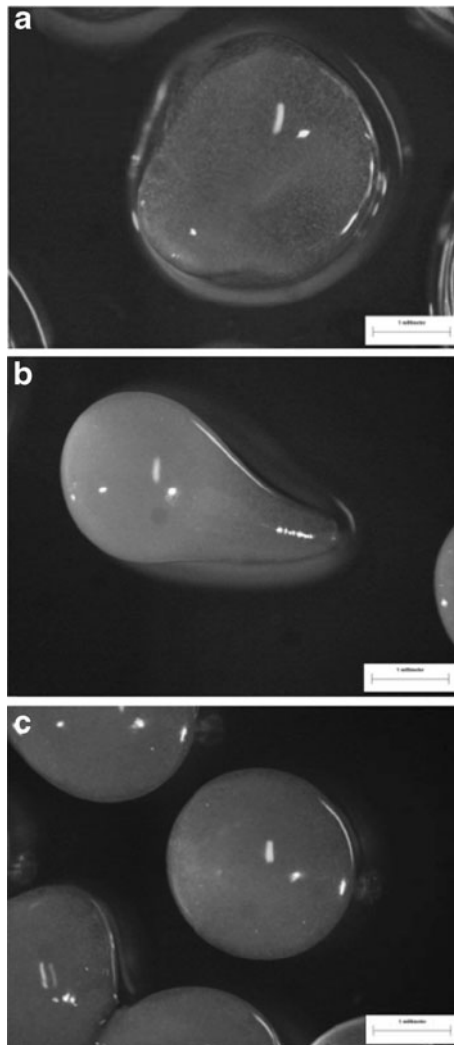
### Determination of Pectin Bead Water Content

The amount of water retained by pectin-SLZ beads (which can be considered as an index of their water affinity) was similar in all tested cases.

### Swelling Studies

The results of swelling study in SGF revealed that both SLZ-loaded beads of HM–LM pectin and SLZ-loaded LM pectin hydrated quickly, with a swelling of 79.8% and 69.2%, respectively, in the first hour. The HM–LM-SLZ beads reached to their maximum swelling within 60 min and then shrunk gradually toward their equilibrium state (Fig. 3a). Similar swelling behavior was found when low DE pectin with or without chitosan coating was immersed in 0.1 M HCl/NaCl buffer solution at pH 1.2 [15]. Also, some authors report that in calcium LM pectin beads loaded with rutin, swelling seems to be higher and buffer-dependent [16].

In addition, in the presence of both SIF and phosphate buffer (pH 6.8 and 7.4), the swelling of HM–LM-SLZ beads was higher than that of LM-SLZ ones (Fig 3b). Swelling studies with HM pectin beads were not determined because of their sticky and soft nature.



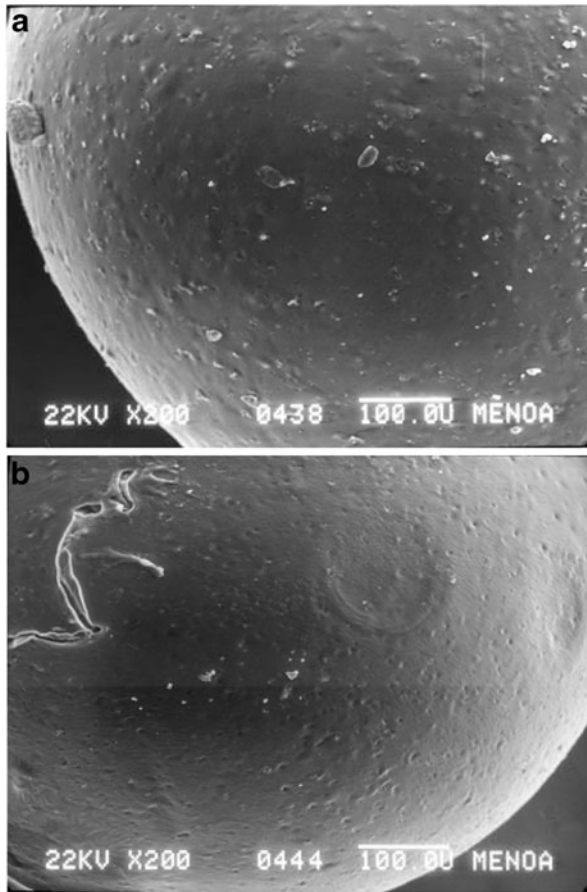
**Fig. 2** Optical microscopy of different pectin beads loaded with sulfasalazine. **a** HM-SLZ, **b** LM-SLZ, **c** HM-LM-SLZ (magnification  $\times 20$ )

#### Encapsulation Efficiency Determination and Drug Release Studies

The SLZ EE was close to 99% in all formulations tested. This SLZ EE is higher than previously reported in both uncoated and chitosan-coated calcium–alginate–*N,O*-carboxymethyl chitosan beads with 65% and 60%, respectively [10]. Other authors described an EE of SLZ from 11% to 13% and 73% to 79% with biodegradable polymers like poly( $\epsilon$ -caprolactone) and poly(lactic-co-glycolic acid) [3].

However, the drug release from pectins gel beads was affected not only by the esterification degree of pectins but also by the blend composition (Fig. 4). The percentage of drug release using a mixture of LM and HM pectins after more than 4 h (255 min) in simulated gastric fluid (pH=1.2), simulated intestinal fluid (pH=6.8), and phosphate buffer (pH=7.4) were 14.97%,



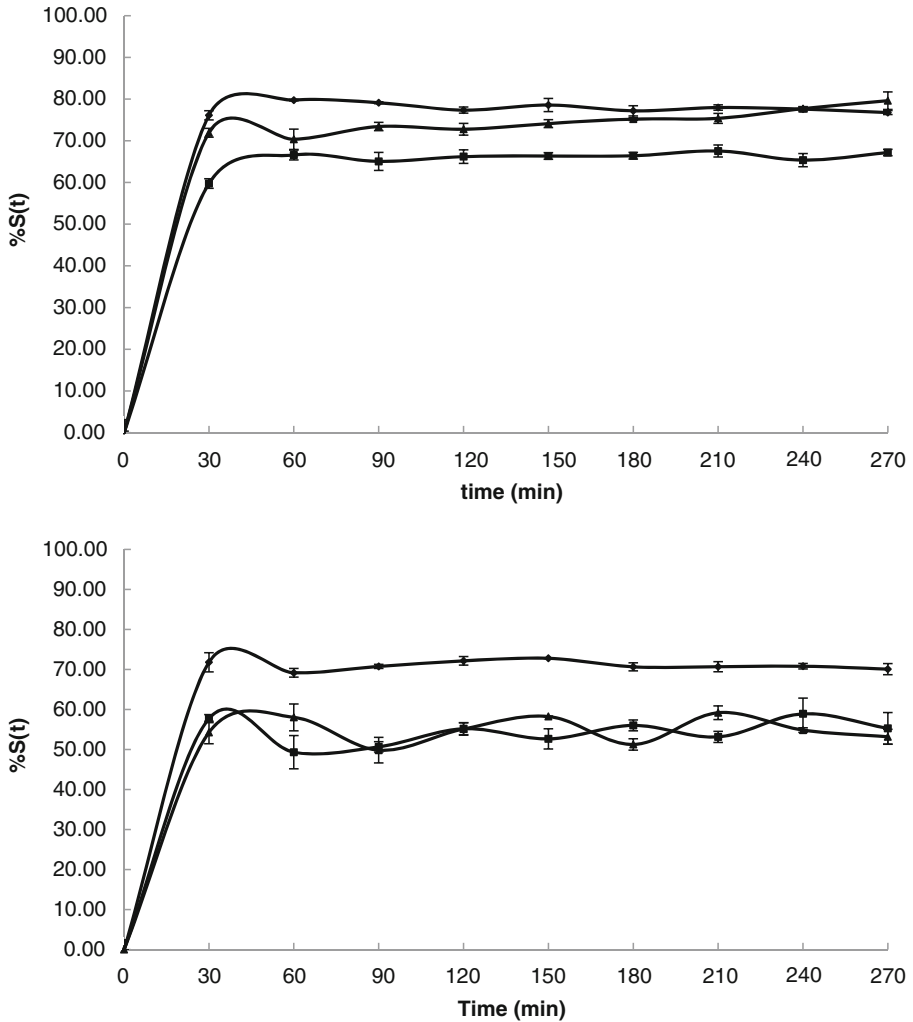


**Fig. 3** SEM of HM–LM–SLZ beads (a) and LM–SLZ beads (b) (magnification  $\times 200$ )

47.04%, and 52.23%, respectively. The SLZ release profiles were also tested with both HM and LM pectins formulations. Interestingly, the cumulative drug release from HM–SLZ pectin bead preparations at either pH 1.2, 6.8, or 7.4 significantly increases comparing with both LM and LM–HM pectin solutions (Fig. 5a–c). This response could be due to a lax structure of the HM pectin hydrogel network. Other authors suggested that drug release from beads made of HM pectins (DE 70%) will be substantially faster, due to a lower and weaker cross-linking, mainly be hydrogen bridges [17].

## Conclusions

The release of SLZ from pectin carriers depends on the esterification degree of pectins as well as the blend composition. Compared with HM pectin beads, HM–LM pectin formulation not only yields almost spherical beads but also significantly decreases the rate of drug release in the presence of SIF and phosphate buffer pH 7.4. In addition, considering the average residence time of a substance in the stomach, about 2 h, the results presented here showed in the present

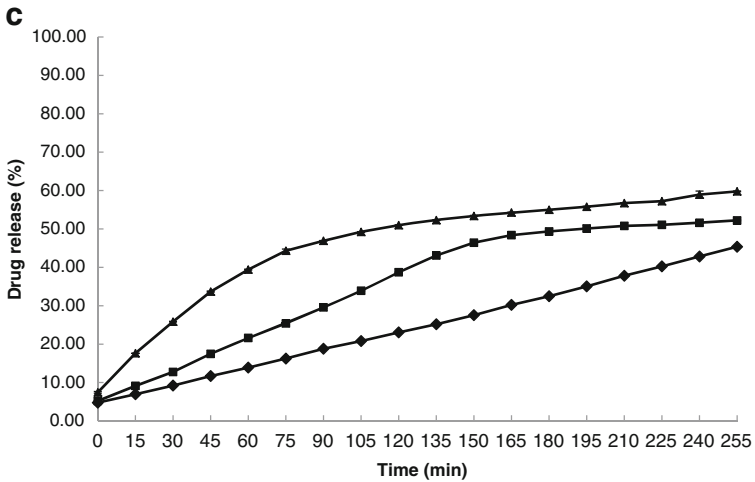
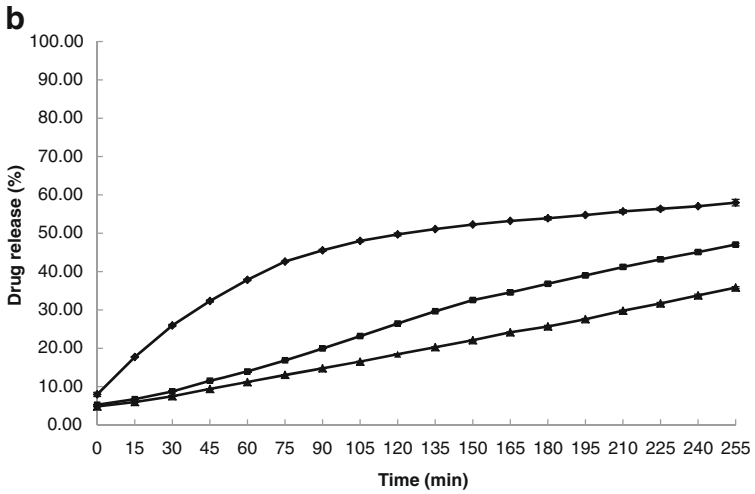
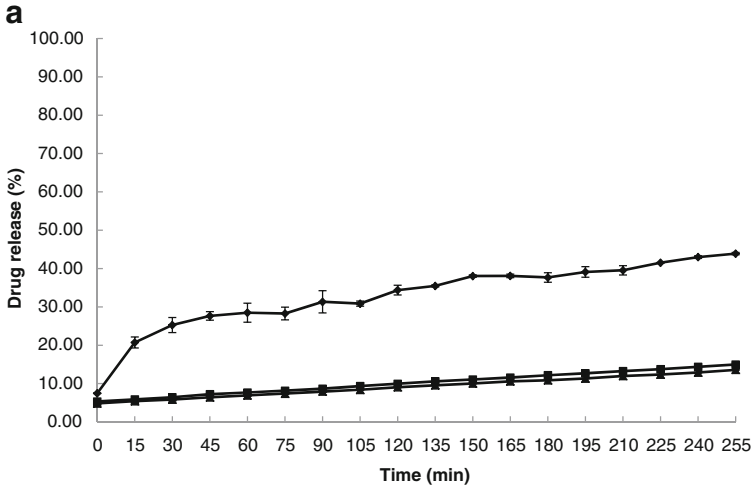


**Fig. 4** Swelling characteristics of HM–LM–SLZ and LM–SLZ pectin beads (a and b, respectively) in simulated gastric fluids (SGF; diamond), simulated intestinal fluids (SIF; circle), and phosphate buffer (pH 7.4; triangle)

work less than 10% at the same time, and about 15% of SLZ was released from the microspheres at pH 1.2.

Based on the results on our work, HM–LM pectin microspheres as a potential biopolymeric carrier can be considered as a serious candidate for colon-specific drug delivery system of SLZ. Studies to determine interaction between SLZ and pectins and SLZ modeling release from the microspheres are under way in our laboratories.

**Fig. 5** Profiles release of SLZ from the beads in SGF (a), SIF (b), and phosphate buffer (c). Symbols: SLZ in 4% HM pectin (diamond), 2% HM+2% LM pectins (square), and 4% LM pectin (triangle)



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