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Original Article

Preparation of Ethylcellulose Coated Gelatin Microspheres as a Multiparticulate Colonic Delivery System for 5-Aminosalicilic Acid

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Abstract

In the long-term management of ulcerative colitis patients, repeat dosing maybe required. Since 5-ASA is largely absorbed from the upper intestine, selective delivery of drugs into the colon may be regarded as a better method of drug delivery with fewer side effects and a higher efficacy. The aim of this study was to prepare and evaluate a double coated multiparticulate system for 5-ASA delivery using gelatin and ethylcellulose as the primary and secondary polymer respectively. Gelatin microspheres containing 5-aminosalicylic acid was produced using the solvent evaporation method. Prepared gelatin microspheres were spherical, free flowing, non-aggregated and showed no degradation in the acidic medium. Entrapment efficacy of microspheres was about 50%. Results showed that drug release was fast and complete and is affected by the amount of core material entrapped. Gelatin microspheres were then coated by ethylcellulose using a coacervation phase separation technique. The idea for this approach was to prepare a delayed drug delivery system, in which, ethylcellulose protects particles for the first 6 h transit through the gastrointestinal tract. However, it was shown that this system could provide a suitable drug release pattern for colonic delivery of active agents, as 30% of the drug was released from the ethylcellulose-coated microcapsules within 6 h, while this amount was 90% of the loaded drug for gelatin microspheres under the same condition.

Keywords: 5-Aminosalicilic acid; Gelatin; Ethyl cellulose; Colon delivery; Microparticles; Microspheres; Microencapsulation.

Introduction

There have been considerable researches in the field of colonic drug delivery for many purposes:

a) Development of new therapeutic agents for the treatment of colonic diseases has required colon- specific delivery systems to maximize the effectiveness of these drugs (1).

b) Introduction of once a day sustained release formulations has required a better understanding of the transit of dosage forms through the colon, and of the colonic absorption of the drug present within them (2).

c) The colon itself is susceptible to many disease states including constipation, irritable bowel syndrome and more serious diseases such as Crohn's disease, ulcerative colitis, Carcinomas and infections. At present these diseases are often poorly and inefficiently managed either by oral drugs, which are largely absorbed before they reach the colon, or by rectal administration, which is less acceptable as a route of administration and rarely succeeds in delivering drugs to the ascending colon. 5-ASA is indicated for the treatment of mild to moderate active ulcerative colitis. In the long-term management of ulcerative colitis delivering drugs to the colon may be regarded as a better method of drug delivery with less side effects and higher efficacy (3).

Using natural polymers such as

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polysaccharides as a controlling agent for delivering of drugs has received much interests in recent years (4, 5). Biocompatibility and biodegradability of these materials provide good advantages for this reason. Natural polymers in fact could obviate toxicity or biodegradability problems (i.e. formation of localized granulomatous inflammation), possibility due to the use of synthetic materials. Gelatin in particular represents a good candidate for the preparation of microspheres. Indeed gelatin has bioadhesive properties which allows the production of drug delivery systems for mucosal delivery i.e. mouth, nasal and gastrointestinal tract. On the contrary, gelatin dissolves rather rapidly in aqueous environments, making its use somewhat difficult for the preparation of long term drug delivery systems (6). This problem may be resolved by coating the gelatin microspheres, using a hydrophobic polymer such as ethylcellulose.

The aim of this study was a) to prepare and characterize gelatin microspheres designed for colonic drug delivery, and b) to prepare and characterize double layer microcapsules to overcome the fast dissolution of gelatin.

Experimental

Materials

5-Amino salicylic acid (ASA) was obtained from Pliva (Zagreb). Sesame Oil was purchased from Aseel, (UAE). Ethylcellulose was obtained from ICN, (USA). Gelatin, Tween 20, Hydrochloric acid, potassium dihydrophosphate, n-hexane, toluene, 2-propanol, sodium hydroxide, and span 80 were purchased from Merck, (Germany).

Preparation of gelatin core microspheres

Drug loaded microspheres were produced as follows. Gelatin was dissolved in water to produce 5%, 7% and 8% W/V solutions. The dispersion of drug into the polymeric solution was aided by sonication for 30 min. The aqueous solution of the drug in gelatin was stirred for further 30 min at 200 rpm. To produce an emulsion of aqueous gelatin solution containing drug molecules in the second oil phase, the aqueous solution was dispersed in sesame oil using an overhead paddle and left stirring for 45 min. When microspheres appeared in the solution (detected by optical microscope), then the temperature of the medium was brought down to 5°C by placing the beaker in an ice bath while 100 ml 2-propanol was added gradually to the system along with and stirring at 1000 rpm for a further 20 min. Microspheres containing the drug particles were formed due to coacervation induced by lowering the temperature of the medium. Subsequently the excess toluene was added to the system to facilitate separation of the prepared microspheres as well as the removal of sesame oil. Prepared microspheres were then collected by filtration, washed with acetone and dried at room temperature. Microspheres with drug to polymer ratios of 20:80, 30:70 and 50:50 were obtained at this stage.

Microspheres coating method

Drug loaded gelatin microspheres were used as a core material for the preparation of double-coated system. A coacervation phase separation method was applied for this step. A known amount of the microspheres having particle size of 100-250 μm was dispersed in an in Ethyl Acetate (25ml) solution containing ethyl cellulose (50, 100, 150 mg) and containing 0.02% W/V span 80. This mixture was agitated for 5 min at 400 rpm. Subsequently 50 ml n-hexane (as the non-solvent) was poured into the polymeric solution containing the core material with the rate of 1 ml/min. The medium was stirred for 60 min to complete the process of microparticles coating. Coated microspheres were then washed with an excess of n-hexane, filtered and dried at room temperature.

Microsphere morphology and particle size determination

The morphology of gelatin microspheres was evaluated by optical microscopy (Prior, England) as well as the scanning electron microscopy (SEM) (Stereoscan 360, Leica Cambridge, UK).

Particle size range and distribution of microspheres were determined using standard sieves.

Drug content and efficacy measurement

To determine drug entrapment within the microspheres, 50 mg of microspheres was dissolved in 100 ml of HCl (0.1 N). After complete dissolution of gelatin, the amount of drug was quantified using a spectrophotometric method at 302 nm in the presence of a blank prepared from microspheres containing all materials except the drug. Drug loading was determined as the percentage of the amount of the drug obtained to the applied amount.

Efficacy of the microspheres preparation method was determined by dividing the amount of the prepared microspheres to the initial amount of the applied material.

Drug entrapment within the coated microspheres was determined by dissolving 50mg of microspheres in 100ml of methylene chloride and HCl. Solute was extracted from the medium by the removal of methylene chloride. The amount of drug was measured spectrophotometrically.

Drug release studies

Profiles of drug release from the prepared microspheres were studied using a USP (Apparatus I) dissolution tester. 100 mg of microspheres was incubated in 500 ml phosphate buffer (pH =7) containing 0.02% W/V tween 20 to aid wetting. The media were agitated at 100 rpm, while maintaining the temperature at 37°C. 5 ml samples were withdrawn from the dissolution medium at regular time intervals and replaced with fresh medium. Concentration of the withdrawn samples was measured spectrophotometrically as mentioned above.

In order to investigate the influence of EC on the release profiles of microspheres, two different dissolution test methods were set in hydrochloric acid (as the gastric medium) for 2 h and then Phosphate buffer as intestinal medium for 22 hours.

Results and discussion

Development of microparticulate drug delivery systems using a combination of polymers has significant advantages over the homogenous polymeric systems (7). Through these systems by the selection of an appropriate combination of core and coat polymers, a microparticulate system for simultaneous entrapment of hydrophilic and hydrophobic drugs is achievable. Indeed, the drug could be entrapped in the core material using the proper characteristics of the core polymer while its disadvantages is improved by the desirable properties of the coating material.

Gelatin is an interesting biomaterial for drug delivery. Degradation of gelatin by natural microorganisms existing in the colon makes it suitable for delivering drugs to the colon. However, its use in oral administration is restricted due to its fast dissolution in the upper part of the gastrointestinal tract and limited capacity for controlling the release of drugs. To overcome these limitations a multiparticulate controlled release system consisting of a hydrophilic gelatin core entrapped within a hydrophobic polymer (ethyl cellulose) was prepared. The purpose of this study was to present an approach for the preparation of gelatin microspheres suitable for oral application. These microcapsules were prepared by different amounts of gelatin and various

Figure 1. Scanning electron microscopy image of gelatin microspheres.

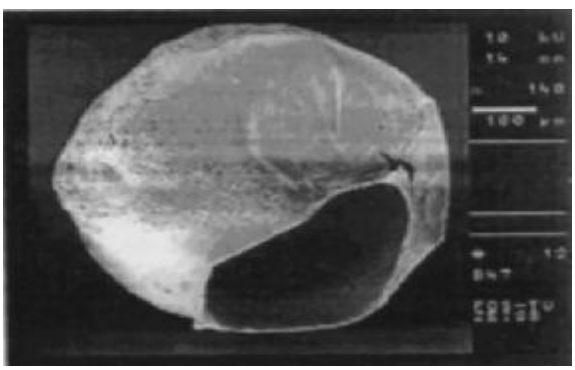

Figure 2. Scanning electron microscopy image of gelatin microspheres after removing from the dissolution medium.

Table 1. Drug microencapsulation efficacy of gelatin and EC-coated microcapsules (n=3).

Drug/gelatin ratio	drug loading (%)	Preparation efficacy (%)	
		Gelatin microspheres	EC-coated microcapsules
20:80	49.25±2.32	87±4.23	67±1.21
30:70	54.27±3.72	87±3.85	68±1.54
50:50	51.36±2.34	91±5.43	68±3.12
Mean	51.62±2.52	88.33±2.03	67.66±19

ratios of core and coat materials. 5-ASA was used as the model drug to investigate the ability of the system for entrapment and controlling drug release in the simulated colon medium.

Production of microspheres under different conditions was investigated. From these experiments it was possible to encapsulate various gelatin concentrations (5, 7, 8%), leading to the formation of particles with different drug to polymer ratio (20:80, 30:70, 50:50).

Microscopic observations (Figure 1) showed that all the dried gelatin microspheres were spherical, free flowing and non-aggregated. The microparticles were stable at low pH values. The morphology of microspheres (as assessed by SEM) did not change significantly following incubation, either in an acidic or neutral medium (figure 2), supporting the suitability of the system for colonic delivery. The size of the microspheres varied between 50 to 400 μm, while 92% of the particles had a size range between 50 to 250 μm. However, about 60% of the microspheres fall within the 100 to 250 μm size range. These results were not much different for the three microsphere preparation conditions applied. Figure 3 shows the particle size distribution of the prepared microspheres.

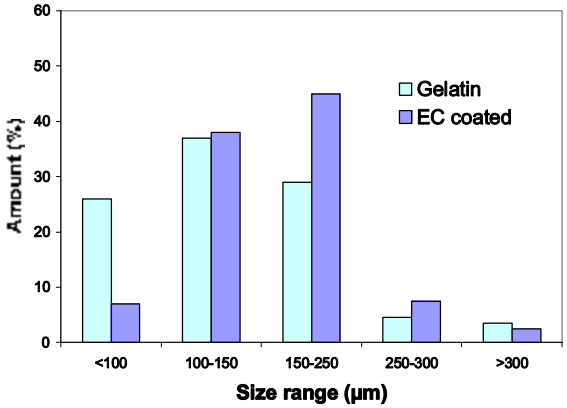

Figure 3. Particle size distribution of gelatin microspheres (50:50) and EC (100mg) coated microcapsules

The entrapment efficiencies and preparation efficacy of different prepared samples consisting various ratios of drug to polymer are shown in table 1. These results show that the preparation efficacy of microspheres was very high irrespective of the processing condition. This is approximately 88% for gelatin microspheres. The drug entrapment was also good in all samples, being about 52% for the primary microspheres. Further more, this amount did not differ for the coated microspheres, showing negligible drug loss during the second reaction.

Figure 4 Shows the pattern of drug release from gelatin microspheres with different drug to polymer ratios in phosphate buffer medium. In another attempt microspheres were placed in the acidic medium for 2 h then the microspheres were transferred to the second medium, which contained phosphate buffer solution for better simulation of the gastrointestinal transit. As swelling is the main factor influencing drug

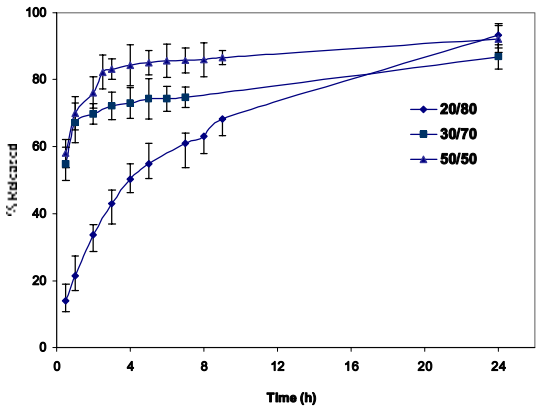

Figure 4. Drug release pattern from the gelatin microspheres with different drug to gelatin ratios in pH 7 phosphate buffer medium (n=3).

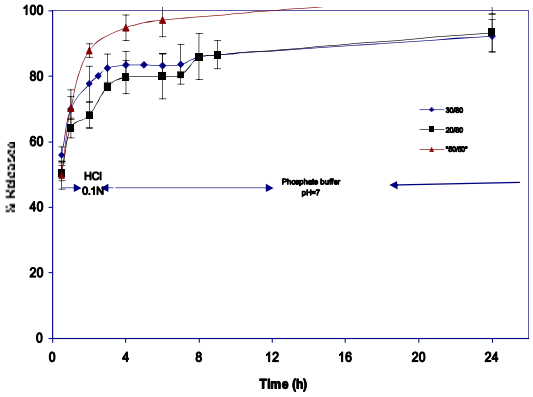

Figure 5. Drug release pattern from gelatin microspheres in hydrochloric acid medium and phosphate buffer medium (n=3)

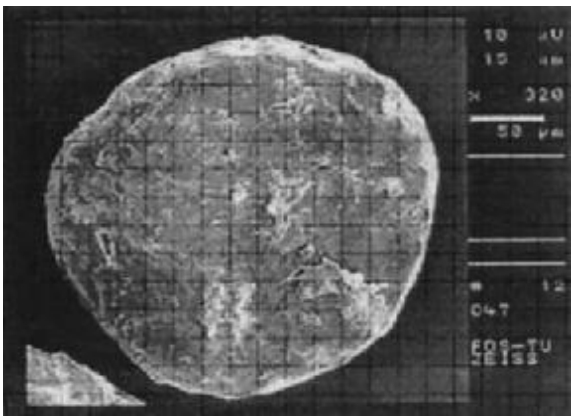

Figure 6. Scanning electron microscopy image of EC-coated microspheres.

release from the microspheres prepared by polysaccharides, leaving this system in the acid medium for 2 h prior to exposure to buffer medium affects drug release. Figure 5 represents behavior of the gelatin microspheres in this condition. Due to the high water uptake of the microspheres, drug release pattern is fast and nearly complete within 6 h, while a large amount of drug is released in the acidic medium before coming into contact with the phosphate buffer medium.

These results also show the influence of core/coat ratio on the *in vitro* behavior of the microspheres. The higher the amount of core material, the faster the in-vitro release rate of the drug. This core to coat dependence of the drug release behavior could be logically explained by the hydrophilicity of gelatin. The probable drug release mechanisms from the gelatin microspheres involve the following processes; i) water penetration into the microspheres, ii) gelatin swelling/gelling and dissolution of the drug and iii) diffusion of the active compound through the gelatin hydrogels. Therefore, the drug release rate would be controlled by the extend and rate of water absorption/swelling of the gelatin included within the microparticles, and the rate of diffusion of drug out of the gel. Gelatin microspheres swell very rapidly and then form a gel-like barrier, which facilitates drug release.

To delay and hinder drug release from microspheres and overcome a fast drug release, ethylcellulose was used to coat the gelatin microspheres.

Ethylcellulose coated microcapsules are spherical, with smooth surfaces. Figure 6 shows the scanning electron microscopic image of these microcapsules. Particle size distribution of the coated microspheres is shown in figure 3. since particles having a size range of 100 to 250 µm were chosen as core for EC coating, about 80% of the prepared microcapsules still have this particle size range. Drug release pattern from the microspheres were studied under the condition mentioned above. As can be seen in figure 7, the drug release pattern has been modified in comparison with the gelatin microspheres. While 90% of the loaded drug was released from the gelatin microspheres in 6 h, about 30% of drug was released under the same condition from ethylcellulose-coated microcapsules. Figure 8 compares the pattern of drug release from gelatin microspheres and ethylcellulose-coated microcapsules.

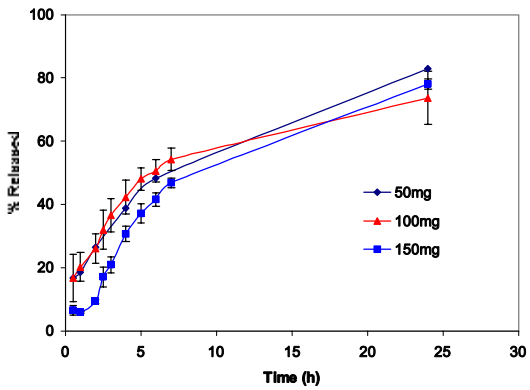

Figure 7. Drug release pattern from microcapsules prepared by EC-coating on gelatin microspheres (n=3)

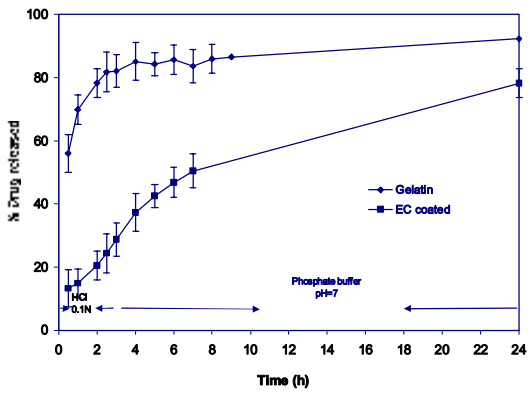

Figure 8. Drug release pattern from gelatin and EC-coated microcapsules in acid and phosphate buffer media (n=3).

Conclusion

As conclusion, it could be said that a drug delivery system prepared by combination of a hydrophobic polymer and a polysaccharide has the capability to be applied as a colonic delivery system. Achieving this potential needs further researches in this area.

Acknowledgement

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