Biodegradable Microspheres Based on Gelatin–Porcine Mucin Admixtures: in Vitro and in Vivo Delivery Studies

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Soluble mucus glycoprotein (S-mucin) processed from the small intestines (ileal region) of freshly slaughtered pigs via homogenization, dialysis, centrifugation and lyophilization and their admixtures with type B gelatin were used to prepare cefaclor-loaded microspheres by the emulsification-crosslinking method. The microspheres were evaluated for the in vitro delivery of cefaclor in both simulated intestinal fluid (SIF) without pancreatin (pH 7.4) and simulated gastric fluid (SGF) without pepsin (pH 1.2). Results obtained indicated that the microspheres formulated were highly mucoadhesive and that release of cefaclor in both release media was non-Fickian and was much higher and more rapid in SGF than in SIF and from microspheres based on gelatin alone when compared to those based on gelatin–porcine mucin admixtures. The mean area under the plasma level versus time curves (AUC) was shown to be dependent on the formulation with values of 172.3 μg·h/ml for the control, 278.5 μg·h/ml for microspheres based on gelatin only and 353.0 μg·h/ml for microspheres formulated with equal parts of gelatin and mucin indicating that the rectal route may provide a therapeutically viable alternative to the oral route for the delivery of cefaclor. Further indications also emerged of a possibility of site-specific delivery of cefaclor to the small intestine through a careful selection of gelatin type and porcine mucin admixtures prior to formulation of the microspheres. On the whole, the inclusion of S-mucin in the composition of the microspheres had an enhancer effect on the release and rectal bioavailability of cefaclor which may be exploited in the design of a rectal delivery system of the drug.

Key words in vitro–in vivo delivery; biodegradable gelatin microsphere; cefaclor; porcine mucin; admixture

Microspheres are one of the multiparticular delivery systems which are formulated to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drugs to specific sites.²–⁹ In the pharmaceutical arena, microspheres have been shown to protect sensitive macromolecules from enzymatic and acid degradation and can offer additional advantages such as limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance.¹⁰–¹⁵ Until recently, mucus had been assumed as an inert blanket, serving as a chemical barrier against potentially injurious chemicals, bacteria and enzymes.¹⁶ It has become clear in recent years, however, that mucus glycoproteins or mucins are capable of interacting in various ways with many biologically important entities such as enzymes, polymers, cations, drugs, viruses, cells surfaces and bacteria.¹⁷,¹⁸ Encapsulation of drugs into mucoadhesive microspheres has been reported to lead to a controlled release of drugs including protein and peptide drugs¹⁹ in addition to other desirable advantages of delivering macromolecules via microspheres. According to origin and pre-treatment of the utilized collagen, two major types of gelatin are commercially produced. Type A gelatin, also known as basic gelatin, is obtained from porcine skin with acidic pre-treatment prior to the extraction process and is known to have an isoelectric point (IEP) of 9.0. The second prevalent gelatin species (type B), also known as acidic gelatin, is extracted from ossein and cuticle split from bovine origin following an alkaline pre-treatment (liming). During this extraction process, the amide groups of asparagines and glutamine are hydrolyzed into carboxyl groups, thus converting many of the residues to aspartate and glutamate.²⁰,²¹ Consequently, the electrostatic nature is affected and in contrast to type A which has an IEP of 9.0, the higher number of carboxyl groups per molecule reduces the IEP of type B gelatin to between 4.8 and 5.2.

Although the rectal route should certainly not be the route of first choice, it can in certain circumstances be of immense benefit to the patient. In spite of its few limitations, the rectal route is still used in many different therapies, intended either for local or for systemic effect. For the attainment of a systemic effect, all orally given drugs including antibiotics, hormones, antihistamines, tranquilizers, analgesics and anti-inflammatory agents can be administered by the rectal route.²² Systemic treatment by the rectal route is of particular value for treating infants, administering drugs such as amphetamine that cause gastric irritation and for treating patients who are unconscious, mentally disturbed or unable to tolerate oral medication because of vomiting or pathological conditions of the alimentary canal. The above background prompted us to explore the rectal route, for the delivery of cefaclor, a broad spectrum, second generation, orally effective cephalosporin. The reported interaction of mucin with many biologically important compounds including polymers spurred our interest to prepare microspheres from admixtures of porcine mucin and gelatin, a pharmaceutical polymer of natural origin. The in vitro and in vivo delivery of cefaclor from these microspheres are also evaluated.

MATERIALS AND METHODS

Materials Cefaclor pure sample (Eli-Lilly, U.S.A.); diethylether, concentrated hydrochloric acid, sodium chloride, glutaraldehyde (BDH, England); sodium hydroxide (Merck, Germany); gelatin and mono-basic potassium phosphate (Sigma Chemical Co., U.S.A.) were used as procured from the manufacturers. Distilled water was obtained from an all-
Microspheres

The apparatus employed for this study was designed to give reproducible measurements and had been employed in our earlier study.23) The test was carried out using a freshly excised hog ileum. The ileum was cut into pieces measuring 12.0 cm (length)×1.5 cm (internal diameter) and each was gently rinsed with chilled saline to free it of its intestinal waste materials and quickly pinned unto a polythene support. A known quantity of the different mucin–gelatin microspheres was weighed out and placed on the trough of the mucus surface and left to hydrate for 15 min to allow for microsphere–mucus interaction to take place. A 250 ml volume of SIF contained in a separatory funnel was allowed to flow over the hydrated microspheres at a rate of 30 ml/min. The weight of microspheres detached (washed out) calculated as a percentage of the original weight of the microspheres was used as an index of mucoadhesion. Five replicates were performed and the mean values were calculated.

Microscopic Examination and Size Determination

Particle size of the microspheres was determined by optical microscopy using a projection microscope (Olympus, Tokyo, Japan). At least, 150 microspheres were dispersed on a slide in a mineral oil plus 1% of a non-ionic surfactant (Tween 65) and their diameter was then sized using suitable objectives.

Drug Entrapment Efficiency of the Microspheres

Accurately weighed microspheres equivalent to 100 mg were suspended in 100 ml of the buffered citrate/phosphate medium (pH 3.0) and allowed to completely swell. A period of 24 h was allowed for complete hydration of the microspheres at room temperature after which the dispersion was vortexed repeatedly to break up the microspheres and cause them to completely discharge their contents. The solution was then filtered and analyzed spectrophotometrically at 290 nm using a digital UV–Vis spectrophotometer (Spectronic 21 D).

In Vitro Drug Release Studies

Dissolution studies were carried out in quadruplicate for all the batches of the microspheres employing the USP XXIII paddle method. The release medium consisted of 500 ml of freshly prepared SIF (pH 7.4±0.10) maintained at 37±0.5 °C and agitated at a constant speed of 50 rpm. An aliquot of the sample was periodically withdrawn at suitable time intervals and the volume of the release medium was kept constant by appropriate replacement after each withdrawal. The withdrawn samples were analyzed spectrophotometrically at 290 nm. The release study was repeated using freshly prepared SGF as the release medium. Four replicate release studies were performed in each case and the mean values were taken.

Pharmacokinetic Studies

Male Wistar rats aged two months with a mean weight of 200±15.0 g were obtained from the Department of Veterinary Pathology and Microbiology of our University. The rats were allowed to acclimatize to the new environmental conditions of our laboratories for one week before use. Three groups of eight animals each were used for the study. An amount of the microspheres equivalent to a dose level of 100 mg of the drug/kg body weight of the rats was carefully transferred into the empty bodies of capsule No. 3. A positive control was set up by enclosing an equal amount of the pure cefaclor powder equivalent to that in the microspheres. By means of the capsules, the drug was administered rectally to the rats. At regular time intervals of 30 min for the first one hour and then subsequently at 1 h in-

Isolation and Purification of Porcine Small-Intestinal Mucus Glycoprotein

The small intestines of freshly slaughtered pigs were obtained from the abattoir of the Animal Science Department in our University. The isolated ileum was dissected starting from the jejunum to the ileocecal sphincter. The intestines, sectioned into short lengths, were flushed through with chilled saline, and the mucosal surface was exposed by longitudinal dissection. By using a microscope slide, the mucus layer was gently scraped off and diluted with four times its volume of chilled distilled water. The gel was homogenized for 2 h at 4 °C and then exhaustively dialysed against distilled water. The dialysate was centrifuged at 10000 rpm for 30 min to yield a supernatant of water-soluble mucus glycoprotein and lower layer of insoluble mucus glycoprotein. The supernatants were collected separately, pooled and lyophilized to obtain flakes of soluble (S) mucin samples which were further powdered and used for the study.

Preparation of Porcine Mucin–Gelatin Admixtures

A 1 g quantity of gelatin was weighed out and dispersed in 10 ml of citrate/phosphate buffer, pH 7.4. Soluble mucin (1 g) was similarly weighed out and mixed thoroughly with the dispersion of gelatin in a beaker. The combination was left to stand for 24 h in order to attain maximum hydration and then homogenized to obtain the polymer admixture. Thus, admixtures of S-mucin and gelatin in ratios of 1 : 1, 1 : 2, 1 : 3 and 1 : 4 were prepared.

Preparation of Stabilized Mucin–Gelatin Microspheres

A 25% (v/v) dispersion of each mucin–gelatin admixture in arachis oil was used. A 1 g quantity of cefaclor was dispersed in 25 ml of mucin–gelatin dispersion and pre-heated to 40 °C. The dispersion was extruded dropwise into pure arachis oil at 40 °C on a thermostatically controlled hot plate-magnetic stirrer. The mixture was stirred at a speed of 500 rpm for 30 min. Enough glutaraldehyde to produce about 1% (v/v) concentration was added and stirring continued for further 30 min. The resulting mixture was centrifuged at 5000 rpm for 10 min to collect the microspheres. The microspheres collected were washed with acetone to remove excess oil and then dried at the ambient temperature of 29±2 °C.

Swelling Studies

The initial weight of the microspheres was recorded and placed in 100 ml of simulated intestinal fluid (SIF) without pancreatin (pH 7.4) and allowed to swell. At regular time intervals, the swollen microspheres were carefully removed using a forcep and blotted dry using a filter paper and weighed. Water sorption was calculated from the difference between the initial weight of the microspheres and the weight at the time of determination. The total time the microspheres spent outside the swelling medium per measurement was less than a minute. The experiment was repeated five times and the mean was calculated. The swelling experiment was further repeated using simulated gastric fluid (SGF) without pepsin (pH 1.2) as the swelling medium.

Determination of the Mucoadhesive Properties of the Microspheres

The apparatus employed for this study was...
tervals, 0.5 ml of blood was sampled from the orbital sinus of the rats.24,25)

**Analysis of Cefaclor in Protein-Free Rat Plasma** The method of Tietz26) was adopted to prepare a protein-free filtrate. From each of the 0.5 ml of blood sampled from the rats, 0.2 ml was added to a test-tube containing 1.8 ml of 3% trichloroacetic acid (TCA). The test-tube was shaken gently to ensure a proper homogenization of the TCA and the blood sample, and allowed to stand for 5—10 min. The test-tube was then centrifuged at 3000 rpm for 10 min after which 1 ml of the clear supernatant layer was collected. The collected portion was analyzed spectrophotometrically without dilution at a wavelength of 290 nm. An absorption spectrum previously run for a solution of cefaclor in TCA did not exhibit any shift from the earlier wavelength of maximum absorption, an indication that no significant interaction occurred between the two compounds.

**Statistical Data Analysis** Statistical data analyses were performed using the Student’s t-test with \( p \leq 0.05 \) as the minimal level of significance.

**RESULTS AND DISCUSSION**

The results of the mucoadhesion of the microspheres on hog everted intestinal tissues as evaluated in SIF are shown in Fig. 1. It is discernible from Fig. 1 that the microspheres formulated exhibited good mucoadhesive properties and showed percentage mucoadhesion as high as 80% in some cases. Mucoadhesion to hog everted intestinal tissue was generally found to be high in the presence of SIF as a washing fluid. This observation may be ascribable to a number of factors. The first factor may be the similarity in the pH of the intestinal tissue and the washing fluid (SIF) which ensured that the integrity of the hog everted intestinal tissue was maintained throughout the duration of the experiment. The second factor may likely be the moderate swelling tendency of the microspheres in the presence of SIF. The microspheres under investigation were formulated with type B gelatin, which is known to be prepared from basic precursors and would, therefore, be expected to show higher swelling in an acidic pH of the SGF, based on ionic interaction, when considered against its moderate swelling in an alkaline environment. This expected enhanced mucoadhesive behavior of our formulation in the presence of SGF was confirmed in our experiments (data not shown). Excessive swelling would, most likely, lead to a more rapid erosion of the microspheres as a result of reduced mucoadhesion. The high mucoadhesive observed in the presence of SIF may also be due to deep penetration of gelatin and gelatin–mucin microspheres into the mucus layer and their adhesion to mucus tissue. This deep penetration would be expected to increase the residence time and improve intimate contact of the microspheres with the wall of the small intestine (adsorption site) which may further significantly improve the degree of absorption of any encapsulated drug and the taking up of the particles by the gut tissue.

It is equally evident from Fig. 1 that, in comparison with microspheres formulated with gelatin alone, those formulated with admixtures of S-mucin and gelatin showed higher mucoadhesion. This may be an indication of a possible enhancement of the mucoadhesive properties of gelatin microspheres by the soluble portion of porcine mucin. It is also deducible from Fig. 1 that the percentage mucoadhesion seems to vary with the mucin–gelatin ratios used in formulating the microspheres. Microsphere prepared with mucin–gelatin in the ratio of 1 : 1 exhibited a consistently high percentage mucoadhesion in SIF. This may, possibly, indicate that 1 : 1 is the optimal ratio of combination of S-mucin and gelatin for enhanced mucoadhesiveness especially when a drug is targeted to adhere to the small intestine for an extended period of time.

The mean particles diameters of the microspheres formulated are presented in Table 1 and ranged from 15.9 to 25.3 μm. This range of particle diameter for microspheres has been found to be suitable for oral, intramuscular and intravenous administration of various classes of drugs.27,28) The size of microspheres is known to play a critical role in determining the route of delivery of various drugs.27) The microspheres formulated in this study might be suitable for all-purpose delivery of various classes of drugs.

Preliminary water uptake studies carried in two different media (SIF and SGF) revealed that microspheres prepared from admixtures of gelatin and S-mucin showed greater swelling tendency especially in SGF when compared to that prepared from gelatin alone. On the whole, water sorption was generally found to be higher in SGF, than in SIF (Figs. 2, 3). There, therefore, seems to be a correlation between the swelling (water sorption) and mucoadhesive properties of the microspheres formulated.

The drug entrapment efficiency of the microspheres is represented as a bar chart (Fig. 4). The drug entrapment efficiency, as is evident from Fig. 4, was dependent on the composition of the microspheres. Microspheres prepared from admixtures of gelatin and S-mucin appeared to entrap greater amounts of cefaclor in comparison with those prepared from

![Image](image_url)
gelatin alone. The wide variation in the drug entrapment efficiency of the microspheres may be as a result of the varying degrees of drug sedimentation and the relative partitioning of cefaclor between the dispersed and continuous phases of the emulsion prior to cross-linking of the polymer admixtures. The drug entrapment efficiency of all batches of the microspheres was in the ranges of 19.5 to 53.5% and higher amounts of cefaclor seemed to have been entrapped by microspheres prepared from admixtures of gelatin and S-mucin than those prepared from gelatin alone.

The release profiles of cefaclor from the microspheres in SIF and SGF are depicted in Figs. 5 and 6 respectively. Release of cefaclor from the microspheres in SIF was generally poor and not up to 34% of the drug was released after four hours except for the microspheres prepared from gelatin alone where up to 90% of the drug was released after 4 h. A burst release of about 20% of cefaclor is evident within the first 10 min of the release experiment especially in SGF. In SIF, however, burst release was less than 10% of the loaded drug. The higher burst release seen in SGF is attributable to the more rapid rate of hydration and swelling of the microspheres in SGF than in SIF as earlier noted. In SGF, however, release of cefaclor was higher and more rapid than in SIF and up to a maximum of 60% of the drug was released after four hours except also for microspheres formulated with gelatin alone where almost 100% of cefaclor was released within the same period. A characteristic feature of the release profile of cefaclor from both release media is the biphasic pattern of release. The first phase of release occurred within the first 4 h of the release experiment while the second phase of release occurred between the 5th and 6th hour of the release experiment. This pattern has previously been reported to be characteristic for gelatin-based microspheres. Much of the drug release observed in both release media for all the formulations except that formulated with gelatin alone occurred between 5 and 6 h of the release experiment which was when complete erosion of the microspheres and subsequent diffusion of cefaclor into the release medium seemed to have taken place. It seems evident also from Figs. 5 and 6 that the release of cefaclor was retarded within the first 4 h in microspheres prepared from admixtures of gelatin and S-mucin when compared with gelatin-only based microspheres.
Table 2. Release Kinetic Parameters of Cefaclor from the Microspheres in Two Release Media

<table>
<thead>
<tr>
<th>Microspheres</th>
<th>SIF</th>
<th>SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n^0 )</td>
<td>( k )</td>
</tr>
<tr>
<td>1:1</td>
<td>0.8465</td>
<td>0.0812</td>
</tr>
<tr>
<td>1:2</td>
<td>0.6786</td>
<td>0.08345</td>
</tr>
<tr>
<td>1:3</td>
<td>0.7308</td>
<td>0.0993</td>
</tr>
<tr>
<td>1:4</td>
<td>0.5806</td>
<td>0.1021</td>
</tr>
<tr>
<td>0:1</td>
<td>0.8308</td>
<td>0.1181</td>
</tr>
</tbody>
</table>

\( n^0 \): release exponent; \( k \): release kinetic constant; \( r \): correlation coefficient. a) The value of \( n \) for each microsphere formulation is the mean of three determinations in each release medium.

It is reasonable, to infer from this observation that in the presence of mucin, the intermolecular network and possibly other characteristics of gelatin and its microspheres, were modified. The more rapid and higher release of cefaclor from the microspheres in SGF, possibly as a result of higher rate of hydration and swelling of the microspheres in SGF than in SIF, may be attributable to the type of gelatin (type B) used in formulating the microspheres. This type of gelatin is known to be prepared from basic precursors and, consequently, would be expected to swell more in an acidic medium such as SGF than in an alkaline environment.

In order to determine the mechanism of release of cefaclor from the microspheres, the Korsmeyer–Peppas’ model was employed and good regression co-efficients were obtained (Table 2). The values of release exponent \( (n) \) ranged between 0.4786 and 0.8465. This indicates that the release of cefaclor from the microspheres occurs by diffusion following non-Fickian transport mechanism in both SGF and SIF.

Further analysis of the release of cefaclor from the microspheres was done using the cube root equation as proposed by Langerbucker. This investigator proposed a cube root treatment for non-disintegrating granules of uniform shape and size. In accordance with this model, the amount of drug remaining undissolved at time \( t \), i.e., \( M_t \) varies with \( t \) according to the following equation:

\[
\frac{M_t}{M_0} = 1 - \frac{t}{T}
\]

where \( M_0 \) is the initial amount of drug present in the granules and \( T \) is the total dissolution time. A straight line is obtained when the cube root of the relative undissolved mass is plotted against time. The dissolution line starts at \((M_t/M_0)=1\), and intersects the time axis at \( t=T \), and has a slope of \(-1/T\). Thus, the Langerbucker cube root model could be used to determine a single constant, \( T \), which is the time required for 100% release of the drug. The \( T \)-values for cefaclor in both SIF and SGF as calculated from representative plots of the cube root equation are presented in Table 3. It is evident from this that there is a much more extended release of cefaclor from all the microspheres in both release media, which is seen as an improvement over the conventional tablet and suspension dosage forms of cefaclor.

The plasma level versus time profiles for cefaclor, after rectal administration to male Wistar rats, are depicted in Fig. 7. The areas under the plasma level versus time curves (AUC) were evaluated based on a non-compartmental pharmacokinetic analysis using the trapezoidal rule. The pharmacokinetic parameters as calculated from Fig. 7 are presented in Table 4. It is evident from Table 4 that the bioavailability of cefaclor via the rectal route was higher (AUC = 353.0 \( \mu g \cdot h/ml \)) from microspheres prepared from an admixture of S-mucin and gelatin (1:1). Microspheres prepared from gelatin alone gave lower AUC value relative to that prepared from its admixtures with S-mucin. Interestingly, the peak plasma concentration followed a trend closely similar to the AUC and this gave further indication as to the bioavailability of the drug studied. The relatively high AUC values obtained for the microspheres may indicate that the absorption of cefaclor was both rapid and complete and that the drug may have been bypassed the hepatic first-pass metabolism. It is worthy of note that adequate precautions were taken to deposit the encapsulated microspheres on the lower or middle portion of the rat’s rectum with the expectation of causing the absorbed drug to drain directly into the general circulation.
via either the lower or middle haemorrhoidal veins. This target may have been realized considering the high AUC values obtained for the microspheres.

It is equally discernible from Table 4 that the bioavailability of cefaclor was generally higher from the microspheres when compared with that of the control. To adjudge whether there was any significant difference in the AUC values of cefaclor from the microspheres and that from the control, the student’s t-test was employed. At 5% level of significance, the AUC values of cefaclor from the bioadhesive microspheres were found to be significantly different from that of the control. The longer $t_{\text{max}}$ values for mucin-gelatin microspheres relative to that for gelatin alone may suggest that the mucin samples modified the characteristics of gelatin microspheres. This modification may have resulted in the prolongation of the in vivo release of cefaclor from the microspheres.

It has been shown in this study that the rectal route could provide a therapeutically viable alternative to the oral route for the delivery of cefaclor, which hitherto, is administered only orally either as tablets/capsules or as suspensions. The findings from this study may be exploited in the design of a rectal delivery system for cefaclor.

Acknowledgements

Technical assistance, in the pharmacokinetic studies, from Dr. J. I. Ihedioha of the Department of Veterinary Pathology and Microbiology of our University is deeply and gratefully acknowledged.

REFERENCES AND NOTES

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