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Comparison of in situ gelling formulations for the oral delivery of cimetidine

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Abstract

Three liquid formulations with in situ gelling properties have been assessed for their potential for the oral delivery of cimetidine. The formulations were dilute solutions of: (a) enzyme-degraded xyloglucan, which form thermally reversible gels on warming to body temperature; (b) gellan gum and; (c) sodium alginate both containing complexed calcium ions that form gels when these ions are released in the acidic environment of the stomach. The in vitro release of cimetidine from gels of each of the compounds followed root-time kinetics over a period of 6 h. Plasma levels of cimetidine after oral administration to rabbits of each of the formulations were compared with those resulting from administration of a commercial cimetidine/alginate suspension with an identical drug loading. In vivo release characteristics of each of the in situ gelling formulations were similar to those of the commercial preparation.

Keywords: Xyloglucan gels; Gellan gels; Alginate gels; In situ gelation; Sustained release; Cimetidine; Oral drug delivery

1. Introduction

Cimetidine was the first H_2-receptor antagonist marketed to control gastric acid secretion and is currently available as a suspension in combination with sodium alginate (for example, Algitec™ and Tagamet™ Dual Action, GlaxoSmithKline), for the suppression of postprandial reflux. In this paper we report an assessment of three liquid formulations that form gels in situ in the stomach and have potential for application as sustained release oral dosage forms for the administration of cimetidine.

The three compounds with in situ gelling characteristics selected for study were derived from natural origin and included xyloglucan, gellan gum and sodium alginate. Xyloglucan polysaccharide derived from tamarind seed is composed of a (1-4)-β-D- glucan backbone chain which has (1-6)-α-D-xylose branches that are partially substituted by (1-2)-β-D-galactoxylose. When xyloglucan is partially degraded by β-galactosidase the resultant product exhibits thermally reversible gelation by the lateral stacking of the rod-like chains. The

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sol-gel transition temperature varies with the degree of galactose elimination (Yuguchi et al., 1997); the material used here had a percentage of galactose removal of 44% and exhibited a thermally reversible transition from sol to gel at temperatures of between 22 and 27°C. We previously reported the potential use of xyloglucan gels for rectal (Miyazaki et al., 1998), intraperitoneal (Suisha et al., 1998) and oral drug delivery (Kawasaki et al., 1999). Its potential application in oral delivery exploits the slow gelation time (several minutes) that, it is proposed, would allow in situ gelation in the stomach following the oral administration of chilled xyloglucan solutions. We recently demonstrated sustained release of indomethacin and diltiazem (Kawasaki et al., 1999) from gels formed in situ in the gastro-intestinal tract of rats and rabbits following the administration of dilute solutions of enzyme degraded xyloglucan.

Gellan gum (commercially available as Gelrite™ or Kelcogel™) is an anionic deacetylated exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucose. It has the characteristic property of temperature dependent and cation-induced gelation (Crescenzi et al., 1990) involving the formation of double helical junction zones followed by aggregation of the double-helical segments to form a three dimensional network by complexation with cations and hydrogen bonding with water (Grasdalen and Smidsroed, 1987; Chanrasekaran et al., 1988; Chanrasekaran and Thailambal, 1990). Much of the interest in the pharmaceutical application of this material has concentrated on its application in ophthalmic drug delivery (Rozier et al., 1989; Sanzgiri et al., 1993; Rozier et al., 1997); aqueous solutions of gellan dropped into the eye undergo transition to the gel state due to the temperature and ionic conditions in the tear fluid. We have previously examined the feasibility of using gellan formulations for the oral sustained delivery of theophylline (Miyazaki et al., 1999). The proposed formulation was a gellan solution containing calcium chloride (as a source of Ca^{++} ions) and sodium citrate, which complexed the free Ca^{++} ions and released them only in the highly acidic environment of the stomach. In this way the formulation remained in liquid form until it reached the stomach, when gelation was instantaneous.

Alginic acid is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues joined by 1,4-glycosidic linkages. The proportion of each block and the arrangement of blocks along the molecule vary depending on the algal source. Dilute aqueous solutions of alginates form firm gels on the addition of di- and tri-valent metal ions by a co-operative process involving consecutive guluronic residues in the G blocks of the alginate chain (Grant et al., 1973; Morris et al., 1973, 1978; Liang et al., 1980). This property has been widely exploited for the fabrication of vehicles for the sustained delivery of bioactive molecules, usually as matrix devises (Nakano and Ogata, 1984; Johnson and Medlin, 1985; Stockwell et al., 1986; Yotsuyanagi et al., 1987; Segi et al., 1989; Nicholson et al., 1990). There have been only few reports on the use of alginates in liquid sustained release preparations for oral administration. Zatz and Woodford (1987), developed a suspension formulation of theophylline which contained sodium alginate and which formed a gel when in contact with simulated gastric fluid. Katayama et al. (1999) reported a liquid sustained release formulation containing sodium alginate intended for the eradication of *Helicobacter pylori* in which in situ gelling was achieved by the separate oral administration of a solution of a calcium salt immediately following that of the sodium alginate solution. We have recently reported (Miyazaki et al., 2000) an alternative strategy to achieve in situ gelation of sodium alginate solutions, which was similar to that described above for the in situ gelation of gellan. In this method gelation of a solution of sodium alginate containing Ca^{++} ions is delayed until the preparation reaches the acid environment of the stomach through complexation of the Ca^{++} ions with sodium citrate. It should be noted that although the commercial preparations cited above contain sodium alginate, they do not include a source of metal ions. It is not, of course, the intention with these commercial preparations

that the alginate should form a gel matrix in the stomach as in the formulations discussed here, but rather should form a raft on the surface so reducing acid regurgitation.

This present study is a comparison of both the in vitro and in vivo release of cimetidine from gels formed by each of the three formulations, from the commercial oral formulation Algitec™ suspension, and also from a solution of cimetidine at pH 5.0.

2. Materials and methods

2.1. Materials

Xyloglucan with a percentage of galactose removal of 44% (Lot. 9530L) was prepared as described previously (Shirakawa et al., 1998) and supplied by Dainippon Pharmaceutical Co., Osaka. Deacetylated gellan gum, Kelcogel™, was supplied by Dainippon Pharmaceutical Co., Osaka, and was used as received. Sodium alginate (Duck Algin™, 350 ± 50 cP for a 1% solution, M/G ratio 0.8–1.0) was supplied by Kibun Food Chemifa Co., Tokyo. Cimetidine was obtained from Wako Pure Chemical Ind. Ltd., Osaka. A commercially available product, Algitec™ suspension (20 mg ml^{-1}), was supplied by GlaxoSmithKline Pharmaceuticals, UK.

2.2. Preparation of sols

Xyloglucan sols of concentrations 0.5, 1.0 and 1.5% w/w were prepared by slowly adding a weighed amount of the enzyme-degraded xyloglucan to cold phosphate buffer pH 5.0. The mixture was slowly homogenized (Nihon Seiki Seisakusho homogenizer type HB) and an appropriate amount of cimetidine was then dissolved in the resulting solution to prepare a 1% w/v sol.

Gellan gum solutions of concentrations 0.25, 0.5 and 1.0% w/v were prepared by adding the gum to ultrapure water containing 0.17% w/v sodium citrate and heating to 90°C while stirring. After cooling to below 40°C appropriate amounts of calcium chloride (0.016% w/v) and cimetidine (1% w/v) were then dissolved in the resulting solution.

Sodium alginate solutions of concentrations 1.0, 1.5 and 2.0% w/v were prepared by adding the alginate to ultrapure water containing 0.25% w/v sodium citrate and 0.075% w/v calcium chloride and heating to 60°C while stirring. Cimetidine was then dissolved in the resulting solution after cooling to below 40°C.

2.3. Measurement of viscosity of sols

The viscosity of sols prepared in water was determined with a cone and plate viscometer (TV-20H, model E, Tokimec Co., Tokyo) at 5 or 20°C using a 1 ml aliquot of the sample.

2.4. Measurement of drug release rate from gels

The release rates of cimetidine were measured by using plastic dialysis cells similar to that described previously (Miyazaki et al., 1984). The capacity of each half-cell was 4 ml and the surface area of the membranes was 2.67 cm^2.

Gels of xyloglucan (prepared in pH 7.4 buffer), gellan (prepared in ultrapure water) or alginate (prepared in ultrapure water) loaded with 1% w/v of drug, were placed in the donor compartment. An equal volume of simulated gastric (pH 1.2) or intestinal (pH 6.8) fluid (as specified for the JP XIII disintegration test) was placed in the receptor compartment. The donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales Co., size 36/32). The assembled cell was shaken horizontally at the rate of 60 strokes per min in an incubator. The total volume of the receptor solution was removed at intervals and replaced by fresh release medium. The drug concentration of the samples was determined using a spectrophotometer at a wavelength of 220 nm.

2.5. Animal experiments

White male rabbits weighing 2.8–3.3 kg were fasted for 24 h prior to the experiments but allowed free access to water. A yoke was used to avoid the possibility of coprophagy, in addition to the fasting process, which ensured that very little food was present in the stomach (from visual

observation). Gels containing cimetidine were produced in situ by oral administration of 8 ml of the appropriate solution containing 80 mg of drug using a stomach sonde needle for rabbits (Natume Seisakusho, KN-342). A stomach sonde needle was also used for oral administration of commercial Algitec™ suspension (80 mg in 4 ml) and aqueous solution at pH 5.0 (80 mg in 8 ml). At given intervals, 0.5 ml blood samples were taken from the ear vein and analysed as described below.

2.6. Determination of cimetidine

The plasma samples were separated by centrifugation and assayed by HPLC (Shimazu LC-10A with a Shimazu SPD-10A detector at a wavelength of 228 nm). The assay of cimetidine was based on the methods described by Russel et al., 1994; Kelly et al., 1995, with minor modifications. To 100 μl of plasma was added 100 μl of ranitidine solution (10 μg ml^{-1}) as internal standard, 100 μl of 1 M sodium hydroxide, 100 μl of saturated solution of potassium carbonate, and 1 ml of ethyl acetate–isoamyl alcohol (96:4) and the sample was vortex-mixed and centrifuged. To 100 μl supernatant was added 100 μl of 0.01 M hydrochloric acid. After shaking and centrifugation, the aqueous phase was passed through a Millipore filter (0.45 μm) and injected onto a 250 × 46 mm i.d. column, packed with Inertsil-ODS2. Elution was carried out with 0.01 M phosphate buffer at pH 6.2 containing 2.5 g l^{-1} heptanesulfonic acid:acetonitrile (75:25) at a rate of 1.0 ml min^{-1} at 40°C.

3. Results and discussion

3.1. Viscosity of sols

Fig. 1 compares the shear dependency of the viscosity of xyloglucan, gellan and alginate sols of a range of concentrations, with Algitec™ suspension. Measurements were performed under conditions representative of those of their proposed administration; the xyloglucan solutions were maintained in the sol form by measurement at 5°C whereas all other formulations were measured at 20°C.

All sols showed a marked increase of viscosity with concentration, higher concentrations exhibiting shear thinning behaviour. The 2.0% w/v alginate sol had a significantly higher viscosity than the Algitec™ suspension at all shear rates, which may be a disadvantage in oral administration. For

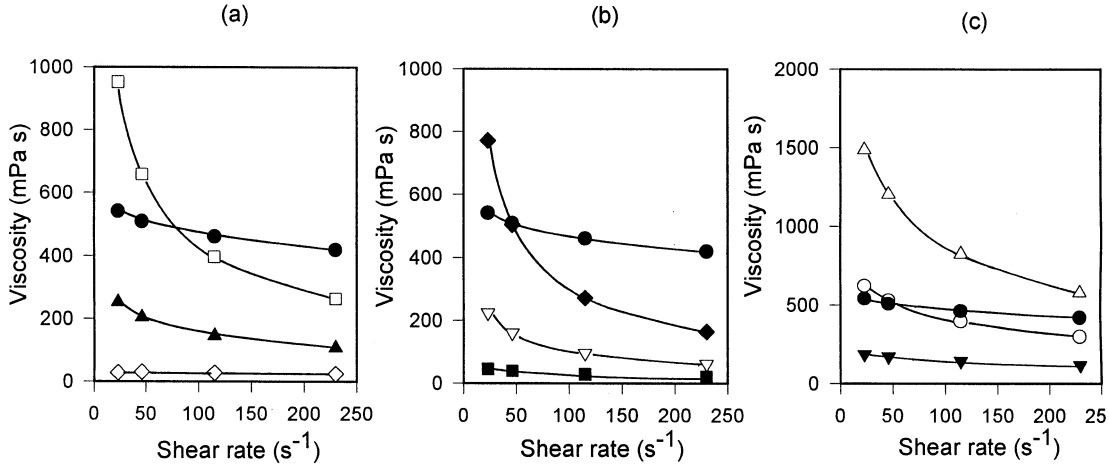

Fig. 1. Comparison of the shear rate dependency of the viscosities of (●) Algitec™ suspension (20°C) and (a) xyloglucan sols of concentrations 0.5 (◇), 1.0 (▲) and 1.5 (□)%w/w (5°C), (b) gellan sols of concentrations (■) 0.25, (▽) 0.5 and (◆) 1.0% w/v (20°C) and (c) alginate sols of concentrations (▼) 1.0, (○) 1.5 and (△) 2.0% w/v (20°C).

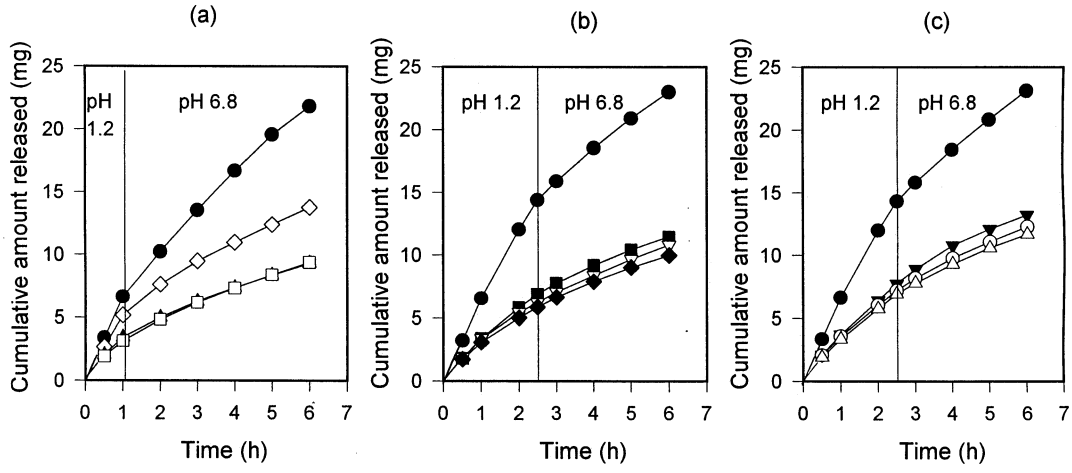

Fig. 2. Cumulative release of cimetidine as a function of time from (a) xyloglucan sols of concentrations 0.5(◇), 1.0(▲) and 1.5(□)%w/w, (b) gellan sols of concentrations (■) 0.25, (▽) 0.5 and (◆) 1.0% w/v and (c) alginate sols of concentrations (▼) 1.0, (○) 1.5 and (△) 2.0% w/v and from (●) an aqueous solution of pH 5.0. Release was into simulated gastric fluid pH 1.2 for the period of time indicated and subsequently into simulated intestinal fluid pH 6.8. Each value is the mean ± S.E. of 4–7 determinations.

this reason we chose to conduct in vivo experiments (Section 3.3) on 1.5% alginate gels. Only the highest concentrations of the other sols at low shear rates had viscosities exceeding that of Algitec™ suspension and their oral administration would not therefore be expected to present any difficulty.

3.2. In vitro drug release

The release profiles of cimetidine from gels of the three materials loaded with 1.0% w/v drug are compared with that from an aqueous solution of pH 5 in Fig. 2. It was not possible to conduct a comparison of the release characteristics with those of Algitec™ suspension because of osmotic effects in the apparatus used. The receptor solutions were changed after a given time interval from simulated gastric fluid at pH 1.2 to a simulated intestinal fluid at pH 6.8 to mimic gastro-intestinal transit. There were no inflections in the release curves for the gels in the region of the pH change despite a reduction in ionisation of the basic group of cimetidine ($pK_a = 6.8$) to 50% at pH 6.8. Although a pH of 1.2 was used as a representation of the gastric acidity, there is evidence that the pH of the rabbit stomach may not be as low as this, and consequently inferences from in vitro to in vivo data should be tempered with caution.

There is evidence in Fig. 2 of a tendency for decreasing release rate with increasing gel concentration for alginate and gellan gels. The more marked difference between release profiles of the 0.5% w/w xyloglucan gels and those of higher xyloglucan concentrations has been noted previously for the release of indomethacin (Kawasaki et al., 1999).

The release data over the whole time period were analysed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. For the initial 50–60% release, the cumulative amount Q of drug released per unit surface area from gels of initial drug concentration C_0 is proportional to the square root of time t:

$$Q = 2C_0(Dt/\pi)^{1/2} \quad (1)$$

Plots of Q vs. $t^{1/2}$ for the release of cimetidine from all gels ($C_0 = 10$ mg ml^{-1}) were linear after a short lag period indicative of diffusion controlled release; a representative plot for gellan gels

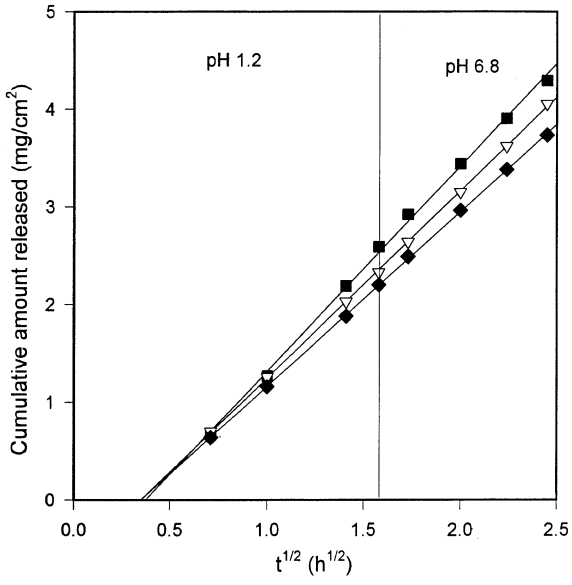

Fig. 3. Cumulative release per unit area, Q, for cimetidine as a function of square root time from gellan gels of concentrations (■) 0.25, (▽) 0.5 and (◆) 1.0% w/v. Release was into simulated gastric fluid pH 1.2 for a period of 2.5 h and subsequently into simulated intestinal fluid pH 6.8. Each value is the mean ± S.E. of 4–7 determinations.

is shown in Fig. 3. Diffusion coefficients, D, calculated from the gradients of these plots are given in Table 1. The values show the expected decrease with increasing gel concentration as a consequence of increased resistance to drug diffusion through the gel matrix. The significantly higher D value for diffusion through 0.5% xyloglucan gels reflects the very weak structure of these gels as was evident from rheological studies reported previously (Kawasaki et al., 1999).

Table 1
Comparison of diffusion coefficients, D, for in vitro release of cimetidine from gels of xyloglucan, gellan and sodium alginate

Dosage form	10^5 D (cm^2 s^{-1})
Xyloglucan (%w/w)	
0.5	1.16
1.0	0.52
1.5	0.55
Gellan (% W/v)	
0.25	0.96
0.50	0.80
1.0	0.70
Sodium alginate (%w/v)	
1.0	1.33
1.5	1.08
2.0	1.00

3.3. In vivo release

The release of cimetidine from gels of each formulation formed in situ in the rabbit stomach following oral administration of 8 ml of the appropriate solution containing 80 mg of cimetidine was monitored by the determination of plasma drug levels. In each case comparison was made with cimetidine levels following oral administration of Algitec™ suspension and aqueous solutions of cimetidine at pH 5. Thermally reversible gelation of xyloglucan sols occurred as chilled sols reached body temperature; gelation of the gellan and alginate sols, which contain calcium ions in complexed form, occurred as a consequence of the release of the calcium ions in the acidic environment of the stomach. Gelation of each of these formulations was confirmed by visual observation of the stomach contents which showed the presence of distinct gel blocks of regular shape.

Despite the differences in the structure of the gels and the mechanism of their gelation, the in vivo release curves from the three gels were similar and resembled that for the commercial suspension (Fig. 4). It should be noted, however, that in order to maintain the cimetidine in solution it was necessary to use an 8 ml volume for the sols compared with 4 ml of the Algitec™ suspension containing the same dose. Absorption of cimetidine from the solution at pH 5.0 was rapid with a peak plasma concentration at 1.38 h. The plasma-concentration curves for all other formulations showed evidence of a more sustained release as noted from the t_{max} values of Table 2. Fig. 4 shows a higher C_{max} for 1.5% w/w xyloglucan than those of the other preparations.

The areas under the plasma concentration–time curve (AUC) and the mean residence times (MRT) were obtained from the plasma concentration-time data for each animal using a computer program for model-independent analysis (Yamaoka et al., 1981) and are summarized in Table

Table 2
Comparison of bioavailability parameters of cimetidine administered from gels of xyloglucan, gellan and sodium alginate formed in situ in rabbit stomach and from Algitec™ suspension and buffer solution pH 5[a]

Dosage form	t_{max} (h)	C_{max} (μg ml^{-1})	AUC (0–8 h) (μg h ml^{-1})	MRT (h)
1.5% xyloglucan	3.50 ± 0.29[b]	1.01 ± 0.32	2.99 ± 0.76	3.77 ± 0.09[b]
1.0% gellan	2.75 ± 0.25	0.79 ± 0.11	2.27 ± 0.14	3.60 ± 0.17[b]
1.5% alginate	3.50 ± 0.29[b]	0.73 ± 0.11	2.46 ± 0.41	3.58 ± 0.25
Algitec™ Susp	3.50 ± 0.29[b]	0.80 ± 0.07	2.48 ± 0.33	3.81 ± 0.32[b]
Solution pH 5	1.38 ± 0.55	1.17 ± 0.18	2.96 ± 0.34	2.58 ± 0.36

[a] Each value represents the mean ± S.E. of four experiments.
[b] $P < 0.05$ compared with pH 5.0 phosphate buffer.

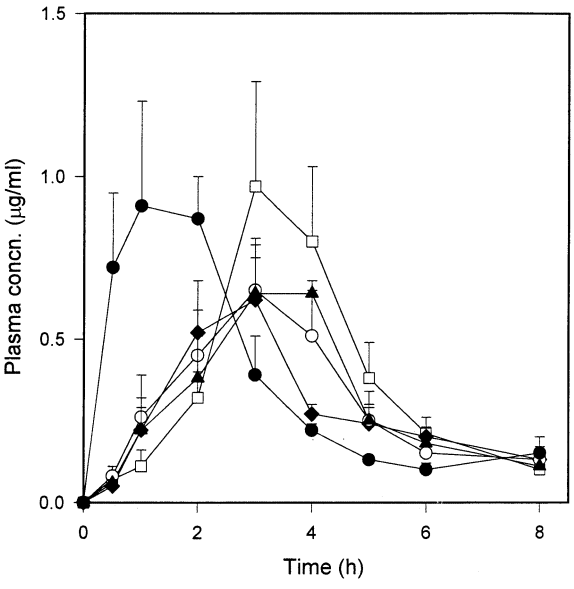

Fig. 4. Plasma concentrations of cimetidine in rabbits after oral administration of (□) 1.5% w/w xyloglucan sols, (◆) 1.0% w/v gellan sols, (○) 1.5% w/v alginate sols, (▲) Algitec™ suspension and from (●) an aqueous solution of pH 5.0. All formulations contained 80 mg cimetidine. Each value represents mean ± S.E. of four determinations.

2. Values of both parameters were similar for each of the gel formulations and also those of the Algitec™ suspension.

4. Concluding remarks

We have demonstrated that similar in vivo release characteristics to those of a commercial preparation for the oral administration of cimetidine (Algitec™ suspension) can be achieved with each of the three in situ gelling formulations. Of these, xyloglucan is of widest application in drug delivery since its gelation does not require the presence of H^+ ions and its use is not restricted by the nature of the drug as is the case with gellan formulations where incorporation of certain drug salts may cause gelation before administration. All of the formulations are homogeneous liquids and do not have the problems associated with the administration of suspensions. In addition, it may be possible to achieve a more sustained release by manipulation of the concentrations of the components of the in situ gelling formulations.

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