pH-Responsive Gelatin Microspheres for Oral Delivery of Anticancer Drug Methotrexate

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SYNOPSIS

Multiple unit delivery dosage forms of biodegradable gelatin microspheres containing the anticancer drug methotrexate (GMM) of various mean particle sizes (1–5, 5–10, and 15–20 μm) were prepared by the polymer dispersion technique and were crosslinked with glutaraldehyde. The GMM were coated with biodegradable natural polymers, namely alginate (AGMM) and chitosan (CGMM), which differ in their pH sensitivity, to obtain two different types of pH dependent delivery systems for oral delivery of methotrexate (MTX). The *in vitro* release profiles of MTX from AGMM and CGMM were determined in simulated gastric medium, intestinal medium, and in media simulating gastrointestinal tract conditions. The effect of the concentration of coating polymer and particle size on the release rate of MTX from both AGMM and CGMM were also studied. Both AGMM and CGMM provided controlled release of MTX following a zero-order release pattern in gastric and intestinal fluids for prolonged periods of time. The release rate of MTX decreased with an increase in concentration of the coating polymer as well as an increase in particle size of the microspheres. Both AGMM and CGMM showed good potential as pH dependent multiple unit delivery systems for the controlled release of MTX in oral administration.
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INTRODUCTION

Oral drug administration is the most popular, convenient, and traditionally preferred mode of delivery of therapeutic agents. Therapeutic drug levels are often achieved by taking multiple and regularly spaced daily doses of medicine. The use of colloidal particulate carriers such as microspheres as an oral delivery system are ideal in providing a constant therapeutic and nontoxic level of the drug and thereby obviating the need to remember to take the pills or tablets frequently. In the oral drug delivery, the delivery system should pass through the gastrointestinal (GI) tract where pH varies widely. In most cases, the delivery system should remain unaffected by the gastric acidity and reach the intestine where drug action or absorption is desired. One primary approach to protect the incorporated drug in the system as well as the system itself from degradation in the gastric juice is to coat the system with biocompatible, nontoxic polymers that are insoluble at acidic pH but dissolve in the alkaline pH of the intestine and release the drug. Modifications of the surface characteristics of microspheres by coating with polymers to alter their body distribution[1-4] and enteric coating of oral drug delivery devices to mask their unpleasant taste or protect them from degradation in the acidic environment of the stomach have been reported by many investigators.[5-7]

Methotrexate (MTX), which is widely used in cancer chemotherapy, causes many toxic side effects such as vomiting, diarrhea, GI ulceration, and liver and kidney damage.[7-9] We reported earlier on the development of gelatin microspheres (GMs) containing MTX for the controlled release of the drug.[10] The present investigation is concerned with the modification of MTX containing microspheres by coating with two biodegradable natural polymers that differ in their pH sensitivity. In this study, two

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natural polymers, namely chitosan and sodium alginate, were chosen for the coating of GMs.

Chitosan is deacetylated chitin and a linear polymer of 2-amino-2-deoxy-beta-D glucan and is abundant in nature as a major hexose of the crustacean skeleton. Increasingly over the last few years, chitosan has been examined for its potential use as a biomaterial in controlled drug delivery systems.[11] Chitosan contains primary and secondary alcoholic groups and its amine group renders the polymer soluble in dilute organic acids. The polymeric cationic character along with its potentially reactive functional groups has given it unique properties for utilization in controlled release systems.[12-15] Because chitosan is easily digestible and is reported to have lipid lowering effects, it is a favorable candidate for oral delivery application.[16-18] Sodium alginate is a linear polymer of β-(1-4)-D-mannosyluronic acid and α-(1-4)-L glucosyluronic acid residues. It has been used widely in the preparation of delivery systems. The polymeric anionic character of alginate renders the polymer suitable for enteric coating of oral delivery systems.[19-23]

These two natural polymers were selected in this study, based on their merits such as nontoxicity, biocompatibility, and *in vivo* degradation properties. By coating with these body friendly polymers, it was possible to obtain two different pH-responsive systems for the delivery of MTX. Work on the modification of MTX loaded GMs by coating with alginate and chitosan, their characterization, and the *in vitro* release profiles of the incorporated MTX in simulated gastric and intestinal fluids is reported herein.

EXPERIMENTAL

Materials

Gelatin (Oxoid, England), glutaraldehyde (25%; Fluka), sodium alginate (Riedel, Germany), calcium chloride (BDH, England), potassium persulfate ($K_2S_2O_8$), and sodium bisulphite ($NaHSO_3$; Loba, India) were used as obtained. Chitosan was supplied by the Central Institute of Fisheries Technology (Cochin, India). MTX was a gift sample from Tamil Nadu Dadha Pharmaceuticals Ltd., India. Polyphosphoric acid was procured from Merck, Germany. Methylmethacrylate (MMA) (Sisco, India) was purified by distillation under reduced pressure. All other reagents used were of analytical grade.

Methods

Preparation of GMs Containing MTX (GMM)

GMMs were prepared by the simple and elegant polymer dispersion technique as reported earlier by us.[10] Polymethylmethacrylate (PMMA) was prepared by the $NaHSO_3$—$K_2S_2O_8$ redox initiation technique from distilled MMA. Briefly, GMs were prepared by dispersing a solution of gelatin in phosphate buffer pH 7.4 using PMMA in organic medium as dispersant. The microspheres were crosslinked with glutaraldehyde saturated toluene. The GMMs of various particle sizes were prepared by varying the concentration of gelatin and PMMA. During the addition of PMMA and crosslinking agent, the solution of gelatin and drug was stirred using a vortex mixer.

Percent Entrapment of MTX in GMs

Indirect Method. The GMMs were given quick successive washings with phosphate buffer pH 7.4. Aliquots from the washings were filtered through a 0.45-μm Millipore filter and assayed spectrophotometrically at 371 nm to determine the surface drug. The amount of MTX entrapped in the microspheres was calculated from the difference in the amount of MTX loaded and the MTX removed from the surface of the GMM.

Direct Method. A known quantity of MTX loaded microspheres were hydrolyzed in 6*N* HCl at 60°C for 20 min. Aliquots from the solution were filtered through a 0.45-μm Millipore filter and assayed spectrophotometrically at 243 nm to determine the amount of MTX entrapped in the GMM.

Polymeric Coating of GMMs

Alginate Coating of GMM. The GMMs were coated by shaking them gently while in contact with solutions of sodium alginate of selected concentrations for 15 min. The coating solution was drained and then the alginate coated GMMs (AGMMs) were crosslinked by the addition of calcium chloride of selected concentration (contact time 5 min). The excess solution was drained and the polymer coated GMs were air dried.

Chitosan Coating of GMM. The GMM were coated with chitosan by shaking them slowly in solutions of selected concentrations of chitosan in acetic acid for 15 min. After draining the chitosan solutions, the chitosan coated GMMs (CGMMs) were cross-

linked by the addition of polyphosphoric acid of appropriate concentrations (contact times 5 mins). The excess solution was drained and the polymer coated GMs were air dried.

Characterization of Polymer Coated GMs

Particle Size Analysis of GMM, AGMM, and CGMM. About 200 GMM, AGMM, and CGMM were randomly selected and their particle sizes were measured using an optical microscope fitted with a micrometer scale (Hertal Reuss, Germany). Their percent frequency was plotted against their particle size.

Optical and Scanning Electron Microscopy (SEM). The surface morphology of GMM, AGMM, and CGMM was studied using optical microscope and scanning electron microscope (Cambridge Steroscan S-150).

In Vitro *Release Studies.* The pH dependent *in vitro* profiles of AGMM and CGMM were determined in simulated gastric fluid (0.1*N* HCl, pH 1.2) and simulated intestinal fluid (0.01*M* phosphate buffer, pH 7.4) at 37°C and 100 rpm using dissolution test apparatus (Veego Model 6RD). Release studies of MTX from AGMM and CGMM were also carried out in 0.1*N* HCl, pH 1.2 for 2 h, followed by a change to 0.01*M* phosphate buffer, pH 7.4 to simulate GI tract conditions. To study the effect of particle size on the rate of drug release, AGMM and CGMM of various mean particle sizes were used in the release experiments. The effect of concentration of coating solution (w/v) on the release rate of MTX in gastric fluid was studied by using AGMM (mean particle size 15–20 μm) coated with various concentrations of sodium alginate and crosslinked with appropriate concentrations of calcium chloride. Similarly, CGMM (mean particle size 15–20 μm) coated with two different concentrations of chitosan solution (w/v) and crosslinked with appropriate concentrations of polyphosphoric acid, was used to study the effect of chitosan concentration on the rate of drug release in intestinal fluid.

RESULTS AND DISCUSSION

Hydrophilic, biodegradable GMs of various mean particle sizes were prepared and crosslinked with glutaraldehyde by the polymer dispersion method. The microspheres were obtained as a free flowing powder. By making appropriate changes in the concentration of PMMA and gelatin it was possible to prepare GMs of various sizes. The surfaces of the GMs were then modified by coating with natural biodegradable polymers. Two types of pH sensitive GMM were obtained by coating with the natural polymers alginate and chitosan, which differ in their pH sensitivity. Different concentrations of the two polymers used in the coating of microspheres (GMM) is given in Table I.

Percent Entrapment of MTX in GMM

Percent entrapment of MTX in GMM and the drug incorporation efficiency of the GMM were calculated. Table II gives the percent entrapment of MTX in GMM of various sizes. About 80% of MTX was entrapped in microspheres of different sizes ranging from 1 to 20 μm. There was a slight increase in the

Table I Compositions of Coating Solutions on Gelatin Microspheres

	Crosslinking Solution			
Mean Particle Size of Microspheres (μm)	Sodium Alginate in H_2O (%)	Calcium Chloride in H_2O (%)	Chitosan in Acetic Acid (%)	Polyphosphoric Acid in H_2O (%)
1–5	2	4	2	4
5–10	2	4	2	4
15–20	2	4	2	4
15–20[a]	3	5	1	3
15–20	5	7	2	4
15–20	8	10	—	—

[a] Range chosen to study the effect of polymer concentration.

Table II Percentage Entrapment of MTX in Gelatin Microspheres of Different Sizes

Mean Particle Size (μm)	MTX Loading (mg)	Surface Drug (mg)	Entrapped Drug (mg)	Encapsulation (%)	μg MTX/mg Microspheres
1–5	20	3.9	16.1	80.5	53.6
15–20	20	3.8	16.2	81.0	54.0
5–10[a]	10	2.4	7.6	76.0	25.3
5–10	15	3.2	11.8	78.6	39.3
5–10	20	4.0	16.0	80.0	53.3

[a] Range chosen for different drug loadings.

amount of MTX that could be entrapped in the system with the increase in the size of the microspheres.

Particle Size Distribution of GMM, AGMM, and CGMM

Figure 1(a–c) shows the particle size distribution of uncoated and coated microspheres of three different particle size ranges, that is, 1–5, 5–10, and 15–20 μm. The particle size of microspheres ranged from 1 to 20 μm. As shown in the figures, the GMM coated with alginate and chitosan solutions did not show any significant increase in size when compared to uncoated GMs. These results clearly indicated that coating GMM using these two natural polymers could not impart any change in the particle size but showed tremendous changes in the dissolution pattern of the microspheres.

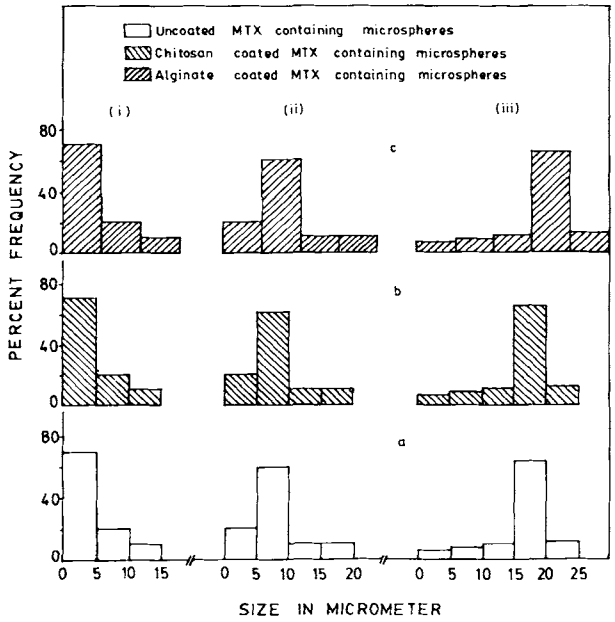


Figure 1 Particle size distribution of gelatin microspheres containing MTX: (a) uncoated GMM of mean particle size (i) 1–5, (ii) 5–10, and (iii) 15–20 μm; (b) CGMM of mean particle size (i) 1–5, (ii) 5–10, and (iii) 15–20 μm; (c) AGMM of mean particle size (i) 1–5, (ii) 5–10, and (iii) 15–20 μm.

Optical Microscopy and SEM Studies

Figures 2 and 3 are the optical photographs of AGMM and CGMM. Figures 4 and 5 show the SEM of AGMM and CGMM. It is evident from the optical and the SEM photographs that microspheres modified by coating with alginate and chitosan appeared spherical and uniform and had solid geometry. It can be seen from the figures that the surface morphology of coated microspheres was not markedly different from uncoated GMM. Therefore the GMMs were coated with only a thin layer of the polymeric membrane, but this was sufficient to cause

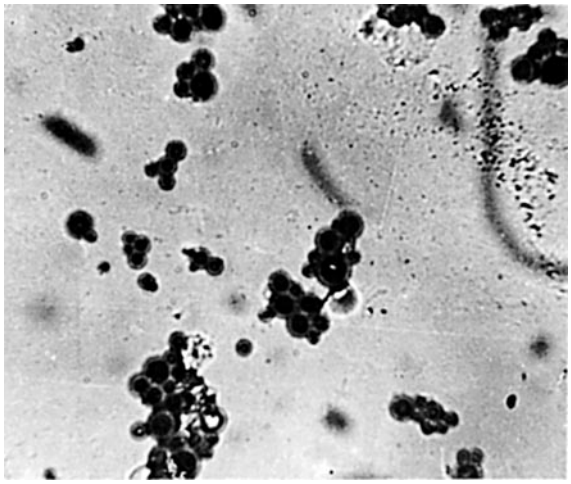


Figure 2 Optical photograph of AGMM.

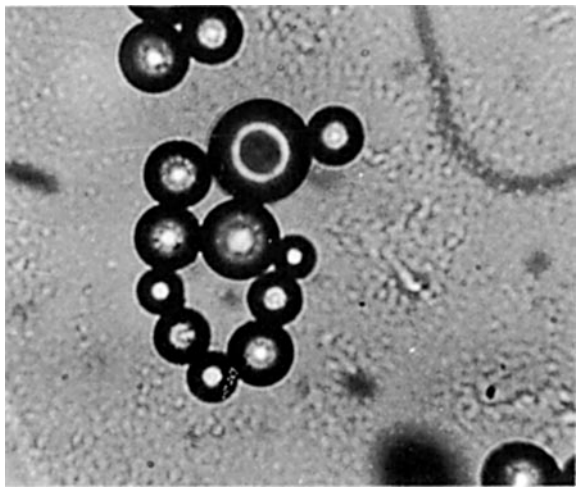


Figure 3 Optical photograph of CGMM.

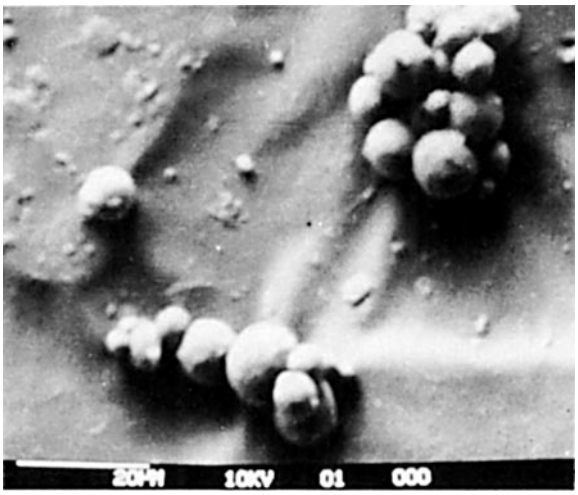


Figure 5 Scanning electron micrograph of CGMM.

tremendous differences in the *in vitro* release profiles of coated GMM when compared to uncoated GMM.

In Vitro Release of MTX

Because the aim of this investigation was to develop pH responsive gelatin microspheres for the controlled release of MTX in the GI tract, the microspheres were coated with polymers that are sensitive to either acidic or alkaline medium. In addition to the pH sensitivity of the coated polymers, the release of the drug depends on the swelling characteristics of the crosslinked polymers and the solubility of the drug in the release medium. Figure 6 is the representative graph in which the release of MTX from AGMM in gastric medium is depicted as a cumulative release profile. All other release data are expressed as rate of MTX release shown in the tables.

Simulated Gastric Fluid

Table III shows the *in vitro* release rates of MTX from AGMM and CGMM of various sizes in simulated gastric fluid. AGMM and CGMM released MTX in a controlled manner following a zero-order fashion for 6–8 and 5–7 days, respectively. AGMM released about 94–98% of MTX and CGMM released about 92–98% of MTX during the experimental period. On the other hand uncoated GMM

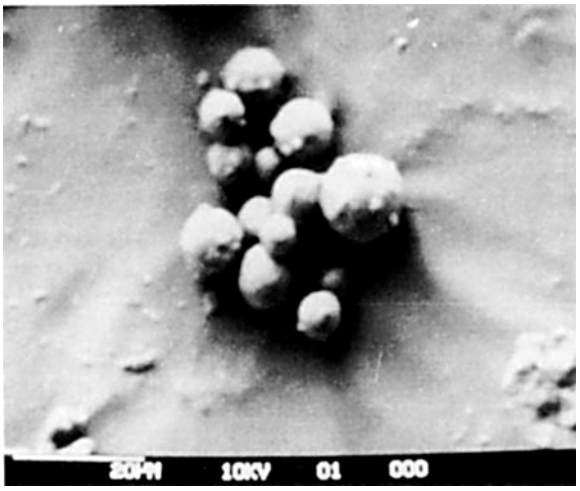


Figure 4 Scanning electron micrograph of AGMM.

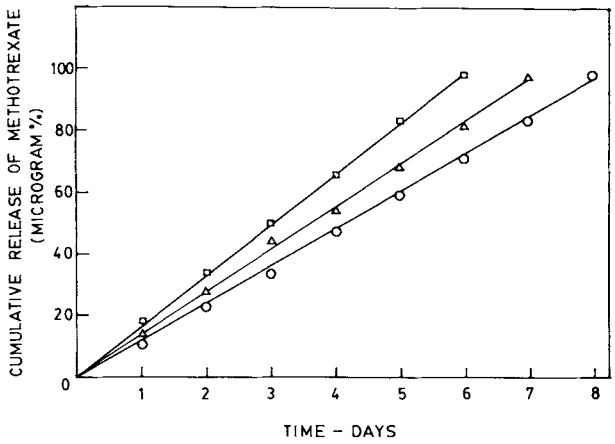


Figure 6 *In vitro* release of MTX in 0.1*N* HCl, pH 1.2 at 37°C from AGMM of mean particle size (□) 1–5, (△) 5–10, and (○) 15–20 μm sodium alginate solution 2% (w/v).

Table III *In Vitro* Release Rate of MTX from AGMM and CGMM of Various Sizes in Gastric and Intestinal Media

	Gastric Medium (μg MTX)						Intestinal Medium (μg MTX)					
	Size of AGMM (μm)			Size of CGMM (μm)			Size of AGMM (μm)			Size of CGMM (μm)		
Days	1–5	5–10	15–20	1–5	5–10	15–20	1–5	5–10	15–20	1–5	5–10	15–20
1	70	130	100	180	150	110	180	90	130	150	110	80
2	270	140	120	180	140	140	190	120	130	150	110	90
3	160	170	110	220	190	150	190	130	150	160	110	90
4	160	110	120	190	130	120	200	120	130	190	130	100
5	180	140	110	210	200	140	190	130	130	130	90	90
6	90	130	140		130	130		120	140	150	130	120
7		120	140			160		200	150	110	100	80
8			120						130		80	100
9											110	130
10												80

released MTX in a controlled manner for 4–6 days in simulated gastric fluid. These results indicated that the alginate and chitosan coats on the microspheres acted as a rate controlling barrier to drug release and thereby prolonged release of MTX when compared to uncoated GMM. The alginate coating is insoluble at pH 1.2 but allowed controlled drug release for an extended period of time. This indicated that when the release studies were continued for a longer time the alginate coating became permeable to the acidic dissolution medium and did not form a diffusion barrier. Penetration of the acidic dissolution medium into the alginate coating caused gradual swelling of the polymer with concomitant dissolution and diffusion of MTX into the medium. On the other hand MTX release from chitosan coated GMM was faster than from AGMM due to solubility of chitosan below pH 6. Further the solubility of MTX in acid pH is also one of the reasons for the gradual release of the drug after swelling of the polymer coating.

Intestinal Fluid

Table III shows the *in vitro* release rates of MTX from AGMM and CGMM coated microspheres of various sizes in simulated intestinal fluid. Release of MTX from AGMM and CGMM was controlled and followed a zero-order release pattern for 5–8 and 7–10 days, respectively. It can be seen from the figure that about 94–98% of MTX was released from AGMM and about 97–98% of MTX was released from CGMM during the release studies. On the other hand the uncoated GMM released MTX in a controlled manner for 5–8 days in simulated intestinal medium. When one compares the *in vitro* release MTX from AGMM and CGMM it is seen that the release of MTX from AGMM was faster than from CGMM due to the solubility of alginate at intestinal pH. Because alginate is highly soluble at pH 7.4, the rate of MTX from alginate coated microspheres was similar to release of MTX from uncoated GMM in intestinal media. On the other hand the chitosan coating formed a diffusion barrier that delayed MTX release for an extended period of time. The solubility of MTX at pH 7.4 also contributed to the release of the drug from the swollen polymer matrix.

Simulated GI Tract Conditions

Table IV shows the controlled and zero order *in vitro* release rates of MTX from AGMM and CGMM of various sizes initially at pH 1.2 for 2 h (simulated gastric fluid) followed by a change to pH 7.4 (simulated intestinal fluid). The release data showed that during the initial 2-h incubation in gastric fluid, only a small percentage of drug was released from both AGMM (about 0.3–1.1% MTX) and CGMM (about 0.6–2.0% MTX) when compared to uncoated GMM (about 6–8% MTX). However, a slightly higher percentage of MTX was released from CGMM than AGMM due to the better permeability of the chitosan coating to acidic dissolution medium when compared to the alginate coating. However, following a change to pH 7.4, MTX release was most rapid from GMM (up to 7 days) as compared to AGMM and CGMM. During the period of release studies in phosphate buffer, about 97–98% of MTX was re-

Table IV *In Vitro* Release Rates of MTX from AGMM and CGMM of Various Sizes in Simulated Gastrointestinal Tract Media

Type of Microsphere	Size (μm)	Gastric Medium (μg MTX)				Intestinal Medium (μg MTX)									
		Minutes				Days									
		30	60	90	120	1	2	3	4	5	6	7	8	9	10
AGMM	1–5	6.5	10	10	11	170	170	90	180	250					
	5–10	2.5	4.5	7.5	8	130	110	140	130	190	100	160			
	15–20	1	1.5	2	3	80	90	140	120	160	90	160	100		
CGMM	1–5	10	14	19	20	140	120	140	180	90	140	160			
	5–10	7	10	10	11	100	200	130	120	90	110	80	100	130	
	15–20	4	5	6	7	160	90	100	140	50	100	150	40	120	110
GMM	1–5	30	46	66	80	230	110	170	200	180					
	5–10	20	40	50	65	120	110	190	180	100	170				
	15–20	10	25	45	60	120	110	180	140	130	150	130			

leased from AGMM and CGMM, respectively. On the other hand if one compares the release from coated microspheres, AGMM released the drug faster than CGMM. This may be attributed to the cationic nature of chitosan, which has low permeability in alkaline medium. This in turn delayed MTX release from gelatin up to 10 days as compared to 8 days for AGMM. The *in vitro* behavior of uncoated and coated microspheres in simulated GI tract conditions is illustrated in Scheme 1.

Effect of Coating Polymer Concentration on Release Rate of Drug

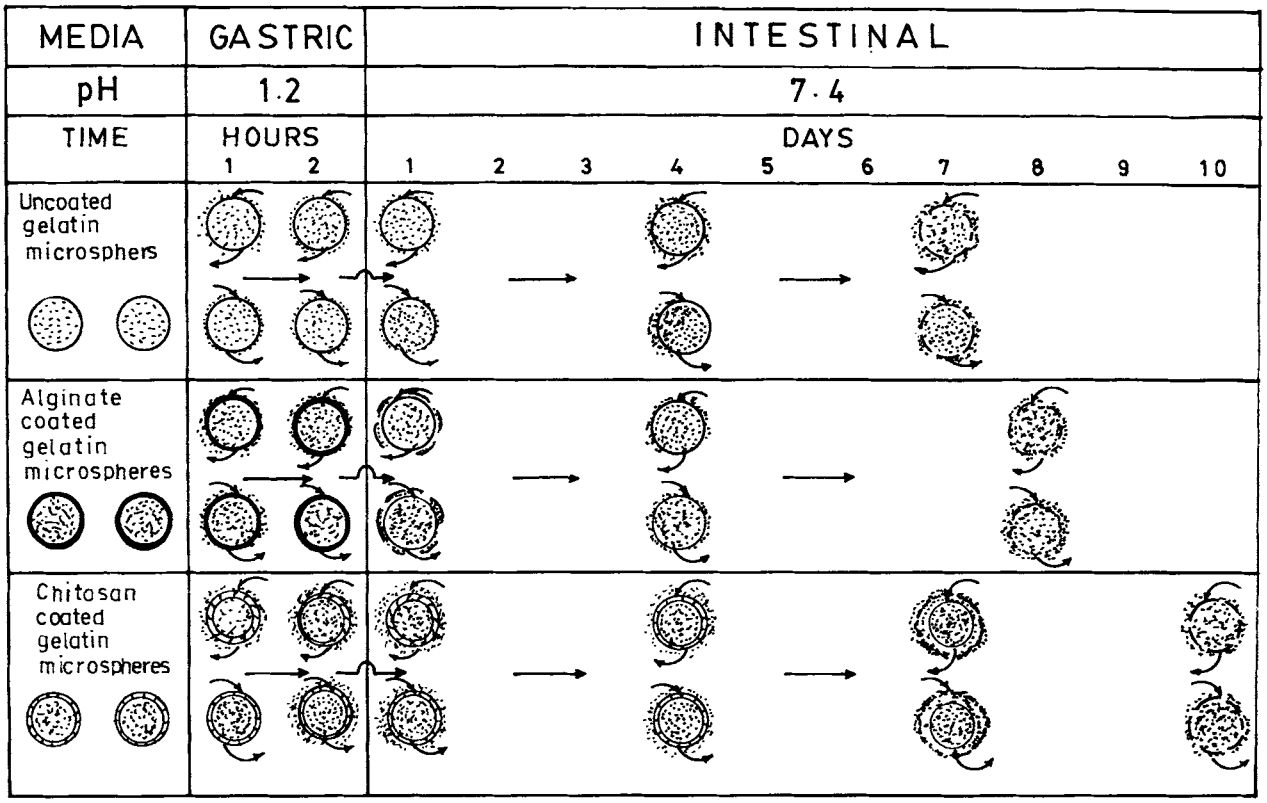
The release data of MTX from AGMM and CGMM at gastric pH and intestinal pH indicated that AGMM provided drug release for a more prolonged time period than CGMM in simulated gastric fluid. However, CGMM provided controlled drug release for more extended time periods than AGMM in simulated intestinal fluid. Because alginate is highly soluble in alkaline pH but protonates to form insoluble alginate in acid pH, the alginate coating on the GMM is certainly useful for prolonging drug release in acid medium. On the other hand, chitosan is soluble at acid pH but insoluble at alkaline pH. Hence the chitosan coat on GMM will be useful to prolong drug release in an alkaline medium. Therefore some experiments were planned to coat GMM with various concentrations of alginate and chitosan and study the release profiles of MTX in gastric fluid and intestinal fluid, respectively.

Effect of Alginate Concentration on Release Rate of MTX. Table V shows the rate of release of MTX *in vitro* from AGMM (mean particle size 15–20 μm) coated with various concentrations of alginate (w/v) in simulated gastric fluid. The controlled release of MTX could be varied from 9 to 11 days by coating GMM with suitably selected concentrations of alginate solutions. During the experimental period of 9–11 days about 97% of MTX was released from AGMM coated with various concentrations of the polymer. The release data indicated that the rate of release of MTX decreased with the increase in the concentration of alginate.

Effect of Chitosan Concentration on Release Rate of MTX. Table V shows the *in vitro* release rates of MTX from GMM coated with two different concentrations of chitosan in simulated intestinal fluid (mean particle size 15–20 μm). The amount of MTX released during the experimental period varied from 97–98% of the drug. Release of MTX could be varied from 9 to 10 days by coating GMM with chitosan solutions of two different concentrations. It was also observed that an increase in chitosan concentration decreased the release rate of MTX.

CONCLUSION

By coating the GMM with two natural biodegradable polymers that differ in their pH sensitivity, it was possible to obtain two different types of pH-respon-



Scheme 1

sive delivery systems for MTX: an acid resistant system (alginate coated GMM) and an alkali resistant system (chitosan coated GMM). Both types of delivery systems provided controlled and extended release of MTX in simulated gastric and intestinal fluids when compared to GMM. Alginate, which is insoluble at acidic pH but highly soluble at alkaline pH, provided more prolonged release of MTX in gastric fluid than chitosan. Chitosan, which is soluble at acidic pH but insoluble at alkaline pH, prolonged the release of MTX in intestinal fluid. From the *in vitro* release studies in simulated GI tract conditions it is evident that the alginate coating protected the GMs containing MTX from degradation when compared to uncoated GMs. CGMM also provided some protection during the 2-h *in vitro* test period in gastric fluid and minimized drug loss when compared to uncoated GMs. After the removal of the microspheres from the gastric fluid (pH 1.2) after 2 h and transfer to the intestinal fluid, the alginate coating quickly dissolved and further release of MTX occurred by gradual erosion of the GMs. In

Table V *In Vitro* Release Rates of MTX from Microspheres Coated with Different Concentrations of Polymers

Microspheres	Concn of Coating Polymer (%)	Days										
		1	2	3	4	5	6	7	8	9	10	11
AGMM (μg MTX)	3	130	80	120	100	110	150	110	60	130		
	5	90	80	110	100	110	70	120	70	120	100	
	8	70	110	90	90	100	70	120	150	120	100	100
CGMM (μg MTX)	1	120	100	110	90	100	100	95	110	100		
	2	80	90	90	110	100	90	90	100	150	90	

the case of CGMM, controlled and continuous drug release took place through the insoluble polymer coat in the alkaline environment of the intestinal fluid. The *in vitro* release studies also indicated that the rate of release of the drug decreased with the increase in the concentration of the coating polymer. This investigation clearly showed that it was possible to control release rate of MTX by selecting appropriate concentrations of the polymers as coating materials or by varying the size of the microspheres. By using a cocktail of GMs of different size ranges as well as microspheres coated with various concentrations the natural polymers alginate and chitosan, it is possible to obtain a viable oral delivery system for MTX in treating cancers.

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