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Microencapsulation of lipophilic drugs in chitosan-coated alginate microspheres

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Abstract

Chitosan-coated alginate microspheres containing a lipophilic marker dissolved in an edible oil, were prepared by emulsification/internal gelation and the potential use as an oral controlled release system investigated. Microsphere formation involved dispersing a lipophilic marker dissolved in soybean oil into an alginate solution containing insoluble calcium carbonate microcrystals. The dispersion was then emulsified in silicone oil to form an O/W/O multiple phase emulsion. Addition of an oil soluble acid released calcium from carbonate complex for gelation of the alginate. Chitosan was then applied as a membrane coat to increase the mechanical strength and stabilize the microspheres in simulated intestinal media. Parameters studied included encapsulation yield, alginate concentration, chitosan molecular weight and membrane formation time. Mean diameters ranging from 500 to 800 μm and encapsulation yields ranging from 60 to 80% were obtained. Minimal marker release was observed under simulated gastric conditions, and rapid release was triggered by transfer into simulated intestinal fluid. Higher overall levels of release were obtained with uncoated microspheres, possibly due to binding of marker to the chitosan membrane coat. However the slower rate of release from coated microspheres was felt better suited as a delivery vehicle for oil soluble drugs.

Keywords: Encapsulation; Alginate; Chitosan; Lipophilic drugs; Drug release

1. Introduction

Drugs are encapsulated for taste and odor masking, to stabilize the drug, improve gastrointestinal (GI) tolerance, and provide sustained-release after oral administration (Deasy 1994). Techniques used to encapsulate lipophilic

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drugs require the use of organic solvents or heat. Solvents are toxic limiting widespread applicability (Alex and Bodmeier, 1990), and vaccines, peptide hormones, vitamins or drugs containing phenols, aromatic amines, aldehydes, ethers and unsaturated aliphatic compounds are heat or oxygen labile. Epinephrine, adrenaline, morphine, apomorphin, ergobasin, eserin, reserpin, ascorbic acid, procaine, phenothiazines and vitamin A are examples of important pharmaceutical products which are readily oxidized in air (Nogueira Prista et al., 1995).

Recently, an emulsification/internal gelation technique has been described (Poncelet et al., 1992, 1995) for immobilizing labile materials on a large scale within alginate microspheres, but the application of this technique for the microencapsulation of drugs has not been described. Hydrophilic alginate gels are often reported to be leaky to various encapsulants, thus would be of interest in controlled release, particularly when applied to hydrophobic macromolecules which would be released by restricting diffusion out of the gel matrix (Robinson and Lee, 1987).

The microencapsulation of an oil soluble dye was demonstrated in this study by means of a multiple phase emulsion. The lipophilic encapsulant dissolved in an oily vehicle was dispersed within alginate sol, then dispersed again in a second oil phase forming an O/W/O emulsion. The oily encapsulant in alginate is then gelled, resulting in alginate microspheres containing immobilized oil droplets. Since alginate gels have a macroporous structure with pore diameters in the order of 10 μm (Overgaard et al., 1991), and in addition may be unstable (Polk et al., 1994), a membrane coat was applied for stability and to reduce bead permeability. Electrostatic interaction of the alginate carboxyl groups with a polycationic amine coat (Huguet et al., 1994), encloses the encapsulant improving drug retention, or potentially release (Polk et al., 1994).

Chitosan is a biocompatible and biodegradable (Chandy and Sharma, 1990) cationic polymer, which has been used to improve or control drug release (Kawashima et al. 1985; Kim and Lee, 1992; Lin and Perng, 1992; Okhamafe et al., 1996). Chitosan membranes have been previously applied to alginate beads formed by droplet extrusion (Huguet et al., 1994). Coating alginate microspheres prepared by emulsification/internal gelation is of interest since it involves a process with industrial-scale potential (Kawashima et al., 1985; Huguet et al., 1994). The objective of this study was to apply the emulsification/internal gelation technique to lipophilic encapsulants, evaluate chitosan coating for improving the encapsulation efficiency, and to explore chitosan coated alginate microspheres as an oral controlled release system for short-life oil-soluble drugs. As a model encapsulant, Sudan orange G, was incorporated into the primary oil for the evaluation of encapsulation efficiency and the in vitro release behavior of chitosan-coated alginate microspheres. Gastrointestinal side effects resulting from direct contact with mucous membranes may be reduced and intestinal absorption improved when a lipophilic drug is administered in an emulsified state (Davis et al., 1985; Uchida et al., 1996). Stabilized emulsions, fixed in an alginate gel matrix would then be a desirable delivery vehicle.

2. Materials and methods

2.1. Materials

Alginate SG 300 was supplied by Systems Bioindustries (Mississauga, ON, Canada) and silicone oil 200 (20 cSt) provided by Dow Corning (Mississauga, ON, Canada). The molecular weight of the alginate was 694 kDa, with a guluronic acid fraction of 0.60 (Quong et al., 1998). $CaCO_3$ was obtained from Omya (Orgon, France), Tween 80 and Span 80 from ICI (Brantford, ON, Canada), and pure soya oil from Le Naturiste (Montreal, QU, Canada), Sudan orange G was purchased from Aldrich (Milwaukee, WI, USA) and chitosan (75, 100 and 300 kDa) was supplied by Pronova (Raymond, WA, USA).

2.2. Microsphere preparation

The emulsification/internal gelation technique to form alginate microspheres has been described

previously (Poncelet et al., 1992, 1995). The procedure was modified to encapsulate soya oil containing a lipophilic marker. Microcrystaline $CaCO_3$ (5% w/v) was mixed into 3–5%(w/v) alginate (Ca/alginate 10% w/w), and the mixture dispersed into soybean oil containing 1.5% (w/v) Sudan orange G. The ratio of alginate to oil was 70/30. A surfactant, consisting of a blend of Span 80 and Tween 80 (60/40 v/v; final HLB = 10.7) was added at 1% (v/v), and the mixture dispersed with high-speed homogenization for 6 min in an ice bath. The resulting oil in alginate emulsion was mixed on an orbital shaker at 150 rpm for 24 h to deaerate.

The O/W emulsion was dispersed into 100 ml of silicone oil using a Caframo type RZR50 mixer (Wiarton, ON, Canada) equipped with a marine impeller. The mixing rate was varied from 400 to 1400 rpm. After 5 min emulsification, 20 ml of silicone oil containing 200 μl glacial acetic acid was added and stirring continued for 5 min to permit the solubilization of the calcium carbonate. A solution of $CaCl_2$ (0.05 M) containing 1% Tween 80 was added to partition and recover the gelled microspheres. Microspheres were washed with 0.05 M $CaCl_2$ containing 1% Tween 80 to remove residual oil.

A chitosan coat was applied by immersing the microspheres into chitosan solution (0.8% w/v) containing 0.05 M $CaCl_2$ (pH 5.5). After 10 and 30 min, microspheres were washed as described previously.

2.3. Microsphere morphology and encapsulation yield

Microsphere morphology was examined microscopically. The internal structure was examined by transmission electron microscope (TEM) by observing dehydrated thin sections prepared with a Cryostat HM 500 (Miles, Elkharty, IN, EUA) using a Philips CM200 microscope.

Volumetric size distribution (volume of microspheres in each diameter by class) of the microspheres was determined by laser light scattering with a 2062-LC particle analyzer (Malvern Instruments) with the mean diameter and standard deviation calculated from the cumulative distribution curve.

Encapsulation yield was determined by dissolving microspheres in 0.055 M sodium citrate, followed by extraction with chloroform. Extracted oil was weighed after chloroform evaporation, and compared to the initial amount of oil encapsulated to give the encapsulation yield. This procedure was also used to release and extract Sudan orange marker. Dye was quantified by absorbance at 388 nm.

2.4. Release studies

Dye release into simulated gastric (pH 1.2) and intestinal (pH 7.5) media (USP XXIII) was determined by mixing 9 g microspheres into 75 ml release media. The suspension was mixed at 150 rpm on an orbital shaker to ensure good contact between microspheres and media. Samples (20 ml) were removed and extracted with chloroform. The volume of each sample was replaced by the same volume of fresh medium. The amount of dye released and extracted into chloroform was determined spectrophotometrically.

3. Results

Soya oil dispersed in alginate resulted in opaque emulsions with droplet diameters ranging to 50 μm. Mean oil droplet diameters were similar for 3, 4 and 5% alginate dispersions at approximately 13 μm. Soya oil/alginate emulsions were then dispersed into silicone oil during the encapsulation step, resulting in spherical and dispersed microspheres as illustrated in Fig. 1. The final chitosan coating step provided a smooth morphology when viewed microscopically. TEM micrographs of uncoated and coated microspheres showed an internal ultrastructure with oil droplets ranging up to 35 μm, dispersed in the alginate matrix as shown in Fig. 2.

Microsphere diameters ranged from 120 to about 1600 μm as seen in Fig. 3. A unimodal distribution was observed with a peak in the range of 500–800 μm. Chitosan coated microspheres showed higher mean diameters compared to uncoated microspheres with the exception of the 75 kDa molecular weight chitosan coat, where

a slightly lower mean diameter was observed (Table 1) for 10 min coating time. A longer coating time (30 min) resulted in a higher mean diameter. Assuming that the increase in microsphere diameter was due to the thickness of the chitosan coat, the membrane thickness increased from 62.5 to 87.5 to 142.5 μm for 75, 100 and 300 kDa chitosans, respectively, for microspheres with mean diameters of 489 μm. The membrane thickness also increased with the coating time for 75 and 100 kDa chitosans as seen in Table 1 and no change in membrane thickness was observed for 300 kDa chitosan beyond 10 min.

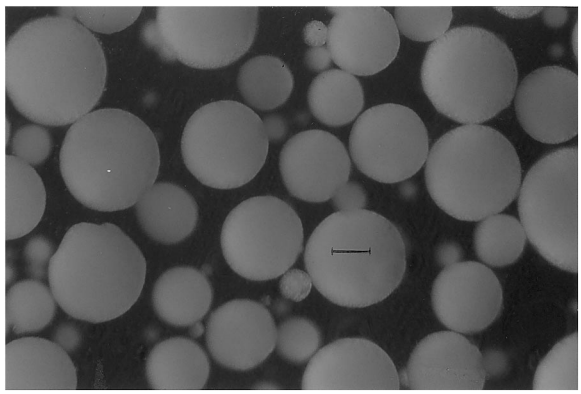

Fig. 1. Photomicrograph of alginate microspheres containing encapsulated soya oil droplets with lypophilic marker. Bar = 100 μm.

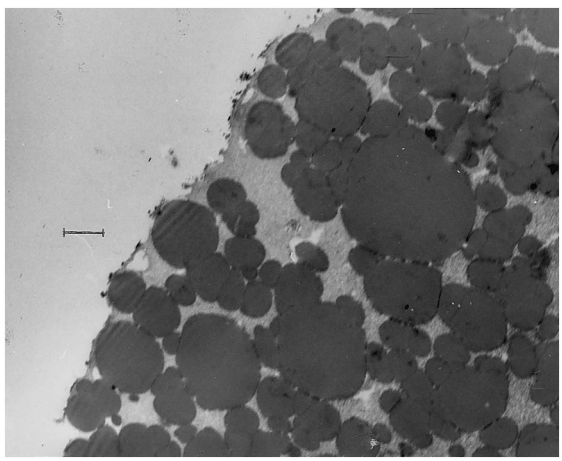

Fig. 2. TEM micrograph of alginate microsphere containing soya oil microdroplets in thin section. Bar = 1 μm.

Microsphere mean diameter depended on the mixer rotational speed during the second stage of emulsification as seen in Fig. 4. Depending on the alginate concentration, mean diameter could be varied between 300 and 700 μm, over a range of rpm between 400 and 1400. Less concentrated alginates resulted in larger diameters for rotational speeds up to 1400 rpm.

The encapsulation yield was determined by liquefying microspheres in citrate and extracting released oil and Sudan orange G into an organic solvent. The yield in Table 2 is the percentage of released and extracted oil, relative to the amount of oil initially encapsulated. Yields measured ranged from 60 to 72% based on released dye, and 70–80% based on released oil. The encapsulation yield approached 80% for alginates coated with the highest molecular weight chitosan. The coating time had little affect on the encapsulation yield.

Release profiles of encapsulated dye from uncoated alginate microspheres in simulated gastric and intestinal fluids are shown in Fig. 5. Initial release within the first hour under gastric conditions was lower than 10% of the total release. In simulated intestinal fluid, dye release was rapid to levels approaching 85% of total release within 10–15 min. No further release of dye was observed after 2 h. Similar levels of release were observed at all alginate concentrations.

Release profiles were also determined for chitosan coated microspheres as shown in Fig. 6. Again, minimal release was observed initially under gastric conditions but rapid release followed onset of simulated intestinal conditions. The total amount of dye released was less than that observed with uncoated microspheres, reaching a maximum of 35% after 2 h. The level of release was inversely related to the molecular weight of chitosan. While dye release was lower in comparison to uncoated microspheres, it reached 90% of the total release after 10 min.

As the coating time affects the membrane thickness, it would then be expected to have an influence on the release profile of encapsulated dye. In Fig. 7, it is seen that for 75 kDa chitosan, a more rapid release of dye was obtained under intestinal conditions for membrane coating times of 10 min

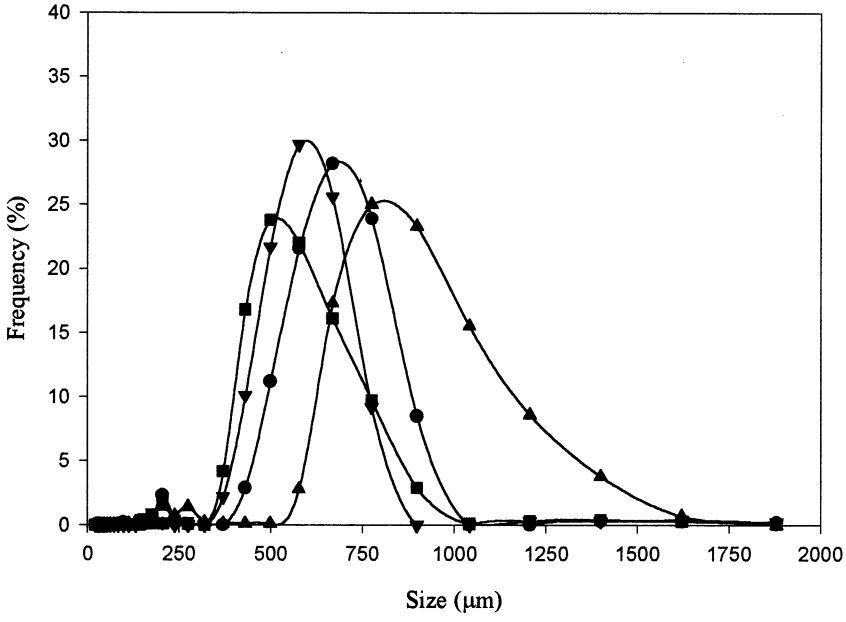

Fig. 3. Size distributions of 3% alginate microspheres (●) and microspheres coated with 75 (■), 100 (▲) and 300 (▼) kDa chitosan.

compared to 30 min. Dye release approached 35 and 18%, respectively. It appears then that the level of release may be controlled to the largest extent by membrane coating, then secondly through appropriate selection of chitosan molecular weight and coating time.

4. Discussion

A multiple-phase emulsion technique for the encapsulation of lipophilic drugs within coated alginates has been described using Sudan orange G as a lipophilic marker dissolved in an edible vegetable oil. The resulting alginate microspheres then contain immobilized oil microdroplets containing the lipophilic drug. Alginate gel then serves as a barrier, retaining oil droplets in discrete pockets within the gel matrix.

Emulsion stability during microsphere formulation was promoted through the use of a surfactant and the recognized stability of alginate interfacial films (Walstra, 1983). The influence of mixing rate during formulation on the microsphere mean diameter obtained with different alginate concentrations suggested a mixing rate limit for each alginate concentration. Higher mixing rates do not result in further reductions in mean diameter. The size distributions of coated and uncoated microspheres have been reported to be related to the alginate physicochemical properties (Poncelet et al., 1992).

Marker and oil encapsulation yields were high, approaching 80%. Losses could have occurred due to incomplete recovery of the microspheres during phase partitioning and washing operations. Dur-

Table 1
Effect of chitosan molecular weight on microsphere diameter (3% alginate, 0.8% chitosan)

Chitosan molecular weight (kDa)	Alginate-coating time (min)	Microsphere mean diameter ± S.D. (μm)
–	–	489 ± 32
75	10	451 ± 23
75	30	614 ± 10
100	10	501 ± 8
100	30	664 ± 6
300	10	778 ± 5
300	30	774 ± 13

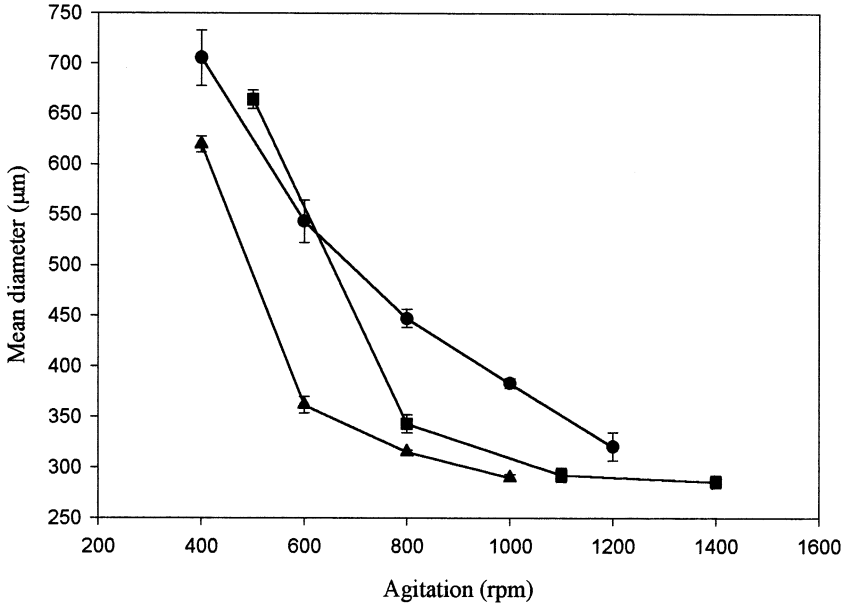

Fig. 4. Mean diameter of microspheres formed at increasing impeller rotational speed for 3 (●), 4 (■) and 5% (▲) alginates. Errors bars represent standard deviations about the mean based on three replicates.

ing formulation, the alginate gel barrier reduces loss of soya oil and encapsulant into the silicone oil external phase, and mutual immiscibility between the internal and external oil phases reduces loss of soya oil during emulsification. Dye solubility in the external oil phase may be responsible for the slightly lower encapsulation efficiency compared to that of the oil. This loss was still minor since the dye is largely insoluble in the aqueous sol (Green, 1991). Chitosan coating provided a modest improvement in encapsulation yield, with the highest yield (80%) observed for the highest molecular weight chitosan. Higher molecular weight chitosans provide thicker membranes, and have been shown to be more impermeable (Polk et al., 1994).

A pH dependent release of dye marker was observed and has been previously reported for protein release from chitosan-coated alginate microspheres (Okhamafe et al., 1996). A low initial dye release in gastric fluid from both uncoated and coated microspheres may be due to the higher

Table 2
Encapsulation yields of oil and dye in alginate and chitosan-coated alginate microspheres (0.8% chitosan)

Alginate (%)	Chitosan molecular weight (kDa)	Chitosan coating time (min)	Oil encapsulation yield (%)	Dye encapsulation yield (%)
3	–	–	70.8 ± 2.6	60.2 ± 3.2
4	–	–	71.5 ± 2.8	60.5 ± 4.4
5	–	–	71.0 ± 2.8	61.5 ± 4.7
3	75	10	73.4 ± 3.1	61.2 ± 5.7
3	75	30	75.9 ± 3.8	63.7 ± 7.9
3	100	10	75.8 ± 3.8	63.3 ± 6.6
3	100	30	75.0 ± 3.9	65.1 ± 6.3
3	300	10	79.3 ± 3.2	71.7 ± 5.1
3	300	30	75.9 ± 3.0	66.3 ± 8.8

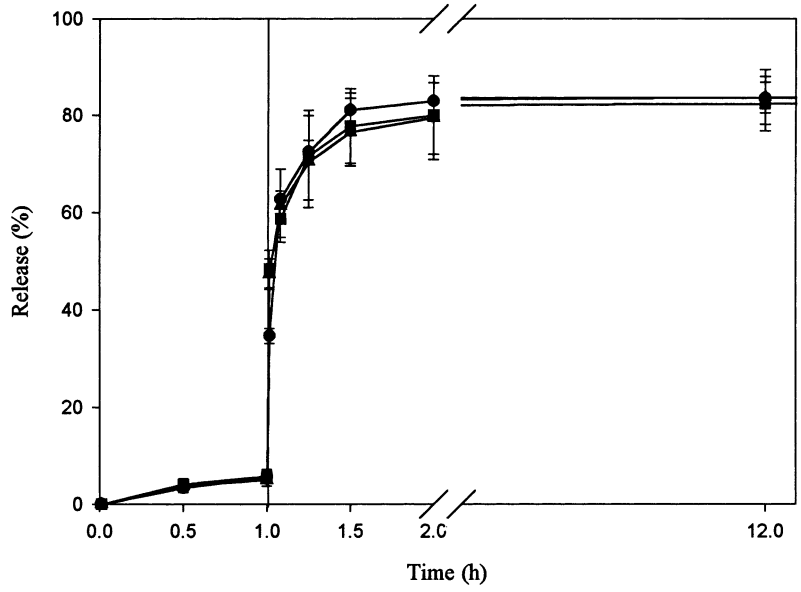

Fig. 5. Sudan orange G release from uncoated 3 (●), 4 (■) and 5% (▲) alginate microspheres after 1 h in simulated gastric fluid and 2 h in simulated intestinal fluid. Errors bars represent standard deviations about the mean based on three replicates.

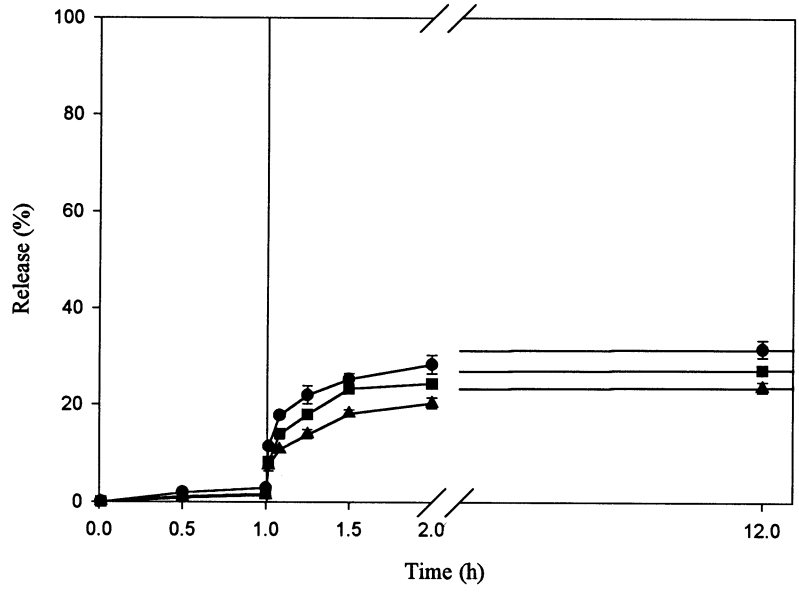

Fig. 6. Sudan orange G release from chitosan coated alginate microspheres in simulated gastric (1 h) and intestinal fluids. Molecular weight of chitosan coat was 75 (●), 100 (■) and 300 (▲) kDa. Error bars represent standard deviations about the mean based on three replicates.

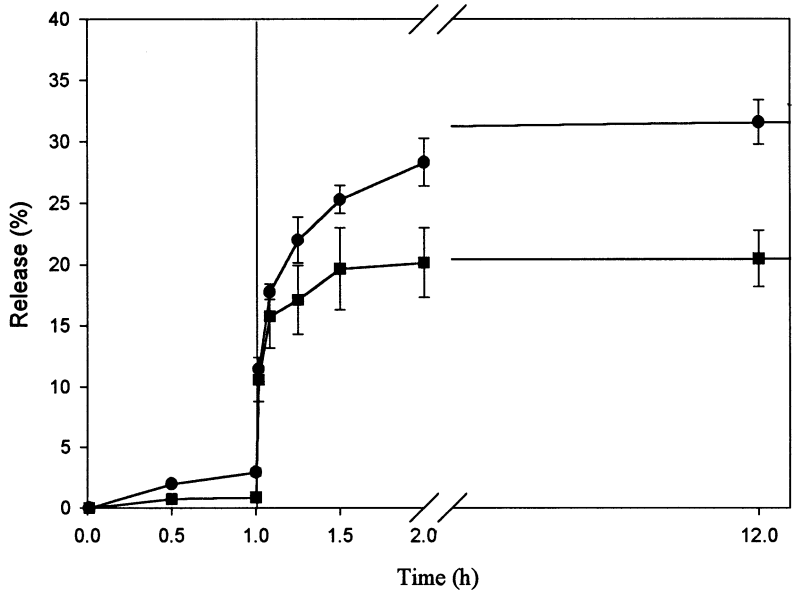

Fig. 7. Effect of chitosan coating time on dye release from chitosan (75 kDa) coated alginate microspheres. Coating times were 10 (●), and 30 (■) min. Error bars represent standard deviations about the mean based on three replicates.

stability of alginates at lower pH (Stockwell and Davis, 1986; Okhamafe et al., 1996). Also low dye solubility in water (Green, 1991) limits diffusion out of the hydrophilic matrix.

In intestinal fluid, alginate matrix erosion leads to a total dye release within 0.5 h whereas in coated microspheres, dye release was limited by membrane diffusion. Chitosan membranes are microporous (Harano et al., 1980; Okhamafe and Goosen, 1993) with the pore size increasing with pH levels above 6 due to electrostatic repulsion (Okhamafe and Goosen, 1993; Okhamafe et al., 1996). For chitosan molecular weights less than 300 kDa, a longer coating time results in thicker membranes reducing the release rate.

An optimum dosage form must deliver drug immediately and in a controlled fashion over 8–10 h to respond to the short half-life of the lipophilic drug's availability (Lee and Min, 1996). Thus it appears necessary to provide a coating to alginate microspheres to ensure prolonged delivery in intestinal media. Chitosan-coated microspheres demonstrated a significantly lower dye release rate, particularly with membranes formed from 300 kDa chitosans. Incomplete encapsulant release suggests the possibility of chitosan-dye binding. Chitosan is a cationic polymer which interacts with anionic compounds, potentially affecting the release rate of the drug (Kristmundsdottir et al., 1995). Chitin and chitosan are also known to function as chelators for transition metal ions (Nagai et al., 1984) and adsorbents for dyes, bromine and iodine (Nagai et al., 1984). Sudan orange G is a moderately acidic dye which in acidic medium forms the diazonium salt (Neckers and Doyle, 1977). These salts are poor electrophiles but show electrophilic substitution with substituted amines (Neckers and Doyle, 1977). Binding may then be attributed to interaction between the diazonium salt and the chitosan amino group. This encapsulant-chitosan interaction may be desirable to control release rate and improve the intestinal drug's bioavailability (Robinson and Lee, 1987; Kristmundsdottir et al., 1995). Drug loaded chitosan microspheres may adhere to the mucin epithelial coating of the gastrointestinal tract, extending gastrointestinal transit time of chitosan ionic bonded drugs, especially the anionic drugs.

In conclusion, an emulsification and internal gelation technique has been described for the po-

tential encapsulation and intestinal delivery of lipophilic drugs. A high encapsulation yield is possible, using a methodology well suited to large scale manufacture.

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