Coated Alginate Microspheres: Factors Influencing the Controlled Delivery of Macromolecules

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SYNOPSIS

A systematic study of the mild alginate/polycation microencapsulation process, as applied to encapsulation of bioactive macromolecules such as proteins, was conducted. When protein drugs were suspended in sodium alginate solution and sprayed into 1.3% buffered calcium chloride to form cross-linked microcapsules, large (up to 90%) losses of encapsulation species were encountered, and moderate to strong protein-alginate interactions caused poor formation of capsules. As a result, a diffusion-filling technique was adopted in which blank alginate beads, coated twice with small amounts of polycation, were formed prior to drug loading. Protein was then loaded into these capsules by stepwise diffusion from solutions of increasing drug concentration. The drug-loaded capsules were coated with a final layer of polycation. In all, three polycation coatings were used, two prior to filling and one after filling. The first coating strongly influenced the size, integrity, and loading capacity of the capsules. Low concentrations of polycation resulted in poorly formed capsules with very low retention of the drug in the final capsule, while very high concentrations prevented the drug from entering the capsule at the filling stage. This first coat also affected the duration of drug release from the capsule and the size of the burst effect. The second coat had less effect on the capsule integrity, but it did influence the drug payload and release profile. The final, sealing-coat had little effect on drug payload and only limited effect on the release profile up to a critical concentration, above which the release profile was not affected. For all coats, increasing polycation concentration decreased the burst effect, and caused the release profile to be more sustained. Encapsulation of a series of dextrans with increasing molecular weight revealed that the release profile was directly related to the molecular weight of the diffusing species, which was more sustained as molecular weight increased. We have shown that the choice of coating parameters in the diffusion-filled, alginate/polycation system is critical for successful drug delivery from these capsules. By carefully choosing the coating parameters, both the drug payload and the release profile can be fine-tuned to meet the desired profile.

INTRODUCTION

Many of the present controlled release devices for *in vivo* delivery of macromolecular drugs involve elaborate preparations, often employing either harsh chemicals, such as organic solvents,[1] or extreme conditions, such as elevated temperatures.[2] These conditions have the potential to destroy the activity of sensitive macromolecular drugs, such as proteins or polypeptides. In addition, many devices require surgical implantation and, in some cases, the matrix remains behind or must be surgically removed after the drug is exhausted.[3] One solution to the problem of surgical implantation of a device is to produce microcapsules which are amenable to injection into the body, usually via the intraperitoneal or subcutaneous route.[4] Microencapsulation techniques that are used for immobilization of viable cells, for example hybridoma cells, are usually mild as a direct result of the fragile nature of living organisms. In

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particular, the naturally occurring copolymer of guluronic and manuronic acid (alginate) is an excellent candidate for microencapsulation under physiological conditions, since it is easily formed into a gel by cross linking with bivalent cations. Alginate has been used to immobilize bacteria,[5] individual protoplasts,[6] and red blood cells.[7] The method was greatly expanded by Lim and Sun who used alginate to encapsulate viable islet cells and then coated the islet-containing microcapsules with a polycationic membrane.[8] This membrane allowed diffusion of glucose and insulin across the capsule wall, but excluded larger molecular weight species, such as those of the immune system (molecular weights of immunoglobulins are in the range of 1.5×10^5), thus forming a protective barrier for the encapsulated islets. Poly-L-lysine/alginate microspheres containing islet cells have demonstrated *in vivo* biocompatibility, relieving streptozotocin-induced diabetes in rats for up to one year after a single intraperitoneal microcapsule injection.[9]

We have investigated this system for use as a sustained delivery device and have conducted a systematic study of the parameters that will influence the use of this technique as a vehicle for the controlled delivery of large molecular weight species.

MATERIALS AND METHODS

Ovalbumin (M wt 45,000), dextrans (M wts 9000 through 73,000), myoglobin (horse heart M wt 18,000), poly-L-lysine HBr (PLL) (M wt 60,000), HEPES(4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid), and anthrone were purchased from Sigma Chemical Company, St. Louis, MO. Alginate LF-60 was obtained from Multichem Corporation, Poly vinyl amine (PVA), (M wt ~ 50,000) was obtained from Polysciences, Warrington, PA, ^{14}C radiolabeled ovalbumin (0.0089 mCi/mg, lot No. 1713-135) and Ultrafluor scintillation fluid were obtained from New England Nuclear, Boston, MA. All other chemicals were Baker Analyzed reagents.

Choice of Encapsulation Species

Ovalbumin was chosen as a model protein with a relatively large molecular weight that was readily available in a radiolabeled form. Release of ovalbumin was followed by measuring ^{14}C decay from 500 μl of solution in 10 mL of Ultrafluor scintillation fluid, using a Tracor-analytical scintillation counter model No. 6892.

Dextrans were used to study the effect on the release profile of the molecular weight of encapsulated species. Dextrans were chosen in preference to proteins since they are available in various molecular weight ranges and, having no charge, effects due to different isoelectric points could be eliminated.

Dextran concentrations were measured using an adaptation of the anthrone method for reducing sugar polysaccharides.[10] Anthrone reagent was made up every 4 days by mixing 200 mg of anthrone with 95 mLs of 75% sulfuric acid and 5 mL of ethanol in a large beaker, with stirring, in an ice bath. The reagent was stored at 4°C in brown glass. Five mL of reagent were added to 1 mL of sample, containing up to 200 μg of dextran, in a large test tube. The solution was vigorously mixed on a vortex mixer. The samples were cooled to 4°C in an ice bath. The covered tubes were placed in a boiling water bath for exactly 10 min, followed by cooling to room temperature. Absorbance at 625 nm was recorded against a water blank. A standard curve up to 200 μg of dextran of the appropriate molecular weight was measured with each set of readings.

Myoglobin was chosen as a model proteinacious species to encapsulate because of the strong 410 nm absorbance peak that it exhibits at pH 7.4. This peak makes it an ideal candidate to follow spectrophotometrically.

Capsule Formation

Initially, capsules containing protein were formed by spraying a mixture of sodium alginate (1.6 wt %) and the protein (4 mg ovalbumin/mL, 0.125 μCi/mg) into a solution of calcium chloride. However, as described later, significant loss of protein was observed, and capsule formation was not good, probably due to protein–alginate interaction.[11] As a result, a modification of the Lim and Sun encapsulation method was used.[12] This involved making blank calcium alginate beads and loading them with the drug by stepwise diffusion from solutions of increasing myoglobin (or dextran) concentration. The blank calcium alginate capsules were treated with solutions of low concentrations of polycation (PVA and PLL) prior to loading. The concentration of cationic polymer was chosen to create a coating that was loose enough to allow the drug to diffuse into the capsule, but also sufficient to slow down the loss of the drug from the loaded capsules at the washing and final coating steps. The protein-loaded capsules were coated with a final cationic skin. Thus capsules were prepared in four steps: primary empty micro-

sphere formation, microsphere precoating, drug loading, and final microcapsule coating.

Primary Microsphere Formation

A 100 mL batch of 1.2% sodium alginate solution was made by gradually shaking the alginate powder directly into the vortex of 80 mL of a rapidly stirred 0.9% saline solution. Vigorous stirring was continued until all of the alginate was dissolved (about 15 min). The alginate concentration was adjusted to 1.2% by the addition of saline. The solution was then cleared of any fine particulate contaminants by centrifugation at 12,000 rpm for 20 min. Alginate was sterilized by heating in a boiling water bath for 20 min. The solution was kept refrigerated when not in use and was freshly made every three days.

Calcium alginate microcapsules were formed by spraying 10 mL of the sodium alginate solution into 250 mL of HEPES buffered calcium chloride (13 mM HEPES, 1.3% $CaCl_2$, pH 7.4) from a 20 mL plastipack syringe through a 22 G needle (Fig. 1). A coaxial stream of sterile air flowing around the needle detached the alginate drops as they emerged from the needle tip, and capsule size was controlled (500–600 μm) by adjusting the air speed with a flow meter in the air line upstream of the syringe needle assembly. Microcapsule size was chosen to facilitate handling of the microcapsules. Alginate flow from the syringe was kept constant by using a modified Razel syringe pump (5 mL per min, using a 20 mL plastipack syringe) to force the alginate solution through the needle.

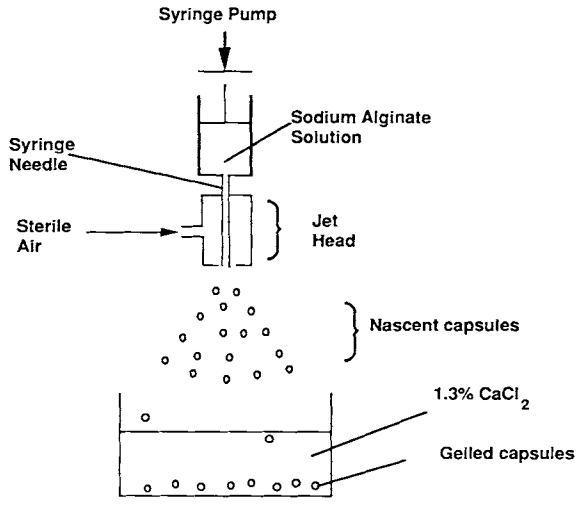

Figure 1 Schematic of spray device used in the preparation of calcium alginate microcapsules.

The calcium alginate beads were allowed to harden for 12 min in fresh, 1.3% buffered $CaCl_2$ solution, and then washed three times in HEPES buffered saline (13 mM HEPES, 0.9% saline, pH 7.4). Washing was with volumes of buffer that were 6 times the volume of the settled beads. The buffer was aspirated from above the settled beads after each wash.

Primary Microsphere Coating

The calcium alginate capsules were coated by gentle shaking for 6 min in contact with selected concentrations of polycationic polymer solutions, made up in HEPES buffered saline (pH 7.4). The volume of polymer solution was equal to the volume of the gelled beads. The first coat consisted of PLL in the presence of 0.2% sodium citrate. The beads were then washed 3 times with saline and coated for 6 min with various concentrations of PVA. After 3 saline washes, the beads were coated for 5 min with a 0.12% solution of sodium alginate and were again washed with saline. This step neutralized the excess positive charge on the surface and prevented clumping of the microcapsules.

Loading of Microspheres with Drug

Microspheres placed in drug solutions with concentrations of 120 mg/mL became crenulated, which was presumably caused by the high osmotic pressure outside the capsule, drawing water out of the capsules before the drug could diffuse into the capsule interior. To avoid this crenulation, microcapsules were loaded in a stepwise fashion by diffusion of the drug from successive solutions with increasing myoglobin concentrations: specifically 24 h exposure to an equal volume of 20 mg/mL solution, followed by two consecutive 12 h exposures to solutions of 120 mg/mL each.

Final Coating of the Loaded Microspheres

The myoglobin solution was drained from the loaded beads through a 50 mL Econocolumn and the microcapsules were briefly washed, in the column, with an equal volume (packed column volume) of hypotonic (0.2%) saline. A coat of PVA was applied to the capsules during a 6 min contact with a large (40 × the packed capsule volume) quantity of PVA solution of the desired concentration. This application was followed by three equal volume saline washes and a coat of an equal volume of 0.12% sodium alginate solution (5 min contact with gentle

shaking.) The coated beads were washed 3 times with saline (0.9%), and used in the release experiments.

Coating Step Concentrations and Nomenclature

Capsule preparation involved three polycation coating steps. The effect of the concentration of polycation in each of these steps was systematically studied using the plan outlined in Table I. For example a capsule designated 1b2a3c will have the following coating parameters: the first coat PLL 0.3%, the second coat PVA 0.066%, and the postfilling PVA coat with concentration 3.4%.

The Effect of Molecular Weight of Encapsulation Species

Capsules loaded with dextran were made in a similar fashion. The concentration of the first coat was kept low (0.03%), which allowed the high molecular weight dextrans to diffuse into the capsule. In addition, citrate was omitted from the solution because it was found to inhibit good capsule formation at this low PLL concentration. The PVA concentration was 0.06%. The second PVA concentration was 0.034%. This corresponds to a capsule designated 1a2a3a.

Release Studies

Release studies were conducted at 37°C in capped scintillation vials containing 10 mLs of phosphate buffered saline at pH 7.4, with 0.02% sodium azide as preservative. The buffer was exchanged at frequent intervals (at least once every 24 h) to mimic the infinite sink conditions of the body. The vials were gently shaken on a rotary shaker. Release of dextran was followed using the modified anthrone method,[10] and myoglobin was monitored in the saline by absorbance at 410 nm. Eight duplicate samples were prepared for each parameter being studied. The standard error about the mean for cumulative percent released was never greater than 2% between the eight duplicates, and the standard error has been omitted from the graphs for clarity. The releasing capsules were contained in a polypropylene mesh cage (constructed in our laboratory using 150 micron mesh size) inside the vials to facilitate transfers to fresh buffer.

Table I Coating Step Concentrations and Nomenclature

Step	Compound	Concentration	Nomenclature
		Preloading Coat	
1	PLL	0.03%	1a
		0.3%	1b
		3.0%	1c
2	PVA	0.066%	2a
		0.66%	2b
		6.6%	2c
		Final Coating (Capsule Filling Stage)	
3	PVA	0.034%	3a
		0.34%	3b
		3.4%	3c

RESULTS

Direct Loading of Drug into Sodium Alginate Solution

When radiolabeled ovalbumin was mixed directly with the sodium alginate solution and sprayed into $CaCl_2$, little protein remained in the capsules at the end of the microencapsulation procedure. Figure 2 shows the loss of radiolabeled ovalbumin at each stage of capsule preparation. Loss was recorded by assaying the quantity of radiolabel (^{14}C) appearing in the wash and coating solutions at each stage. Over 60% of the ovalbumin was lost to the $CaCl_2$ solution at the first spraying step, and by the end of the process less than 10% of the drug was in the microcapsules. Other proteins, such as bovine serum albumin (data not shown) interacted more strongly with the alginate, resulting in very poorly formed capsules. This situation led us to adopt the diffusion method of loading empty calcium alginate capsules followed by a final cationic coating.

Diffusion-Loaded Capsules

Size and Shape

Although all capsules were made from identical alginate core beads, the coated, drug-loaded beads were found to differ greatly in size and integrity, depending upon the coating concentrations that were used. The most important factor in determining the final size and integrity of a given bead was the concentration of PLL in the first coating step. Figure 3 is a series of photomicrographs of myoglobin-loaded beads taken at the same magnification (75 ×). The capsules shown in Figure 3 (i) (coats 1a2c3b) and Figure 3 (ii) (coats 1b2c3b) differ only in a tenfold

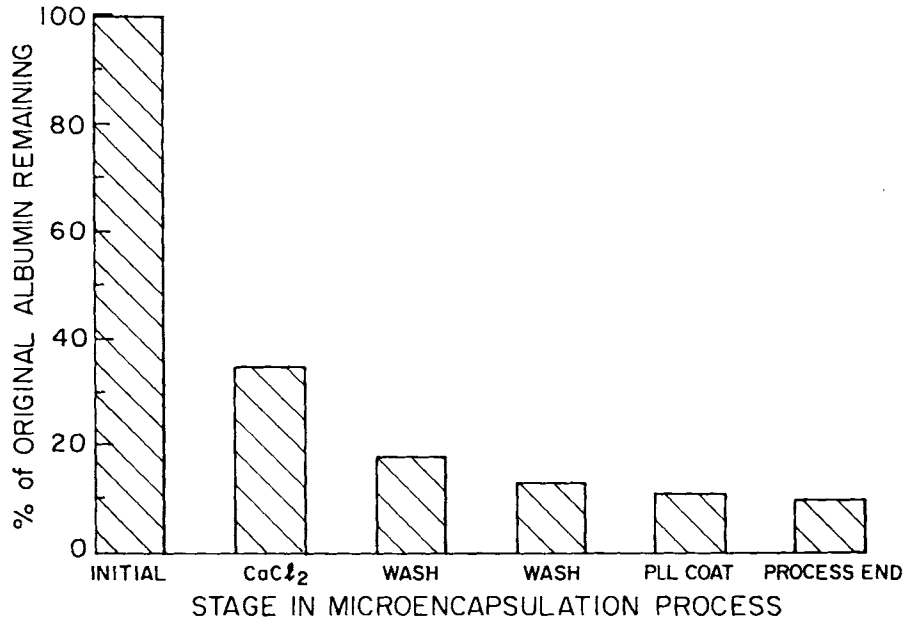

Figure 2 Retention of ovalbumin inside capsules at various stages of preparation by direct spraying into calcium chloride solution.

increase in the concentration of the initial coating solution. Beads prepared with the lowest initial PLL concentration (1a, 0.03%) were poorly formed, and a large number of the beads collapsed at the PVA coating stage. When preparing dextran-loaded beads, we found that improved bead integrity resulted when we omitted citrate from the solution at these low PLL concentrations. This is best seen when comparing the series prepared with the same initial PLL concentration (0.3%), [Fig. 3 (ii), which is (1b2c3b), Fig. 3 (iii), which is (1b2b3a), and Fig. 3 (iv), which is (1b2b3c)]. Note that these capsules have a similar size.

The first coat also has a crucial role in the ability of the capsule to take up protein at the filling stage. Figure 4 compares micrographs of capsules with the highest initial PLL coating concentration (1c) with similar capsules prepared with an intermediate PLL concentration (1b). The 1c series capsules were small and crenulated (even though they were loaded in the usual manner by exposure to a low concentration of the drug in the first loading solution), suggesting that the myoglobin encountered resistance when diffusing into the capsule and that the high osmotic pressure in the solution outside the capsules, created at the second loading stage with 120 mg myoglobin/mL, caused water to be drawn out of the capsules. The capsules did not regain their spherical shape after the final coating step with PVA.

Payload of Drug Loaded into the Capsules

All values of drug payload are quoted as mg myoglobin/mL equivalent of alginate, that is, the total amount of drug that was released from that quantity of beads that were made from one mL of original sodium alginate. This allows for normalization with respect to variation in bead size. Quantities of myoglobin released from the beads were determined from the 410 nm absorbance in the release buffer using an experimentally determined extinction coefficient for myoglobin in PBS of 4.26 $(mg/mL)^{-1}$. The results are listed in Table II.

Assuming that the myoglobin concentration reached equilibrium within the capsules at the loading stages, the maximum concentration inside the capsules would reach 92 mg/mL. The highest value that remained after the loading stage and coating with PVA was 27 mg/mL, a 30% retention of the drug. Most of the drug that was lost from the loaded capsules was lost to the first wash in hypotonic (0.2%) saline.

Choice of the correct concentration of polycation in the first (PLL) coating was critical to successful drug loading. When the concentration was low (0.03%), the poor formation of the beads resulted in minimal retention of myoglobin/mL equivalent (example, **1a**2c3b, 8 mg, as compared with **1b**2c3b, 27 mg). Equally poor was a high initial coating concentration (3.0%, the crenulated capsules), which

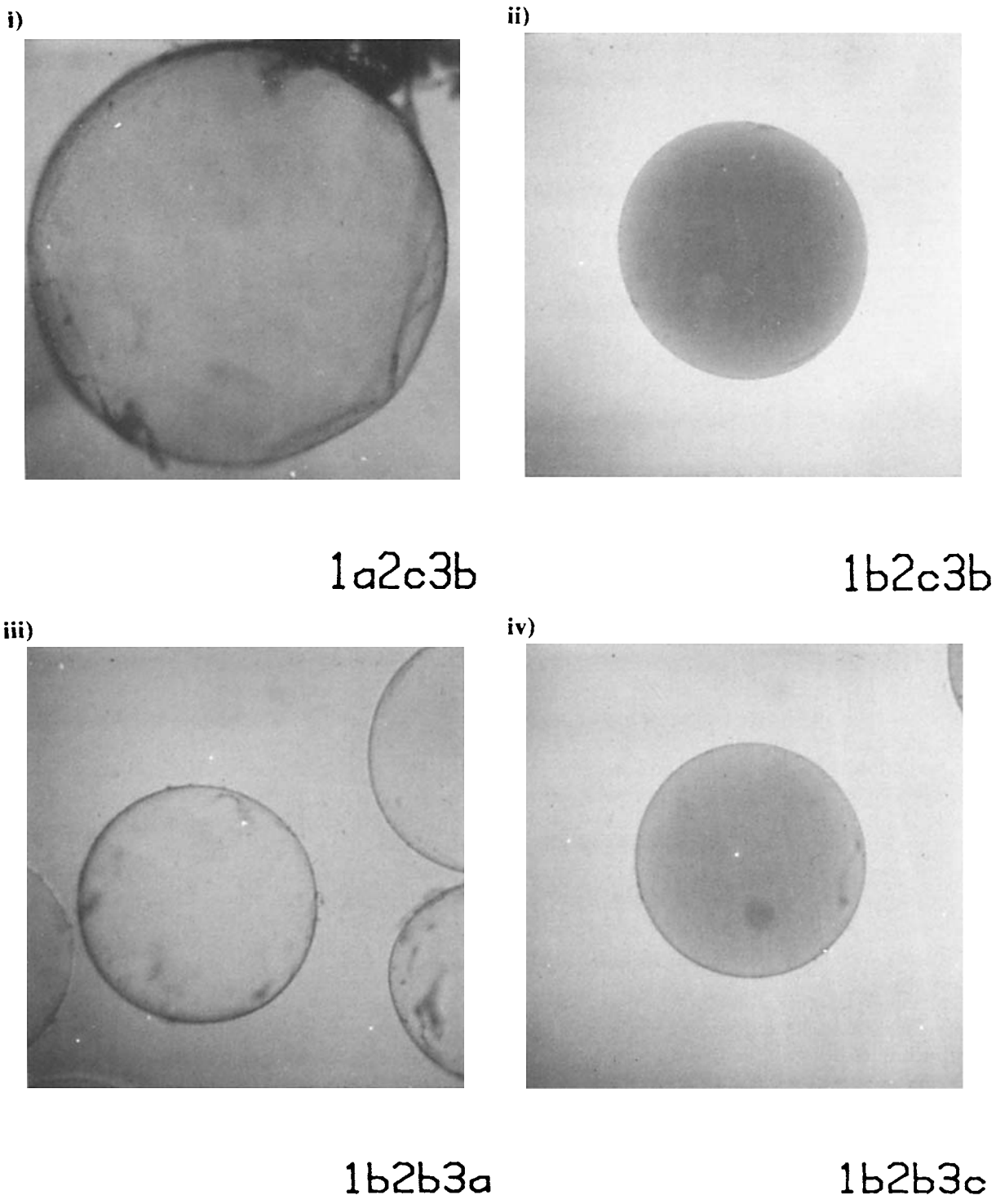

Figure 3 Photomicrographs of myoglobin-loaded capsules showing the effect that varying the coating concentration has on the size and integrity of the capsules. (i) 0.03% PLL, 6.6% PVA, 0.34% PVA (1a2c3b); (ii) 0.3% PLL, 6.6% PVA, 0.34% PVA (1b2c3b), (iii) 0.3% PLL, 0.66% PVA, 0.034% PVA (1b2b3a); (iv) 0.3% PLL, 0.66% PVA, 3.4% PVA (1b2b3c) (75 ×). The microspheres are between 200 and 600 μm.

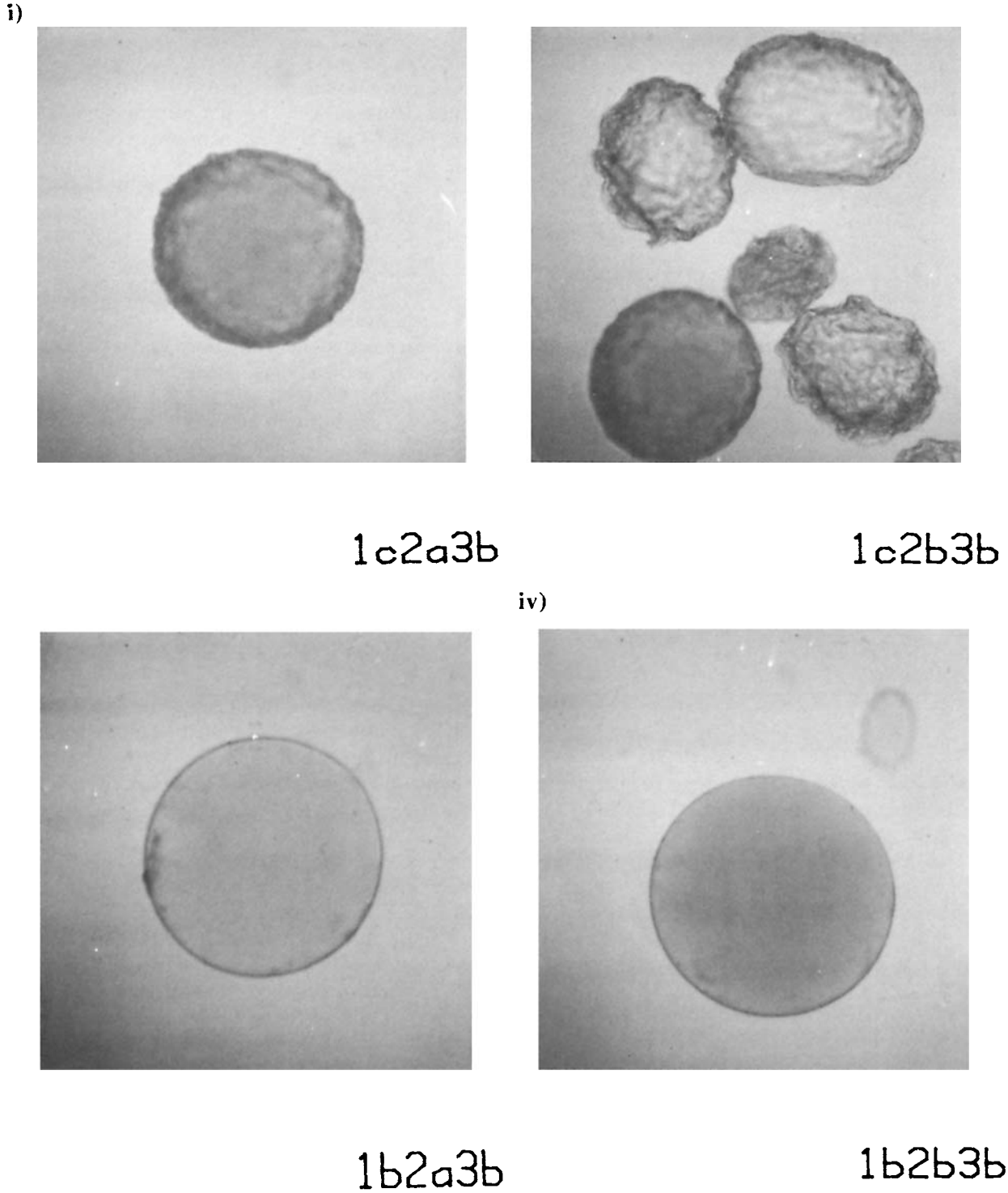

Figure 4 Photomicrographs of myoglobin-loaded capsules showing the effect of the concentration of the first coating solution. (i) 3.0% PLL, 0.066% PVA, 0.34% PVA (1c2a3b); (ii) 3.0% PLL, 0.66% PVA, 0.34% PVA (1c2b3b); (iii) 0.3% PLL, 0.066% PVA, 0.34% PVA (1b2a3b); (iv) 0.3% PLL, 0.66% PVA, 0.34% PVA (1b2b3b) (75 ×). The microspheres are between 200 and 600 μm.

Table II Drug Loading in Capsules as a Function of Capsule Preparation

Capsule Coating	Protein (mg/mL)
First Coat	
1*a*2c3b	8
1*b*2c3b	27
1*c*2a3b	2
1*b*2a3b	13
1*c*2b3b	3
1*b*2b3b	20
Second Coat	
1b2*a*3b	13
1b2*b*3b	20
1b2*c*3b	27
Third Coat	
1b2a3*a*	15
1b2a3*b*	13
1b2a3*c*	14
1b2c3*a*	26
1b2c3*b*	24
1b2c3*c*	23

restricted the myoglobin from entering the capsules (**1c**2a3b, 2 mg, as compared with **1b**2a3b, 13 mg and **1c**2b3b, 3 mg, as compared with **1b**2b3b, 20 mg). The intermediate concentration (0.3%), was optimal as seen in the capsules **1b**2c3b, 27 mg.

For the second (PVA), preloading coating step, better retention of the drug was achieved as the concentration of PVA increased. This is seen in the series: 1b2x3b, x = a 13 mg, x = b 20 mg, x = c 24 mg.

The concentration of the final, post loading coat had the least effect of all the coats. This is in agreement with the observation that most of the myoglobin was lost from the capsules in the first stage after loading, before the final coat was applied. For example, in the 1b2a3x series, x = a contained 15 mg, x = b contained 13 mg, and x = c contained 14 mg, all similar amounts. In the series 1b2c3x, x = a contained 26 mg, x = b contained 24 mg, and x = c contained 23 mg.

The best results for high payload of myoglobin are achieved with an intermediate first coat (PLL) concentration and a high PVA concentration prior to loading.

Release Profiles

The Effect of the First Coating. In Figure 5 (i) the release profiles for capsules with a ten fold concentration difference in the first coating step (1a2c3b and 1b2c3b) are shown. Increasing the concentration of this first coat resulted in a less pronounced burst effect (burst effect is defined here as the percentage released in the first few h, 15% as compared with 60%), and a more sustained release (50% released at 18 h as compared with 0.6 h).

Release profiles for capsules with a high initial PLL concentration (the capsules that were poorly formed and appeared crenulated under the light microscope), show the same trend [Fig. 5 (ii)], with a more sustained release as compared with capsules that were coated with a lower PLL concentration (1c2a3b as compared with 1b2a3b). Although the higher initial coating concentration does delay the release of the drug (50% released in 13 h with 1c2a3b as compared with 2 h for 1b2a3b), it must be recalled that the payload is very low and the actual amounts of drug being released are very small (50% release represents 1 mg of the drug for 1c2a3b as compared with 6.5 mg of the drug for 1b2a3b).

These results support the conclusion that the preloading, first coating step has a marked effect on the final release profile of the drug; increasing the concentration reduces the burst effect and produces a more sustained release in any given series. These results are consistent with a diffusion release mechanism.

The Effect of the Second Preloading Coating. Figure 6 indicates that, as with the first coating, increasing the concentration of the second coating (1b2x3b series shown here) both reduces the burst effect and leads to a more sustained release profile. In addition, as mentioned previously, the second coat has an effect on the final payload of the drug, which increased from 13 mg/mL equivalent through 20 mg to 24 mg/mL.

The Effect of the Final Polycation Coat. Figure 7 (i) shows the effect on the release profile of increasing the final (PVA) coating concentration for the series 1b2b3x. The greatest effect is seen between the lowest concentration that was used, and a ten-fold increase in that concentration. Above this value, concentration increases do not have a great effect. This result is mirrored in the 1b2c3x series, Figure 7 (ii). These results suggest that since there are a finite number of accessible carboxylate groups from the alginate at the capsule surface, there is an upper

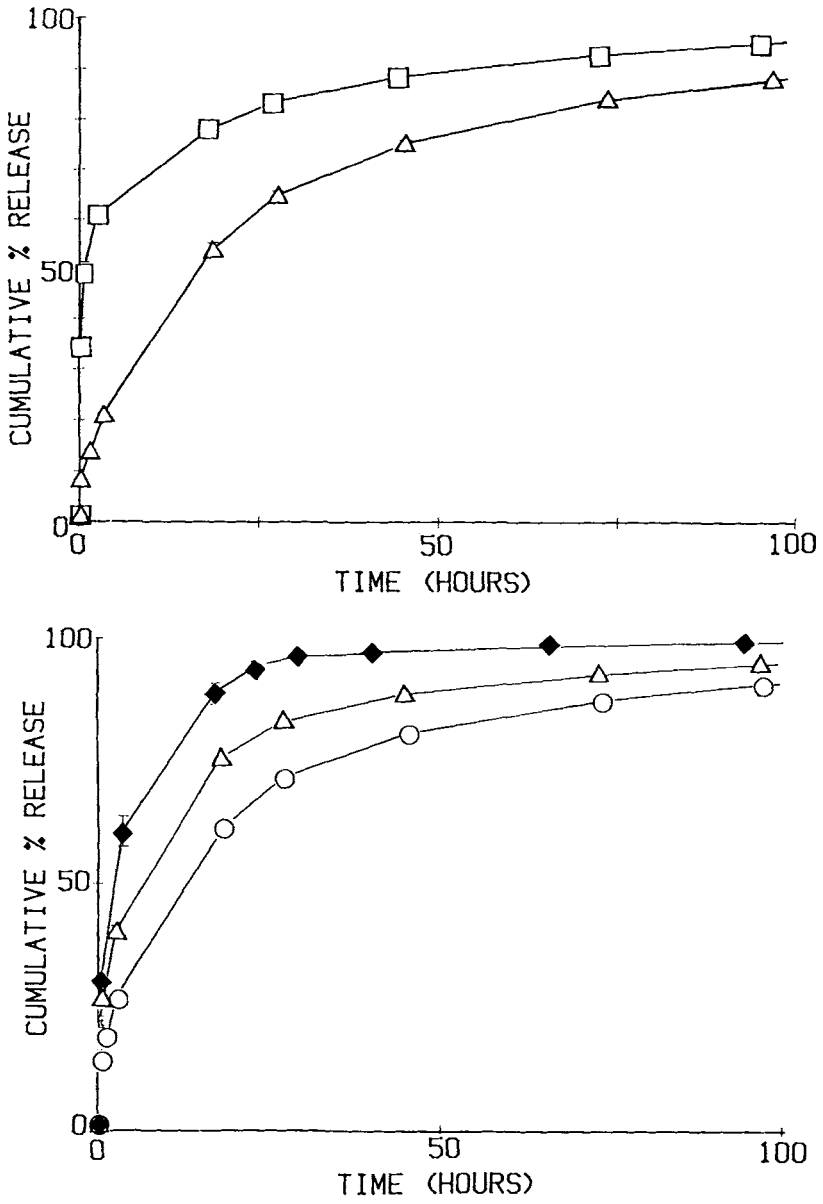

Figure 5 Myoglobin release profile as a function of the first coating. (i) -□- 0.03% PLL, 6.66% PVA, 0.34% PVA (acb), -△- 0.3% PLL, 6.66% PVA, 0.34% PVA (bcb); (ii) -◆- 0.3% PLL, 0.066% PVA, 0.34% PVA (bab), -△- 3.0% PLL, 0.066% PVA, 0.34% PVA (cab), -○- 3.0% PLL, 0.66% PVA, 0.34% PVA (cbb). (All points are the mean of eight samples, SEM in the range 0.1–1.2%.)

limit to the amount of polycation that can bind to the capsule.

Release as a Function of the Molecular Weight of the Encapsulated Species

A series of capsules were made containing dextrans of increasing molecular weights. The conditions were similar to those used in Figure 3 with the following changes: the concentration of the first coat was 0.03%, citrate was omitted from the solution, PVA concentration was 0.06%, and the second PVA concentration was 0.034%.

The molecular weight of the dextrans that were loaded into the capsules increased from 9000 to 40,000. Dextrans of molecular weights 73,000 and greater would not diffuse into the blank capsules.

Figure 8 represents release profiles for the series

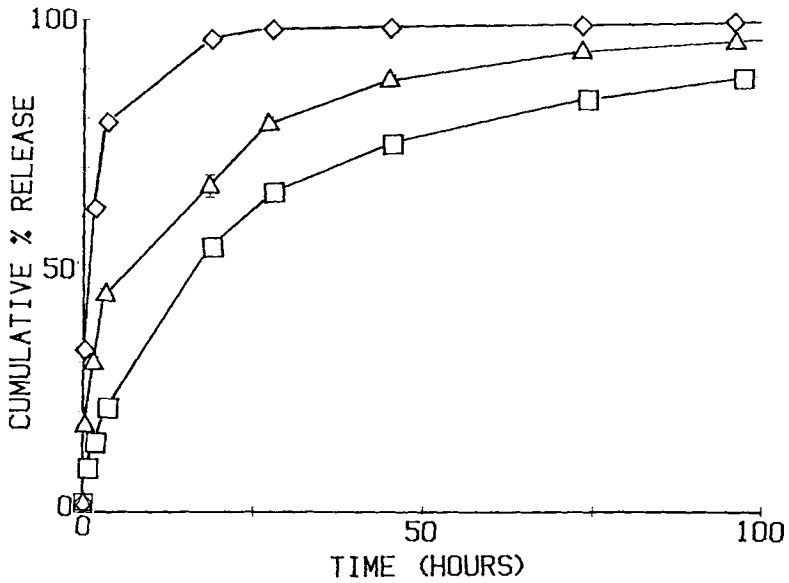

Figure 6 Myoglobin release profile as a function of the second coating. -◇- 0.3% PLL, 0.066% PVA, 0.34% PVA (bab), -△- 0.3% PLL, 0.66% PVA, 0.34% PVA (bbb), -□- 0.3% PLL, 6.6% PVA, 0.34% PVA (bcb).

of dextrans. In all cases the release is far more sustained than for myoglobin, (molecular weight 18,000). The nonionic nature of the dextran may be partly responsible for this. The drug payload is not expected to interact with the capsule core (carboxylate groups on the alginic acid), and hence the final polycation coating can interact in a tighter fashion with the capsule. However, too close a comparison between the two species should not be drawn since the molecular shapes of the two molecules differ greatly; one is a linear polysaccharide and the other a globular protein. Release of dextran from the capsules is molecular weight dependent; the higher the molecular weight of the dextran, the more sustained is the release profile. According to the theory of Polson, the diffusion coefficient of large unhydrated molecules varies with the inverse of the cube root of the molecular weight.[14] Plotting the cumulative percent release for a time point chosen in the more linear region, after the influence of any burst-type effect (78 h), against the inverse of the cube root of molecular weight of diffusing species generates the straight line plot as shown in Figure 9, which tends to support the idea of a diffusion mechanism for release.

DISCUSSION

The calcium alginate/polycation system of microencapsulation has been used extensively as a mild method for encapsulation of viable cells.[5-9] It has been proven successful *in vivo* for the encapsulation of viable islet cells, which have demonstrated long term (over one year) ability to control chemically-induced diabetes when implanted intraperitoneally in rats.[9] Careful choice of the polycationic membrane has been demonstrated to result in production of biocompatible capsules.[13] The system would therefore appear to offer many advantages for use as a drug delivery system, especially for drugs such as proteins and peptides that may be sensitive to harsher microencapsulation procedures. However, it is important to identify the parameters that influence the release profile (or capsule permeability) for bioactive molecules encapsulated by this method. In addition, such information would be valuable in the design of new encapsulated cell systems. Capsule permeability determines both the ability of substances secreted by the encapsulated cell to escape from within the capsule (e.g., hormone from a hormone-secreting cell line), and the ease with which external molecules, such as trigger molecules (glucose in the case of encapsulated islet cells), or deleterious substances, such as immunoglobulins, can enter the capsule interior.

We have conducted a series of investigations that have identified many of the parameters that influence the delivery of macromolecules from alginate/polycation microspheres. Early in our study we encountered problems with loss of drug payload during the spraying stage of microcapsule preparation,

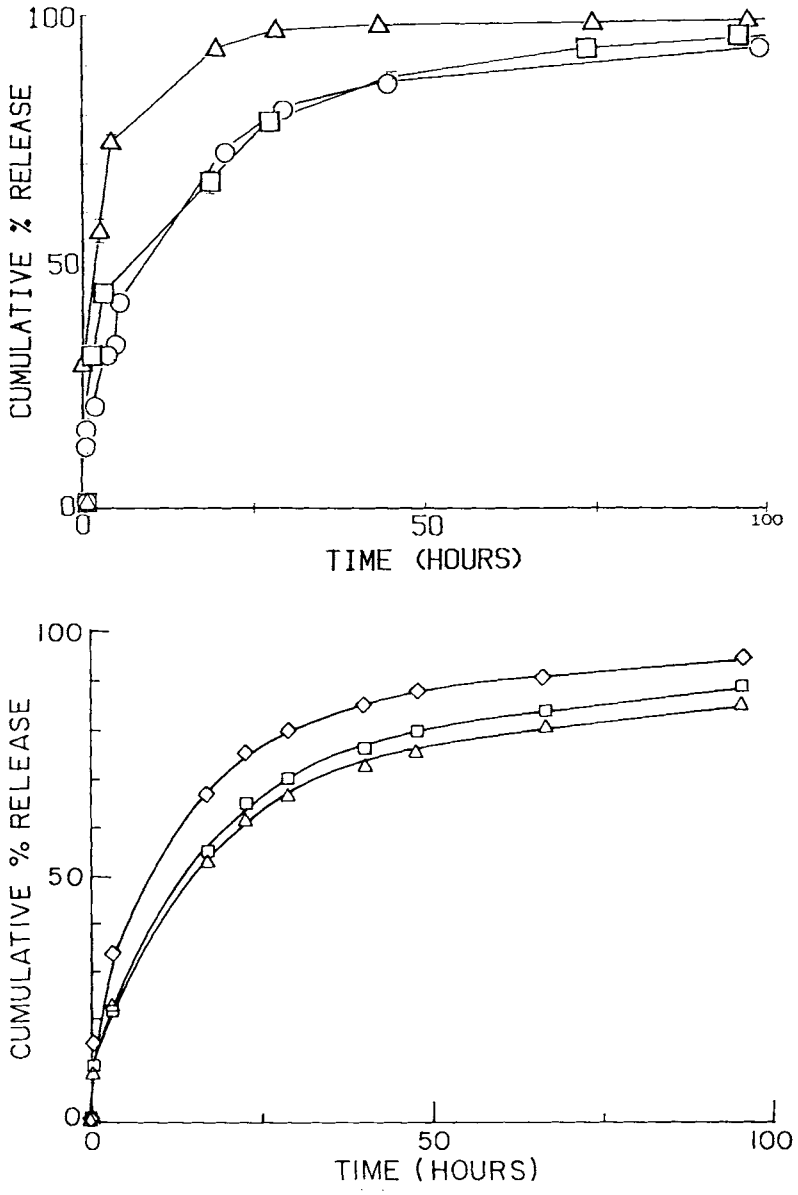

Figure 7 Myoglobin release profile as a function of the final coating. (i) -△- 0.3% PLL, 0.66% PVA, 0.034% PVA (bba), -□- 0.3% PLL, 0.66% PVA, 0.34% PVA (bbb), -○- 0.3% PLL, 0.66% PVA, 3.4% PVA (bbc); (ii) -◇- 0.3% PLL, 6.6% PVA, 0.034% PVA (bca), -□- 0.3% PLL, 6.6% PVA, 0.34% PVA (bcb), -△- 0.3% PLL, 6.6% PVA, 3.4% PVA (bcc).

problems which were exacerbated in the case of proteins that interacted with the alginate resulting in poorly formed capsules. These problems were especially noticeable for proteins with a high surface charge (e.g., bovine serum albumin). As a result we used a modification of the original encapsulation technique, which involved loading proteins by diffusion into preformed capsules, which were then further coated with polycation.

A systematic study of the effects of altering the capsule coating parameters showed that the release profile and drug payload were heavily influenced by these coating steps. The first coating step, prior to capsule filling, strongly influenced the size, integrity, and loading capacity of the capsules. The second coat affected both the release profile and the duration of the burst effect. The final, postloading step had a small but finite effect on the final release profile. In all cases increasing concentration of the coating solution resulted in a more prolonged release.

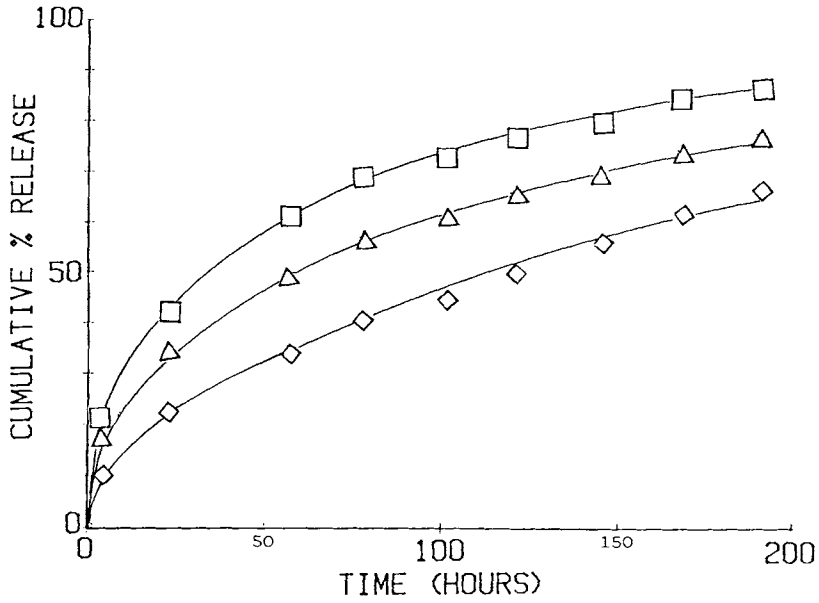

Figure 8 Dextran release profile as a function of molecular weight of dextran, -□- 9000, -△- 18,000, -◇- 40,000 for capsules 0.03% PLL, 0.6% PVA, 0.034% PVA (aaa).

In addition, there was an upper limit to the concentration that could be employed in the first step; too high a concentration led to a coat that inhibited free diffusion of the drug into the capsule at the filling stage. The concentration of polycation at which the drug will be excluded depends on the molecular weight of the drug. In the series of dextrans with increasing molecular weights, drug release appeared to be diffusion controlled (Fig. 9).

From our study of the various factors involved in the preparation of coated alginate microcarriers, we have shown that both drug loading and release rate

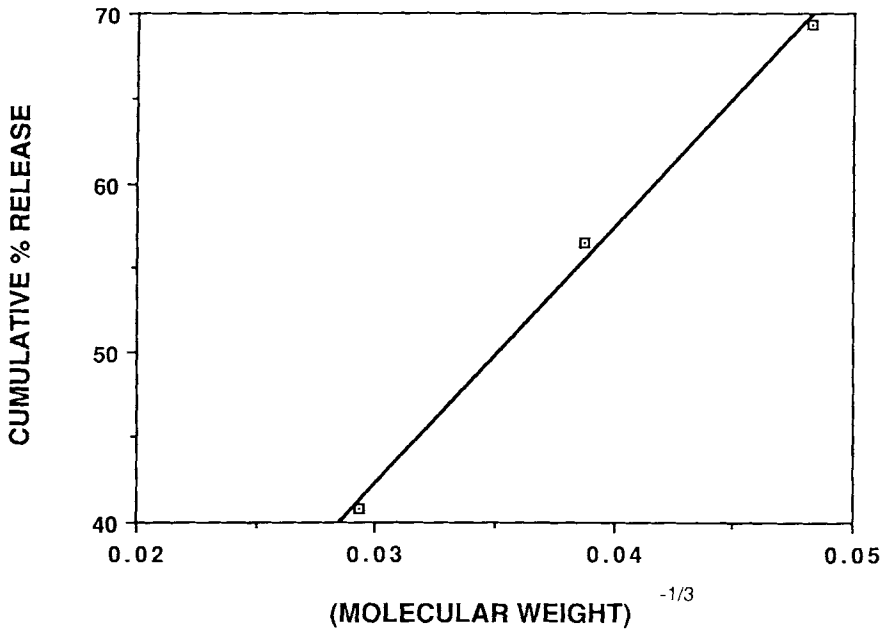

Figure 9 Cumulative percent released at 77 h for dextran of molecular weight 9000, 18,000, and 40,000, plotted against the inverse cube root of molecular weight.

can be manipulated and fine tuned by careful choice of the various coating parameters. This could be used in production of either uniform batches of capsules, with a single defined release profile, or mixing of batches, with different release profiles to yield preparations releasing as "tiny time pills," similar to the coated spansule capsules. Unlike the spansules, however, the microcapsules would be capable of releasing molecules for long time periods (e.g., days to weeks) and the mild encapsulation techniques employed permit the encapsulation and release of high molecular weight species.

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