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CASEIN MICROBEADS AS A CONTROLLED PARENTERAL DRUG DELIVERY SYSTEM

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ABSTRACT

The formulation of a novel controlled release Parenteral dosage form was the goal of this study. Microbeads of crosslinked casein containing Diethylstilboestrol (DEST), as a model drug, have been prepared using emulsion polymerization technique. The effect of different concentrations of Glutaraldehyde, as a cross linking agent, on particle size, particle size distribution, shape of microbeads, drug content as well as the rate of drug release from the microbeads were studied. Spherical microbeads with low particle size distribution and high drug load were obtained as glutaraldehyde concentration increased. Furthermore, the release rate from the microbeads were decreasing with the increase of the concentration of the cross linking agent. The effect of pH of casein solution as well as the amount of added drug on the characteristics of the microbeads were also investigated.The biodegradability of casein microbeads prepared at glutaraldehyde concentration 2.37% were tested in α-chymotrypsin solution. The microbeads showed a distinct signs of biodegradation within few days of incubation. No signs of adverse effect were noticed when drug free casein microbeads were injected intraprotenially in mice. It was concluded that casein microbeads could be considered as a good candidate for the preparation of a dependable parenteral time release dosage form.

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INTRODUCTION

The formulation of controlled release drug dosage forms has gained great interest (1). A dependable parenteral time release dosage form would eliminate many problems that may be associated with oral administration (2). Microencapsulation is considered as an ideal system for this parenteral dosage form (3). It is advantageous in this case to use a matrix that should not produce adverse immunological reaction, biodegradable (4-6), readily available and relatively inexpensive (4). Albumin have been chosen for the formulation of such dosage forms and have been characterized by many investigators (3-8).

Casein is an amphoteric phosphoprotein occurring in milk which is the main commercial source. Casein could be administered intravenously as hydrolysate in the form of sterile solution in concentration up to 10% (9). Because casein has been reported to bind with some drugs (10, 11), the release of drug from a formulated casein matrix is expected to be controlled by binding of the drug to the polymeric matrix, the diffusion of drug through the casein matrix and/or biodegradation, if any, of the matrix by proteolytic enzymes.

The manufacturing of medicated cross-linked casein microbeads that could be used as a controlled parenteral drug delivery system together with an insight into those parameters that tend to affect its characteristics was the aim of this study.

MATERIALS AND METHODS

Materials

Casein powder and castor oil were purchased from BDH Chemicals Ltd.(Poole, England). Diethylstilboestrol (DEST) and glutaraldehyde 25% v/v solution in water were supplied by Merck Co. (Darmstadt, Germany). α-Chymotrypsin was obtained from laboratories Leurquin (Paris, France). All other reagents and solvents were of analytical grade.

Preparation of Casein Microbeads

In this study, some of the methods described for the preparation of albumin beads were tried with casein (2, 3, 6, 8, 12-15). It was clear that these methods are not always successful with casein.

A modification of the method described by Proven et al. (14) was successful and allowed for the preparation of casein microbeads. In this method emulsion

polymerization technique was used. Six milliliters of freshly prepared casein solution (12.5% w/v) in a buffer containing dispersed drug particles were added to a mixture of 30 ml castor oil and 20 ml toluene at appropriate stirring speed (1200 rpm) using an overhead stirrer.

Saturated solution of glutaraldehyde in toluene was prepared by mixing equal volumes of the aqueous glutaraldehyde solution and toluene in a separating funnel followed by shaking for ten minutes. The upper toluene layer containing glutaraldehyde was separated. The concentration of glutaraldehyde in toluene was determined and found to be 1.43% v/v. A suitable volume of the toluene solution was pipetted dropwise to the casein dispersion.

The dispersion was stirred until the cross-linking reaction was completed (8-12 hours). The formed suspension of microbeads was washed of oil three successive times each with 5 ml toluene followed by centrifugation at 3000 rpm for 2 minutes. The microbeads were then washed three times with 5 ml of diethylether and centrifuged each time at 3000 rpm for 2 minutes. Between each wash the supernatent was discarded and the microbeads resuspended in fresh solvent. At the end of washing the microbeads suspension were filtered at reduced pressure and air dried at room temperature. Finally yellowish orange free flowing fine particles were obtained. Drug content as well as the characteristics of the beads were controlled by varying the amount of glutaraldehyde used in the reaction mixture, pH of casein solution and the amount of added drug.

Determination of Drug Loading

A method similar to that described by Jun and Lai (12) was used. 100 mg of microbeads were triturated and triplicate samples of 20 mg of the triturate were vortexed with 1 ml of dimethylformamide for 40 minutes. Thereafter, 100 ml of ethyl alcohol was added to the solution and shaked for another 40 minutes. The solution was completed to 250 ml. Ten ml aliquot was filtered through 0.22 um membrane filter (Millipore Corp., Bedford, USA) and assayed spectrophotometrically for its content of DEST.

Measurement of Size Distribution of Casein Microbeads

Casein microbeads were observed by light microscopy (Reichert, Austria) at 500 times magnification, which revealed beads with drug particles embedded in the matrix. The particle sizes of symmetric particles were measured using the above microscope by spreading a

minute quantity of microbeads on a clean glass slide. The mean diameter of 50-100 microbeads were measured and recorded. The range of size for asymmetric particles were determined using standard sieves ranging from 53-180 um. The effect of different variables on particle size and size distribution of microbeads was determined.

In-vitro Release from Casein Microbeads

In-vitro release of DEST from casein microbeads was monitored in phosphate buffer pH 7.4. Accurately weighed microbeads (200 mg) were placed into a basket covered with a 10 um opening nylon cloth. The basket was immersed in 750 ml of dissolution media at 37+1°C and rotated at 50 rpm. Five ml samples were withdrawn from the dissolution media at predetermined time intervals and equal volume of fresh media were replaced immediately. The samples were assayed spectrophotometrically for released DEST. Dissolution experiments were triplicated. No significant amount of degradation product of DEST have been detected under condition of preparation and dissolution of casein microbeads as was checked by HPLC.

Measurement of Biodegradability and Biocompatibility of Casein Microbeads

The biodegradability of a 20 mg sample of drug free casein microspheres prepared with 2.37% glutaraldehyde was examined by mixing with 5 ml of isotonic saline solution containing 10 mg (900 E.A.U.) of α-Chymotrypsin. The suspension was then incubated at 37°C for 96 hours. The resulting microspheres morphologies were observed every 24 hours and photographed with a stereomicroscope (Kyowa Co.,Tokyo, Japan). For biocompatibility study, about 2 mg of drug free microbeads suspended in 0.2 ml isotonic saline were injected intrapretonially in each of eight mice. The behaviors of the animals were observed for one week.

RESULTS AND DISCUSSION

Characteristics of Casein Microbeads

The physical characteristics of the prepared casein microbeads are summarized in Tables 1, 2 and 3 where, the effect of glutaraldehyde concentration, pH of casein solution and the amount of added drug were studied.

It is clear that the change of glutaraldehyde concentration has a pronounced effect on the characteristics of the prepared microbeads. The effect of glutaraldehyde concentrations namely, 0.59, 1.18 and 2.37% were examined using casein solution of pH 7.4. Table 1 shows that at glutaraldehyde concentration 0.59% the obtained microbeads were not perfectly spherical and having irregular surface while, concentrations 1.18% and 2.37% gave regular spherical microbeads with nearly smooth surface, as were examined by the light microscope. It seems that minimal structure alteration could be obtained at high glutaraldehyde concentration. The irregular microbeads showed particle size range of 53-90 um (as was determined by a series of sieves), while the spherical microbeads showed mean particle size (±SD) of 42.3±7.0 and 78.1±3.18 um (as examined by light microscopy) using glutaraldehyde concentration 1.18 and 2.37%, respectively. From Table 1, it can be observed that the distribution of particle size is smaller at high glutaraldehyde concentration.

Change in pH of the casein solution, also, showed a significant effect on the characteristics of casein microbeads (Table 2). The effect of pH were studied at a constant glutaraldehyde concentration (2.37% v/v). In acidic media (acetate buffer pH 4.0) the microbeads were irregular with a broadly distributed particle size (90-180 um). Using phosphate buffer, pH 7.4, triethanolamine/HCl buffer, pH 8.6, and carbonate buffer, pH 10.0, the obtained beads were spherical in shape with a narrow particle size distribution. Particle sizes were 78.1±3.1, 152.8±4.93 and 212±6.2 um respectively.

The isoelectric point of casein is 4.6 which means that at pH 4 the protein is positively charged while at pH 7.4, 8.6 and 10 the molecules should have a negative charge. The above results indicate that the change in hydrogen ion concentration may change the rate of reaction between the cross-linking agent (glutaraldehyde in this case) and casein where negative charges enhance the reaction and leads to the formation of smooth spherical microbeads.

Drug Loading

The effect of variables on drug loading were also examined. Table 1 shows that as the concentration of glutaraldehyde increased the drug loading was also increased. The drug loading was 8.82, 17.45 and 42.95% at 0.59, 1.18 and 2.37% glutaraldehyde concentration, respectively. The increase of drug loading might be due to improved drug uptake within the microbeads network

TABLE 1

Effect of Glutaraldehyde Concentration On The Characteristics Of Casein Microbeads*.

Glutaraldehyde concentration (% v/v)	Particle size (um)	Drug[a] content%	Shape
0.59	53-90[b]	8.82	irregular
1.18	42.3±7.00[c]	17.45	spherical
2.37	78.1±3.18[c]	42.95	spherical

* Prepared with casein solution pH 7.4 and 300 mg of added DEST.
a. Mean of three experiments.
b. Determined by sieves method.
c. determined by light microscope.

TABLE 2

Effect Of pH Of Casein Solution On The Characteristics Of Casein Microbeads*

PH	Buffer	Particle size(um)	Drug[a] content(%)	Shape
4	Acetate	90-180[b]	10.19	Irregular
7.5	Phosphate	78.1±3.18[c]	42.95	spherical
8.6	Triethanolamine/HCl	152.8±4.93[c]	58.59	spherical
10.0	Carbonate	>180um[c]	-----	spherical

* Prepared at glutaraldehyde concentration 2.37% v/v with 300 mg of added DEST
a. Mean of three experiments.
b. Determined by sieves method.
c. Determined by light microscope.

structure that is enhanced by the effect of glutaraldehyde.

The change of pH of casein solution was another factor that may affect drug loading. For microbeads prepared with 2.37% glutaraldehyde with 300 mg added drug, the loading was 10.19, 42.95 and 58.59% at pH 4, 7.4 and 8.6, respectively. This finding indicated that high drug loading is possible at high pH of aqueous casein solution.

To study the effect of the amount of drug added on microbeads loading, the beads were prepared using 150-300 mg of DEST suspended in the aqueous phase of casein. At glutaraldehyde concentration of 2.37% the drug content showed an increase as the amount of added drug is raised. The drug contents were 10.26, 19.74, 38.85 and 42.95% at 150, 200, 250 and 300 mg of added DEST, respectively (Table 3). Table 3 also shows that the mean size of microbeads are affected by drug load where average particle size was 29.5± 1.53, 53.9±4.0, 61.5±4.76 and 78.1±3.18 at 150, 200, 250 and 300 mg of DEST, respectively. It appears that the increase in drug loading gives larger mean particle sizes.

In all cases drug loading was ranging between 8.82 and 58.59%. It is believed that the loss of drug occurs during the production of casein microbeads by a partitioning of DEST into the dispersed oil phase during emulsification and during washing of the formed microbeads. It was clear that at rapid reaction rate (high concentration of glutaraldehyde), the best drug loading could be obtained at high pH of casein solution and on adding large amount of drug.

In-vitro Release

Casein microbeads containing DEST and prepared at different concentration of glutaraldehyde at pH 7.4 were subjected to in-vitro release study (Figure 1). The release kinetics was found to follow Higuchi model (16) where straight lines were obtained when the percent drug released were plotted versus the square root of time. This relation indicated a release process in which diffusion through a growing path length is the rate determining step (6, 17).

The diffusion through the casein matrix was shown to be dependent on the density of that matrix and this can explain the slow release of DEST from the microbeads as the concentration of glutaraldehyde increased. This means that the concentration of glutaraldehyde not only affect drug content and particle size but also the release characteristics of the drug from the microspheres. A lag time for the wetting of the beads and for a break through of the diffusion barrier

TABLE 3

Effect Of Amount Of Added DEST On The Characteristics Of Casein Microbeads*.

Amount of DEST (mg)	Particle size[a] (um)	Drug content[b] (%)	Shape
150	29.5±1.53	10.26	spherical
200	53.9±4.00	19.74	spherical
250	61.5±4.76	38.85	spherical
300	78.1±3.18	42.95	spherical

* Prepared at glutaraldehyde concentration of 2.37% v/v and pH 7.4.
a. Determined by light microscope.
b. Mean of three experiments.

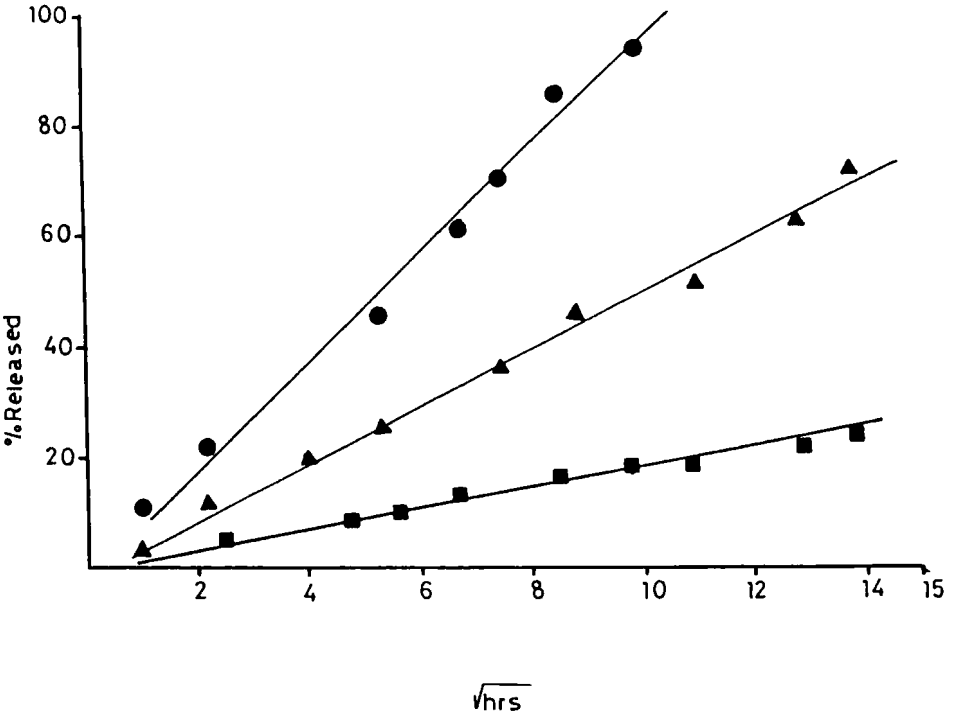

Fig. 1.

Release of diethylstilboestrol from casein microbeads prepared with different glutaraldehyde concentrations, (●) 0.59%, (▲) 1.18% and (■) 2.37% v/v at 37+1°C.

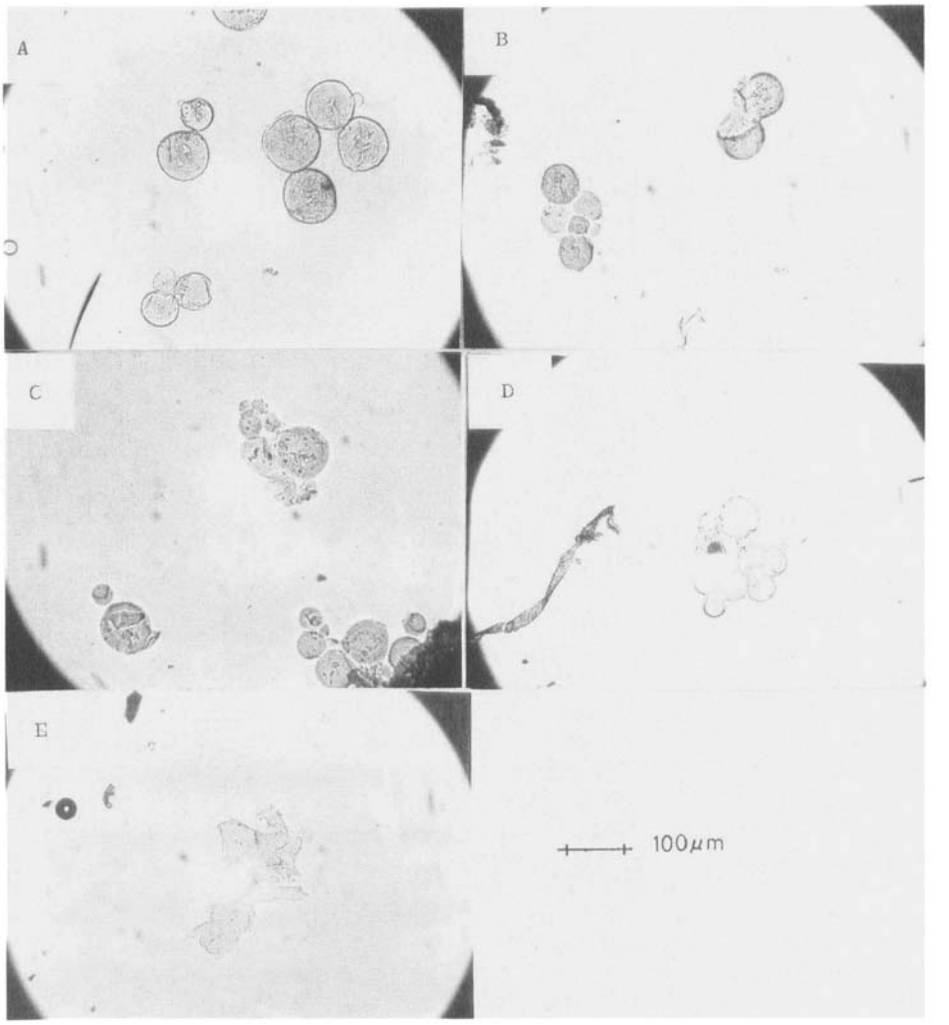

Fig. 2.

Micrograph of casein microbeads prepared with 2.37% glutaraldehyde treated with α-chymotrypsin (A) no treatment; (B) 1 day after treatment; (C) 2 days after treatment; (D) 3 days after treatment; (E) 4 days after treatment.

was also noticed in all cases. Within 96 hours the release was 100% for microbeads prepared with glutaraldehyde at concentration 0.59% while, at 1.18 and 2.37% the released amounts were 49 and 18%, respectively. At 192 hours the release was 72.0 and 24.6% at glutaraldehyde concentration 1.18 and 2.37%, respectively.

Biodegradability and Biocompatibility of Casein Microbeads

Biodegradability is a primary concern in design of parenteral drug delivery system. In this study drug-free microbeads formulated with different concentrations of glutaraldehyde were digested with α-chymotrypsin within four days. Figure 2 shows photographs taken for casein microspheres prepared with 2.37% glutaraldehyde with no enzyme treatment and at 1, 2, 3 and 4 days after treatment with α-Chymotrypsin. The photographs show the slow and gradual biodegradability of the microbeads. The microspheres lost their regular shape, gradually become rough with an irregular surface and then were digested. Thus, the casein microspheres proved to be suitable as a parenteral dosage form, as they have a rigid structure that does not resist biodegradation.

Biocompatibility of the microbeads prepared with a glutaraldehyde concentration of 2.37% was preliminary tested in animals. No sign of any abnormal behaviors were noticed when microbeads were injected intraprotenially in mice, indicating a possibility of biocompatibility. It is believed that further in-vivo study on drug release and biocompatibility is necessary in the future.

In conclusion, the obtained results show that casein microbeads could be considered as a promising candidate for controlled release parenteral formulation. The characteristics of these microbeads such as particle size, drug loading and release rate can be controlled for product optimization.

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