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# BIODEGRADABLE MICROSPHERES OF CURCUMIN FOR TREATMENT OF INFLAMMATION

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**Abstract :** Curcumin, a natural constituent of *Curcuma longa* (turmeric, CAS 458-37-7) was formulated as prolonged release biodegradable microspheres for treatment of inflammation. Natural biodegradable polymers, namely, bovine serum albumin and chitosan were used to encapsulate curcumin to form a depot forming drug delivery system. Microspheres were prepared by emulsion-solvent evaporation method coupled with chemical cross-linking of the natural polymers. Curcumin could be encapsulated into the biodegradable carriers upto an extent of 79.49 and 39.66% respectively with albumin and chitosan. Different drug:polymer ratios did not affect the mean particle size or particle size distribution significantly. However, the concentration of the crosslinking agent had remarkable influence on the drug release. *In-vitro* release studies indicated a biphasic drug release pattern, characterized by a typical burst-effect followed by a slow release which continued for several days. Evaluation of antiinflammatory activity using Freund's adjuvant induced arthritic model in Wistar rats revealed significant difference between both the formulations, albumin microspheres and chitosan microspheres as well as against control.

It was evident from the present study that the curcumin biodegradable microspheres could be successfully employed as prolonged release drug delivery system for better therapeutic management of inflammation as compared to oral or subcutaneous route.

**Key words :** curcumin, biodegradable, albumin, chitosan, microspheres, antiinflammatory

## INTRODUCTION

Free radical reactions participate in inflammatory processes by inducing chemotactic activity, by virtue of their relations with arachidonic acid metabolism and by inhibiting the serum inhibitor of leukocytic proteases (1). Curcumin (diferuloyl methane), an important constituent of *Curcuma longa* (turmeric CAS 458-37-7), is a potent antioxidant capable of scavenging oxygen free radicals like superoxide anion

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and hydroxyl radicals and the powerful antiinflammatory activity of curcumin is a result of its antioxidant property (2).

During the past decade, considerable progress has been made in the field of controlled drug delivery. Microspheres, as used in drug delivery are discrete, micrometer-sized spherical particles containing an entrapped drug. They can be prepared from a variety of carrier materials of both natural and synthetic origin. The main advantage of controlled drug delivery systems seems to be maintenance of therapeutically optimum drug concentrations in the plasma through zero-order release without significant fluctuations and eliminating the need for frequent single dose administrations. Parenteral administration of a drug in a microencapsulated formulation into a subcutaneous tissue results in the formation of a depot at the site of injection. This depot acts as a drug reservoir which releases the drug continuously at a rate determined to a large extent by the characteristics of the formulation, leading to the sustained absorption of the drug thereby reducing inherent disadvantages of conventional parenteral drug administration.

In the present investigation, curcumin was chosen as a model anti-inflammatory agent, as much emphasis is given to drugs of natural origin in recent years. Poorly water soluble and photosensitive nature of the curcumin makes it worthwhile formulating as novel delivery system. We have also chosen the biocompatible polymers of natural origin (bovine serum albumin - a naturally occurring protein and chitosan - a naturally available polysaccharide) to facilitate the usage of naturally available resources in drug delivery research. Both the chosen polymers have a long history in biomedical research and variety of delivery systems have been designed employing these biopolymers (3–12).

## METHODS

Curcumin was purchased from Loba Chemie, Chennai; bovine serum albumin (BSA) and Freund's complete adjuvant from Sigma Chemicals Co, USA. Chitosan was a gift sample from Fisheries Research Institute, Trivandrum. All the other chemicals and reagents used were of analytical grade. For pharmacodynamic studies, adult male Wistar rats (200-215 g), were obtained from Central Animal Housing Facility, Department of Pharmacology, KMC, Manipal.

### Preparation of curcumin loaded chitosan microspheres

Chitosan microspheres were prepared as described earlier (10) with slight modification. A 2% w/v solution of chitosan was prepared in 5% w/v citric acid aqueous solution with the assistance of heat. A known quantity of curcumin was dispersed in polymer solution with the aid of a homogenizer. This suspension was dropped into the liquid paraffin bulk oil phase containing 1% v/v Span 80 under continuous stirring using a homogenizer at 2000 rpm to form a water-in-oil (w/o) emulsion. Four ml of the crosslinking agent (gluteraldehyde saturated toluene, $T_{GS}$) was added drop wise and the emulsion was agitated at constant rotation (1000-1500 rpm) for specified period (4-5 hours) at ambient temperature. The microspheres formed were separated from

the reaction mixture by centrifugation (2500 rpm), washed several times with n-hexane until the oil phase was removed completely and finally dried at room temperature in a desiccater. Three D:P (drug:polymer) ratios such as 1:4, 1:2, and 1:1 were employed to prepare different formulations C1, C2 and C3 respectively.

**Preparation of curcumin loaded BSA microspheres**

A technique for manufacturing BSA microspheres has been developed by modification of the emulsion cross-linking method described by Gupta et al (4). BSA (200 mg) was dissolved in 1 ml of distilled water at room temperature and a known quantity of drug was dispersed by homogenization. The dispersion was transferred into a beaker containing liquid paraffin with 1% v/v Span 80. Stirring was maintained at a steady speed of 2000 rpm and 1 ml of $T_{GS}$ was added drop wise. Agitation was continued at room temperature until cross-linking completed (4–5 hours). The suspension was centrifuged and microspheres were collected; washed several times with n-hexane until the oil phase was removed completely and finally dried at room temperature in a desiccater. Three D:P (drug:polymer) ratios such as 1:4, 1:2 and 1:1 were employed to prepare different formulations A1, A2 and A3 respectively.

**Physicochemical characterization**

The size of the microspheres was determined by light microscopy using calibrated eye piece micrometer. Three hundred microspheres were counted and mean diameter and the size distribution characteristics were computed.

To determine the encapsulation efficiency and drug content, 100 mg of curcumin loaded BSA microspheres were digested in 10 ml 0.1N HCI for 48 hours in a shaker bath. The suspension was then transferred into a separating funnel and 10 ml octanol was added to selectively extract curcumin. The octanol layer was separated, diluted suitably and curcumin concentration was determined by measuring the absorbance at 434 nm against suitable blank prepared using microspheres without curcumin. Similarly, curcumin loaded chitosan microspheres were digested in 10 ml of 5% w/v citric acid solution; curcumin was extracted into 10 ml octanol and drug concentration was estimated spectrophotometrically at 434 nm.

***In-vitro* release studies**

Microspheres containing known amount of the drug were taken in 5 vials containing 10 ml of octanol maintained at a constant shaking of 60 oscillations per minute and ambient temperature. Samples were withdrawn at periodic time intervals with replacement of fresh release medium. The drug concentration in the samples was measured using UV-spectrophotometer (UV 240, Graphicord, Shimadzu, Tokyo, Japan) at 434 nm after suitable dilution.

***In-vivo* studies**

Pharmacodynamic evaluation of the drug

in the formulation was carried out using Freund's adjuvant induced arthritic model. Animals were divided in five groups of six each. Chronic inflammatory condition was induced in one of the hind paws of adult male Wistar rats (200-215 g) by an intradermal injection (0.1 ml) of Freund's complete adjuvant into subplantar surface. The edema produced was regularly monitored and assessed by measuring the volume of the injected paw using a mercury plethysmometer. Treatment schedule is given in table 2. On eighth day of inducing edema, paw volumes were recorded as initial values and curcumin loaded chitosan and BSA microspheres were given as single subcutaneous (s.c) injections after suspending in 0.3% w/v sodium carboxymethyl cellulose. Paw volumes were recorded on 10th day post-treatment and antiinflammatory activity was expressed as percentage inhibition of inflammation (primary response) (13). Statistical analysis was performed by student's t-test.

## RESULTS

Curcumin loaded BSA and chitosan microspheres exhibited good morphological characteristics with smooth boundary and mostly spherical ones as observed by light microscopy. However, occasional deformed and discrete particles were seen in both cases. The size distribution analysis revealed highest frequency of the microspheres in the vicinity of 20-30 μm. Chitosan microspheres exhibited larger diameter than their BSA counterparts at corresponding D:P (drug and polymer) ratios (Table I). The percentage yield of the product ranged between 55 to 80% and was always higher for BSA than for chitosan. From the table I, it is apparent that the content of curcumin in the chitosan microspheres is significantly lower than in the equivalent BSA microspheres. BSA microspheres exhibited higher drug encapsulation efficiency ranging from 69 to 80% with chitosan microspheres in the range of 30 to 40% at comparable D:P ratios.

TABLE I: Physicochemical characterization of curcumin loaded microspheres.

| *Formulation* | *D:P ratio* | *Encapsulation efficiency (%)* | *Mean particle size (μm)* | *Drug content per 100 mg microspheres* |
|---|---|---|---|---|
| A1 | 1:4 | 79.49 | 22.83 | 15.89 |
| A2 | 1:2 | 69.68 | 23.92 | 23.21 |
| A3 | 1:1 | 72.77 | 28.51 | 36.35 |
| C1 | 1:4 | 32.96 | 29.23 | 6.59 |
| C2 | 1:2 | 30.17 | 25.01 | 10.05 |
| C3 | 1:1 | 39.66 | 33.33 | 19.83 |

A = Albumin microspheres, C = Chitosan microspheres
D:P Ratio = Drug : Polymer ratio

***In-vitro* release studies**

The release pattern of curcumin was observed as biphasic (Fig. 1 and 2) characterized by an initial burst effect followed by a slow release. A secondary burst was observed in case of 1:1 ratio towards the tailend from albumin microspheres while no secondary burst effect was observed with chitosan microspheres with 1:1 D:P ratio. The trend of drug release pattern was comparable for both the polymers however, chitosan microspheres released the loaded curcumin at a slower rate compared to the BSA counterparts. D:P ratio had a remarkable influence on drug release with higher polymer ratios retarding the drug release. We examined the polymers at single corsslinking density as per their molecular weights (BSA -47,000 Da; chitosan - 1 to 3 x 105 Da). No

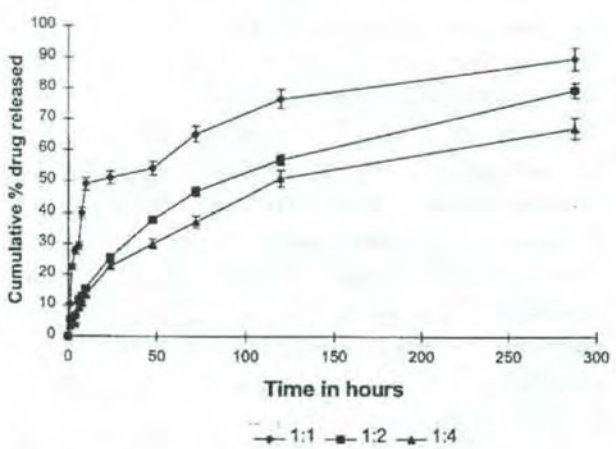


Fig. 1: *In vitro* release of curcumin from BSA microspheres.

*In-vitro* release studies from albumin microspheres was done by placing microspheres containing known amount of drug in 5 vials containing 10 ml of octanol maintained at a constant shaking of 60 oscillations per minute and ambient temperature. Values are expressed as mean ± S.D. of three trials.

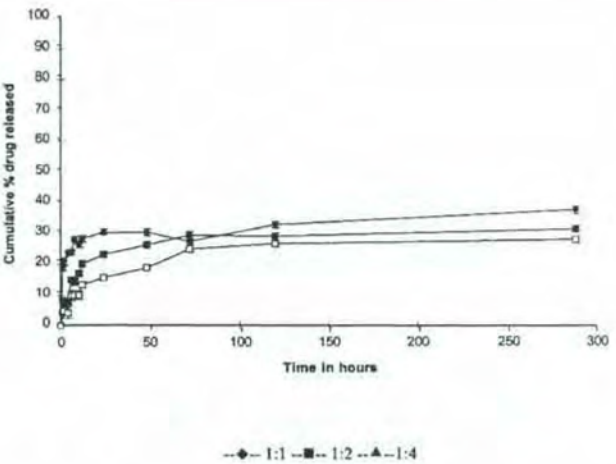


Fig. 2: *In vitro* release of curcumin from chitosan microspheres.

*In-vitro* release studies from chitosan microspheres was done by placing microspheres containing known amount of drug in 5 vials containing 10 ml of octanol maintained at a constant shaking of 60 oscillations per minute and ambient temperature. Values are expressed as mean ± S.D. of three trials.

flakes or aggregates were observed in both cases ruling out possible bridging between the microspheres formed and the products formed were free flowing.

The result of pharmacodynamic activity of curcumin formulations in both the carriers are depicted in the Table II. When curcumin was given orally, its anti-inflammatory response was very minimal. Maximal inhibition of inflammatory response was observed in case of BSA formulations which was significantly ($P<0.05$) higher compared to chitosan formulations as well as curcumin given as injection. There was no significant difference observed between the groups treated with curcumin loaded chitosan microspheres and plain curcumin injection.

TABLE II: Anti-inflammatory activity of curumin loaded microspheres.

| *Group* | *Treatment* | *Dose mg/kg* | *Change in paw volume (mL)* | *% inhibition* |
|---|---|---|---|---|
| 1 | Control (0.3% CMC Na) | ------- | –0.136 | –15.1341 |
| 2 | Curumin (s.c. injection) | 100 | 0.163 | 17.65 |
| 3 | Curumin (oral) | 100 | 0.096 | 11.32 |
| 4 | Albumin microspheres (A3) (s.c. injection) | 100 | 0.396 | 35.86* |
| 5 | Chitosan microspheres (C3) (s.c. injection) | 100 | 0.17 | 15.93 |

Number of animals per group = 6 rats
*Significant difference ($P<0.05$) compared to curcumin loaded microspheres and plain curcumin injection
CMC.Na = Carboxy methyl cellulose sodium

## DISCUSSION

Curcumin could be microencapsulated into BSA and chitosan even though the extent of encapsulation varied significantly. The products formed, when observed under microscope, exhibited discrete particles with some microspheres showing deformed surface/ boundary and a wide size distribution range (from 6 to 90 μm). Occasionally, microspheres obtained from natural polymers are not perfectly spherical because of variations in molecular weight and other chemical properties of the polymer itself. Also, because of the high viscosity of the liquid paraffin, the microspheres formed, experience greater resistance (hence get deformed) while the internal phase evaporates leaving behind spherical walls of the polymer within which the drug is encapsulated. As the internal phase is aqueous in both the cases, its rate of evaporation is also very slow giving rise to deformed boundaries. Wide size distribution range is also because of the higher viscosity of the external oil phase and higher rate of shear. Discrete patches of precipitated drug on the surface of the microspheres were discernible by their vivid yellow color under light microscope.

We have used 5% w/v citric acid aqueous solution for solubilizing chitosan as it forms soluble salt with acids. Even though most of the reported methods used for preparation of chitosan microspheres employ acetic acid as the solvent, its use in biomedicine is limited by its toxicity aspect. Citric acid enjoys better tolerance and safety index compared to other mineral acids, hence it was taken up. The viscosity measurements of the solution of chitosan in citric acid (120 cps for 1% w/v solution in 5% w/v citric acid) showed optimum fluidity compared to solution in acetic acid (175 cps for 1% w/v solution in 6% v/v acetic acid). The higher viscosity of acetic acid solution of the polymer reduced the ease of handling the solution for formulation procedure. The citric acid as a solvent offered optimum viscosity for suspending the drug for preparation of w/o emulsion and also

facilitated the formation of smooth walled microcapsules. We opted for chemical crosslinking procedure as control of the degree of crosslinking is easily variable and reproducible from batch to batch. Gluteraldehyde saturated toluene ($T_{GS}$) was employed rather than aqueous solution of gluteraldehyde as the former offer uniform distribution of the crosslinking agent throughout the continuous oil phase. The aqueous solution of gluteraldehyde, due to non-miscibility in the oil phase, results in non-uniformly crosslinked product leading to erratic entrapment and drug release kinetics. The curcumin was loaded into BSA microspheres as a concentrated suspension ranging from 5 to 20% w/v where as same loaded into chitosan microspheres in the range of 0.5 to 2% w/v. Due to this discrepancy, there was significant difference in the encapsulation efficiency between the two formulations. Also, the total polymer available for encapsulation was much less for chitosan formulation (1% w/v) compared to BSA formulation (20% w/v). It also could be assumed that, BSA having many reactive groups distributed throughout the surface, may get involved in weak hydrophobic interactions with curcumin, which could be one of the reasons for higher degree of encapsulation.

During the in-vitro release studies, the initial burst effect corresponds to the release of the drug located on or near the surface of the microspheres or release of poorly entrapped drug. The washings with n-hexane did not result in removal of the adsorbed drug particles. The subsequent slow release period is due to the release medium being diffused into the polymer matrix, whereby the drug diffuses out of the microspheres. The secondary burst of albumin microspheres with 1:1 D:P ratio indicated the erosion of the polymeric matrix due to continuous efflux of the drug followed by dissolution of the polymer into the release medium. The rate of drug release increased with decrease in the polymer ratio in the formulation. The chitosan formulations released drug pay load much slower than the BSA counterparts because of higher molecular weight of the former and also higher degree of crosslinking density (6.66% v/v for chitosan against 1.66% v/v for BSA). Insignificant intra-batch and inter-batch variations with respect to in-vitro drug release pattern indicate uniform degree of crosslinking throughout the encapsulation procedure.

We selected the formulation with highest rate of drug release (D:P = 1:1) so as to project the pharmacodynamic profile at initial stage itself. We compared the activity with curcumin given orally and as subcutaneous (s.c.) injection by suspending in 0.3% w/v sodium carboxymethyl cellulose at a dose level of 100 mg/kg body weight, which is the ED50 of curcumin (14). The route of administration was s.c. which favours prolonged drug release than intravenous or intramuscular routes because of diffused vascularity of adipose tissue and is ideal for a depot forming formulation. The reasons for significantly ($P<0.05$) increased anti-inflammatory response in case of BSA formulations can be explained on the basis of total amount of drug release during the period of study. As predicted from the *in-vitro* drug release data, 84% of the encapsulated curcumin was released from BSA

formulation against 36% in case of chitosan microspheres. This percentage would be much higher in-vivo due to presence of enzymes and other tissue fluids at the injection site which favour faster extraction of the drug from the formulation. Oral route, which is not a preferred route of administration for curcumin (as it is least absorbed from GIT) offered minimal protection against Freund's adjuvant induced rat paw edema. The s.c. injection of curcumin also produced insignificant reduction in inflammation though the reduction in paw volume initially was greater than polymeric microspheres which could be due to greater amount of curcumin available in plain injection. In case of microspheres, though the initial reduction (first two days post-treatment) of paw volume was not significant, the total inhibition of inflammatory process at the end of 10 days was much higher than plain injection. BSA microspheres, due to smaller diameter, offered greater surface for drug extraction from the depot and hence greater pharmacodynamic activity. Total amount of curcumin available was extended over the study period resulting in improved efficacy. With chitosan microspheres, the rate of drug release was slower as explained earlier and total amount available at the end of 10 days could be comparable to that of plain injection. Improved anti-inflammatory activity may be expected from chitosan formulation if the duration of study is further increased. We took 10th day post-treatment as the end point as extrapolated from the *in-vitro* kinetics and formulation parameters.

Curcumin is a potent antiinflammatory agent acting through scavenging oxygen free radicals produced as a consequence of reactions occurring due to inflammation process. Inhibition of pathological free radical reactions has been supposed to account for part of the action of the known antiinflammatory agents including NSAIDs. We have reported improved radioprotection activity for microencapsulated curcumin in synthetic biodegradable polymer earlier (15). Incorporation of curcumin into natural biodegradable polymers not only improves the pharmacodynamic efficacy but also reduces the cost of therapy due to reduction in total dosing and lesser cost of such polymers compared to synthetic ones.

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