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Research Article

PREPARATION AND EVALUATION OF BIODEGRADABLE ALBUMIN MICROSPHERES OF KETOROLAC TROMETHAMINE

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ABSTRACT
The objective of the present study is to prepare sustained-release ketorolac tromethamine microspheres of bovine serum albumin in different ratios by the emulsion cross-linking method using epichlorohydrin. The prepared microspheres were subjected to various physicochemical evaluation and *in vitro* release studies. The drug release from microspheres of 1:5 ratios is the most constant and prolonged drug release is diffusion followed by erosion. The characteristics of the prepared microspheres are conducive to the formulation of the sustained release drug delivery system.

KEYWORDS: Microspheres, Ketorolac tromethamine, Epichlorohydrin.

INTRODUCTION
Microspheres are the colloidal drug delivery system. Microspheres are characteristically free-flowing powders consisting of proteins/synthetic polymers that are biodegradable in nature and ideally having a particle size less than 200 µm. Biodegradable microspheres can be utilized to direct drugs to certain organs through capillary blockade. Its success depends on the size of the microspheres used and on the mode of administration (intravenous/intra-arterial)[1]. Microsphere carrier systems made from the biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[2,3]. They have varied applications and are prepared using assorted polymers[4].
Ketorolac (KT) is a well known non-steroidal anti-inflammatory drug with potent analgesic activity. It is used in the form of Tromethamine salt due to its water-insolubility[5]. Ketorolac is currently administered intramuscularly and orally in multiple divided doses for short-term management of post-operative pain[6]. Intramuscular injection is the preferred route of administration (30 mg four times a day). It is administered for moderate to severe pain management, even though patient compliance is rather low for this route. The drug is also administered via the oral route as a conventional tablet (10 mg four times a day) for management of mild to moderate pain[7]. In addition to the limitations in the available routes of administration, the half life of KT ranges from 4-6 h[5]. Therefore, frequent dosing is required to alleviate pain in postoperative patients due to its short half-life. To avoid an invasive drug delivery technique (i.e. intramuscular injection) and to decrease the gastrointestinal side effects produced by the oral tablets, there is a need for an alternative noninvasive mode of delivery for KT. The new delivery system should also provide sustain in the release of this medication to assist patient compliance. Many trials have been conducted to formulate KT into dosage forms other than IM injection or oral tablet. Roy and Manoukian[8] have reported the transdermal feasibility of ketorolac formulated in transdermal patches. In another study, nasal formulation of ketorolac has been reported by Santus[9].

Albumin microspheres are biodegradable particles that can be produced in a size range of 1 to 200 μm in diameter, by either physical or chemical solidification of an albumin emulsion in an organic phase[10]. Bovine serum albumin (BSA) is widely used for microsphere preparation because it is nonantigenic, biodegradable, free from toxicity, able to control the physicochemical characteristics of the microspheres produced, and readily available. Albumin-based drug delivery systems are popular for the treatment of inflammation and arthritis because albumin has a tendency to deposit at the inflamed joints[11-13]. Albumin is a major plasma protein constituent, accounting for 55% of the total protein in human plasma. Albumin microspheres are metabolized in the body, and the size of particles, degree of stabilization, and site of metabolism are the main factors influencing the extent of metabolism. Drug release from the microspheres can be controlled by the extent and nature of cross-linking, size, and drug incorporation level in the microspheres.

Thus, in the present study KT-loaded microspheres were prepared using biodegradable carrier albumin by emulsification-crosslinking technique, using epichlorohydrin as crosslinking agent. The microspheres were characterized in terms of particle size, percentage yield, percentage drug encapsulation, scanning electron microscopy, infra-red spectroscopy, and *in-vitro* drug release studies.

MATERIALS AND METHODS

Ketorolac tromethamine (KT) was procured as a gift sample from Ranbaxy pharmaceutical, Dewas. Bovine serum albumin (BSA), and Epichlorohydrin were purchased from S.D. Fine Chem. Ltd., India. Liquid paraffin, Span-80 and n-hexane were purchased from Rankem, New Delhi. All reagents used were of analytical reagent grade.

Preparation of microspheres of KT

Albumin microspheres were prepared by slight modification of emulsion cross-linking method earlier reported by Mathew et al[14]. Drug (200 mg) was dissolved in BSA solution in water (20%, 1-2 mL) using a cyclo-mixer. This mixture was added dropwise to liquid paraffin (50 mL), while stirring the whole system at 300 rpm. One percent wt/vol Span 80 was added as surfactant to the oil phase. After 15 minutes of stirring, epichlorohydrin was added as a chemical cross-linking agent. Stirring was continued for the required cross-linking duration (4-12 hours). The cross-linked albumin microspheres were separated from the oil phase by filtration and were washed with n-hexane (75 mL) to remove the excess oil. The microspheres obtained were then dried in a vaccum desiccator to get free-flowing microspheres in a powder form. By varying the drug: polymer ratio, six batches of microspheres were prepared (Table 1)

PHYSICOCHEMICAL EVALUATION OF THE MICROSPHERES

Percentage production yield

Total amount of microspheres obtained were weighed individually for each batch and the percentage yield was calculated taking into consideration the weight of drug and polymer[15]. Yields of production of different formulations were calculated by using the formula:

$$\% \text{ Yield of Production} = \text{practical yield/theoretical yield} \times 100$$

Size analysis

It is carried out by using a compound microscope at ×45. Dried microspheres were first redispersed in distilled water and placed in a glass slide and the number of divisions of the calibrated eye piece was counted by a micrometer using a stage micrometer. The average size of the particles was determined[16].

Melting point

A small amount of the microspheres was taken and they were ground to remove the coating material and then subjected to melting point determination[17].

Entrapment efficiency

To determine the amount of KT encapsulated in microspheres, a known weight of microspheres was weighed into screw-capped vials with 0.1 N HCl and digested for 24 hours on a magnetic stirrer in order to extract the entrapped drug completely. The absorbance was noted at 323 nm using UV spectrophotometer (UV 1700 Schimadzu, Japan) after diluting suitably with distilled water. Blank microspheres treated in a similar manner were used as the blank[14]. The percentage of encapsulation efficiency was calculated by the following formula.

$$\% \text{ EE} = [\text{ED/AD}] \times 100$$

Where, %EE is the percentage entrapment efficiency; ED is the amount of entraped drug; and AD is the amount of added drug.

Scanning Electron Microscopy (SEM) analysis

Scanning electron microscopy (SEM) is helpful to examine microspheres' shape and surface characteristics in order to correlate other determined characteristics such as surface area and bulk density. The microspheres were placed on one side of an adhesive stub, and the stub was then coated with conductive gold with sputter coater attached to the instrument. The microspheres were then examined under JSM 5310 SEM at 15 to 20 kV (JEOL Ltd, Tokyo, Japan).

***In-vitro* drug release studies**

Release profile of the pure drug and selected formulations were studied through dialysis bag. Five milligrams of the pure drug or microspheres equivalent to 5 mg was placed inside the dialysis bag and 4 mL of phosphate buffer saline (PBS, pH 7.4) was added to the bag. This was then suspended in 50 mL PBS contained in a beaker. At specific time intervals, 1 mL of the sample was withdrawn from the receptor compartment, and the volume was made up with distilled water. The absorbance was noted at 323 nm after suitable dilution in UV spectrophotometer (UV 1700 Schimadzu). One milliliter of fresh PBS was added to the receptor compartment after withdrawal of the sample to compensate for the loss caused by removal of sample. Each experiment was conducted in triplicate[14].

RESULT AND DISCUSSION

In the present study, BSA microspheres loaded with KT were prepared by the emulsion cross-linking method described by Mathew *et al.,* with modification in cross-linking agent and rpm. Microspheres of drug polymer ratio 1:1 and 1:2 could not be obtained, indicating the insufficiency of the proportion of the polymer for the formation of the microspheres while microspheres of the drug polymer ratio 1:6 shows clumping. Microspheres of all the other three batches were discrete and free flowing and were evaluated further.

Physical characteristics of microspheres BSA-3, BSA-4 and BSA-5 were shown (Table 2). The melting points of the free drug and the drug in the microspheres were found to be the same (166°C), indicating that there is no change in the nature of the entrapped drug due to the process of formulation of the microsphere. The yield of microspheres was obtained in range of (83-92%) while the size of microspheres prepared in this study was in the range of 9.078 to 22.453 μm. It was observed that as the amount of polymer increased in the microspheres the particle size also increased proportionally. The increase in the particle size observed with increase in polymer concentration was due to increase in viscosity of droplet[18]. The microspheres were analyzed for the encapsulation efficiency and was found to be encapsulated 68-82% in two batches (BSA-3 and 4), and 89% in one batch (BSA-5), which shows that if there is an increase in the concentration of the polymer, the encapsulation efficiency also increases. From the encapsulation efficiency data, we can state that there is no wastage of the drug and hence this method is economical. SEM analysis of microspheres revealed that all microspheres prepared were spherical in shape and is having porous outer skins (Figures 1).

The dissolution of the pure drug was complete within 50 min, indicating that the solubility of the drug in the dissolution medium is not a limiting factor or a constraint to drug release. The drug release from all the batches (BSA-3 to 5) is sustained over 24 h. A biphasic release pattern was observed with all the batches, which is a slow first phase followed by a rapid release (Table 3). This is in concurrence with the observations made with the release from the biodegradable microspheres. The second rapid release after 17–21 h may be attributed to the increased permeability of the microspheres and facilitated diffusion of the drug through the newly generated pores and surfaces and also the degradation of the polymer, which might have enhanced the release. As the polymer ratio increased, the time taken for the change in the release behavior was also prolonged, although relatively high microspheres were obtained in BSA-3 and its release was unusually high. This may be due to the lower polymer proportion. A perfect positive correlation was observed with the release behavior in all the batches, showing a constant controlled delivery except the slight 1st-h burst effect. This might have been due to the un-entrapped drug.

CONCLUSION

BSA microspheres loaded with KT were prepared by the emulsion cross-linking method with modification in cross-linking agent and rpm. As the amount of polymer increased in the microspheres the particle size also increased proportionally. The increase in the particle size observed with increase in polymer concentration was due to increase in viscosity of droplet. The dissolution of the pure drug was complete within 50 min, indicating that the solubility of the drug in the dissolution medium is not a limiting factor or a constraint to drug release. The drug release from all the batches is sustained over 24 h. A perfect positive correlation was observed with the release behavior in all the batches, showing a constant controlled delivery of drug.

REFERENCES

1. Longo WE, Iwata H, Lindheimer TA, Goldberg EP. Preparation of hydrophilic albumin microspheres using polymeric dispersions agents. J Pharm Sci 1982; 71: 1323-1328.
2. Viernstein H, Christian P, Luftensteiner. Statistical experimental desigh based studies on placebo and mitoxantrone-loaded albumin microspheres. Int J Pharm 1998; 171: 87-99.
3. Narayani R, Rao KP. Controlled release of anticancer drug methotrexate from biodegradable gelatin microspheres. J Microencapsul 1994; 11: 69-77.
4. Chowdary KPR, Rao YS. Mucoadhesive microspheres for controlled drug delivery. Biol Pharm Bull 2004; 27:1717-1724.
5. Buckley MMT, Brogden RN. Ketorolac: a review of its Pharmacodynamic and pharmacokinetic properties, and therapeutic potential. Drugs 1990; 39: 86-109.
6. Olmedo MV, Galvez R, Vallecillo M. Double-blind parallel comparison of multiple doses of kerorolac, ketoprofen and placebo administered orally to patients with postoperative dental pain. Pain 2001; 90: 135-141.
7. Morszczak EJ, Jung D, Yee J, Bynum L, Sevelius H. Ketorolac tromethamine pharmacokinetics and metabolism after intravenous, intramuscular and oral administration in humans and animals. Pharmacotherapy 1990; 10: 33S-39S.
8. Roy SD, Manoukian E. Transdermal delivery of ketorolac tromethamine: Permeation enhancement, device design and pharmacokinetics in healthy humans. J Pharm Sci 1995; 84:1190-1196.
9. Santus G, Rivolta R, Bottoni G. Nasal formulations of ketorolac tromethamine: technological evaluation, bioavailability and tolerability in rabbits. Il Pharmaco 1993; 48:1709-1723.
10. Gupta PK, Hung CT. Albumin microspheres. 1. Physico-chemical characteristics. J Microencapsul 1989; 4: 427-462.
11. Bahukudumbi P, Carson KH, Rice-Ficht AC, Andrews MJ. On the diameter and size distributions of bovine serum albumin (BSA)-based microspheres. J Microencapsul 2004; 21:787-803.
12. Bernard NG, Shaw SM, Kessler WV, Landolt RR, Peck GE, Dockerty GH. Distribution and degradation of iodine-125 albumin microspheres and technetium-99m sulphur colloid. Can J Pharm Sci 1980; 15:30-34.
13. Zolle I, Rhodes BA, Wagner HN. Preparation of metabolizable radioactive human serum albumin microspheres for studies of the circulation. Int J Appl Radiat Isot 1970; 21:155-167.
14. Mathew ST, Devi SG, KV S. Formulation and evaluation of ketorolac tromethamine-loaded albumin microspheres for potential intramuscular administration. AAPS Pharm Sci Tech 2007; 8: E54-E60.
15. Yadav AV, Mote HH. Development of biodegradable starch microsphere for intra-nasal delivery. Indian J Pharm Sci 2008; 2:170-174.
16. Capan Y, Jiang G, Giovangali S, Na KH, Deluca PP. Preparation and characterization of Poly (D,L-Lactide-co-Glycolide) microspheres for controlled release of human growth harmone. AAPS Pharm Sci Tech 2003; 4:1-10.
17. Jayaprakash S, Mohamed HS, Mohamed F, Kulaturanpillai K, Abhijith, Nagarajan M. Preparation and evaluation of biodegradable microspheres of methotrexate. Asian J Pharm 2009; 1:26-29.
18. Pisal S, Shelke V, Mahadik K, Kadam S. Effect of organogels component on *in vitro* nasal delivery of propanolol hydrochloride. AAPS Pharm Sci Tech 2004; 4:1-9.

Table 1: Composition of KT microspheres of bovine serum albumin

Sl. No.	Batch Code	Drug: Polymer ratio
01	BSA-1	1:1
02	BSA-2	1:2
03	BSA-3	1:3
04	BSA-4	1:4
05	BSA-5	1:5
06	BSA-6	1:6

Table 2: Physical characteristics of prepared microspheres of KT

Formulation code	Product yield (%)	Size distribution (μm)	Entrapment efficiency (%)
BSA-3	83	9.078	68
BSA-4	88	16.321	82
BSA-5	92	22.453	89

Table 3: Data of the biphasic release pattern of KT from the microspheres

Formulation code	Cumulative % release of KT in first phase		Cumulative % release of KT at the 24th h
	Time (h)	Cumulative % release	
BSA-3	17	29.16	95.64
BSA-4	17	24.70	87.20
BSA-5	17	25.45	72.02

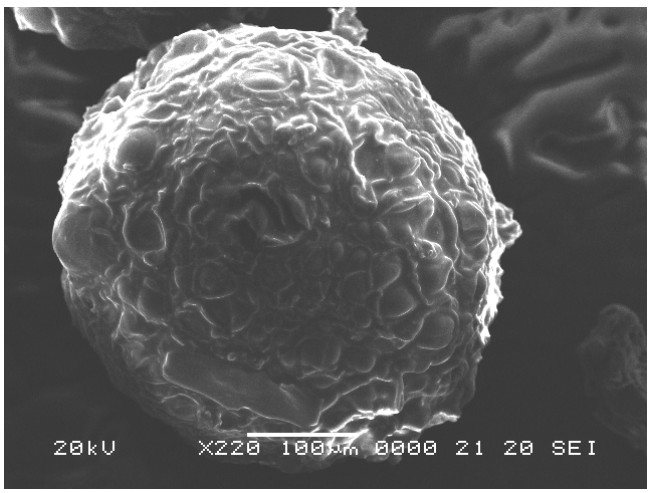

Figure 1: Scanning electron photomicrograph of bovine serum albumin microspheres containing KT

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