Formulation and Evaluation of Ketorolac Tromethamine-loaded Albumin Microspheres for Potential Intramuscular Administration

Submitted: May 30, 2006; Accepted: August 10, 2006; Published: February 23, 2007

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ABSTRACT

The objective of this work was to prepare and evaluate ketorolac tromethamine-loaded albumin microspheres using a factorial design. Albumin microspheres were prepared by emulsion cross-linking method. Selected formulations were characterized for their entrapment efficiency, particle size, surface morphology, and release behavior. Analysis of variance (ANOVA) for entrapment efficiency indicated that entrapment efficiency is best fitted to a response surface linear model. From the statistical analysis it was observed that as the drug:polymer (D:P) ratio and volume of glutaraldehyde increased, there was a significant increase in the encapsulation efficiency. Scanning electron microscopy of the microspheres revealed a spherical, nonporous and uniform appearance, with a smooth surface. Based on the entrapment efficiency and physical appearance, 9 formulations were selected for release study. The maximum particle size observed was below 40 μm. The release pattern was biphasic, characterized by an initial burst effect followed by a slow release. All selected microspheres, except those having less polymer proportion (D:P ratio is 1:1), exhibited a prolonged release for almost 24 hours. On comparing r^2 values for Higuchi and Peppas kinetic models, different batches of microspheres showed Fickian, non-Fickian, and diffusion kinetics. The release mechanism was regulated by D:P ratio and amount of cross-linking agent. From the experimental data obtained with respect to particle size and extent of drug release, it could be concluded that the prepared microspheres are useful for once-a-day intramuscular administration of ketorolac tromethamine.

KEYWORDS: Ketorolac tromethamine, albumin microspheres, intramuscular administration, Higuchi and Peppas kinetic models.

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INTRODUCTION

Ketorolac tromethamine (KT), a nonsteroidal anti-inflammatory drug (NSAID), is indicated for the short-term management of severe acute pain that requires analgesia at the opioid level. It is one of the commonly used drugs for the treatment of pain that is inexpensive, safe, and well tolerated.[1] Ketorolac (free acid) is sparingly soluble in water and, therefore, it is marketed in the form of tromethamine salt, which increases its solubility in water.[2]

Ketorolac tromethamine is a potent NSAID analgesic, and its administration carries many risks when administered as a conventional oral dosage form. The major side effect is gastrointestinal (GI) complications including peptic ulcers, and GI bleeding or perforations. KT is available for oral, intravenous (IV), or intramuscular (IM) administration. The half-life of ketorolac tromethamine is around 6 hours. The recommended daily dose of KT injection is 120 mg in divided doses. Frequent dosing of the drug may reduce patient compliance. Moreover, the majority of adverse reactions to KT are dose related, and it is strictly advised not to exceed this limit. Hence the need to have a controlled drug delivery system for KT is necessary.

A trend in NSAID development has been to improve therapeutic efficacy and reduce the severity of upper GI side effects through altering dosage forms of NSAIDs by modifying release of the formulations to optimize drug delivery. These formulations are designed to increase patient compliance through a prolonged effect and to reduce adverse effects through lowered peak plasma concentrations. Many controlled-release dosage forms are designed to release the drug at a predetermined rate, thus maintaining relatively constant drug levels in the plasma for an extended period of time. Several benefits may result from the use of such formulations. Reduction of frequency of dosing, lowered adverse effects, and improved patient compliance are considered the primary advantages of controlled-release dosage forms. One such formulation uses polymeric microspheres as carriers of drugs. Many authors have reported that nanoparticles and microparticles have a tendency to accumulate in the inflamed areas of the body. It has been reported that microspheres of NSAIDs

reduce the GI toxic effects and exhibit sustained action, thus increasing patient and therapeutic compliance.[3]

The use of biodegradable polymeric microparticulate systems is an interesting application in the control of drug release and targeting. The yield, drug content, and particle size distribution depend on different factors such as the nature of the polymer, and the formulative and preparative methods.

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.[4] 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.[5-7] 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.

In the present study, preparation and characterization of KT-loaded albumin microspheres was performed. Many process variables can influence the characteristics of the resultant albumin microspheres. It is difficult to assess the effect of the variables individually or in combination. The effects of 3 variables, namely: (1) drug:albumin ratio, (2) amount of glutaraldehyde, and (3) duration of cross-linking, on the encapsulation efficiency of albumin microspheres were studied using a factorial design. The objective of the study was to prepare KT-loaded albumin microspheres using a factorial design and to characterize the microspheres for encapsulation efficiency, release mechanism, particle size, and surface morphology.[8,9]

A technique of 3^3 factorial design taking 3 prime selected variables at 3 different levels affecting the entrapment efficiency was used to design the experimental batches for the preparation of KT-loaded albumin microspheres by chemical cross-linking method. A factorial design evaluating 3 factors at all combinations for each factor would result in a full-factorial design consisting of $3^3 = 27$ runs. The addition of center points allows for detection of nonlinearity in the responses. The total number of runs becomes 27 + 5 runs = 32 runs. The center points were run 6 times to get an estimate of experimental or pure error. F test was used to compare the variance among the treatment means with the variance of individuals within the specific treatments.

Materials and Methods

Materials

Ketorolac tromethamine, United States Pharmacopeia (USP), was a gift sample from Dr Reddy's Laboratories, Hyderabad, India; BSA fraction V (analytical grade) was purchased from Thomas Baker Chemicals, Mumbai, India. All other chemicals and solvents were of analytical reagent grade.

Preparation of Microspheres

Albumin microspheres were prepared by slight modification of emulsion cross-linking method earlier reported by Kumar et al.[10] Three independent formulation variables were taken at its 3 levels; low, medium, and high. Values of these selected variables at different levels are shown in Table 1. All other factors, namely, rpm (2500), strength of glutaraldehyde (25%), and concentration of surfactant were kept constant. 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 2500 rpm. One percent wt/vol Span 80 was added as surfactant to the oil phase. After 15 minutes of stirring, glutaraldehyde-saturated toluene (Gst) 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 at room temperature. All experiments were performed under subdued light conditions to prevent photodegradation of KT.

Statistical Design

A commercially available software program was used (Design Expert, Version 7, Stat-Ease Inc, Minneapolis, MN). The experimental design chosen was response surface, 3-factor, 3-level factorial; 32 formulations were prepared. Run order was randomized to protect against the effects of time-related variables and also to satisfy the statistical requirement of independence of observations.

Analysis of variance (ANOVA) and all statistical analyses were also performed using the Design Expert software. Calculation of the effects was performed; half-normal plots

Table 1. Different Variables and Their Levels

Variables	Levels		
	Low	Medium	High
A (drug:albumin ratio)	1:0.5	1:1	1:2
B (amount of glutaraldehyde, mL)	0.5	1	2
C (duration of cross-linking, hours)	4	8	12

were then plotted. The significant effects would constitute the model. The F value was then calculated by comparing the treatment variance with the error variance. The multiple correlation coefficient was calculated, which is a measure of the amount of variation about the mean, which is explained by the model. All assumptions underlying the ANOVA were checked. For statistical purposes, the assumption was made that residuals are normally distributed and independent with constant variance.

Determination of Encapsulation Efficiency and Percentage Yield of Microspheres

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 322 nm using a double-beam spectrophotometer (UV 1601 Schimadzu, Kyoto, Japan) after diluting suitably with distilled water. Blank microspheres treated in a similar manner were used as the blank. The percentage of encapsulation efficiency was calculated by the following formula.

$$\%EE = \left(\frac{ED}{AD}\right) \times 100 \qquad (1)$$

where $\%EE$ is the percentage encapsulation efficiency; ED is the amount of encapsulated drug; and AD is the amount of added drug.

The percentage encapsulation was determined to evaluate the effect of 3 independent factors: drug:albumin ratio, duration of cross-linking, and volume of glutaraldehyde on the drug encapsulation. Based on the encapsulation efficiency and physical appearance, 9 formulations were selected for further study.

The percentage yield of microspheres was calculated using the following formula.

$$\text{Percentage Yield} = \left(\frac{\text{Practical Yield}}{\text{Theoretical Yield}}\right) \times 100 \quad (2)$$

Surface Morphology and Characteristics

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 840A SEM at 15 to 20 kV (JEOL Ltd, Tokyo, Japan).

Particle Size Analysis

The size of the albumin microspheres was analyzed by laser particle size analyzer (Malvern Instruments, Mastersizer 2000, Malvern, UK) using n-Hexane as dispersant. The sample was vortexed for 1 minute before sampling. The samples were then sonicated in a sonicator attached to the instrument throughout the process, and the duration of sonication was kept constant for all samples. The obscuration ranged from 4% to 6%. Volume of distribution was plotted using a computer program supplied by the manufacturer. Polydispersity was calculated by the following equation:

$$\text{Polydispersity} = (D_{0.9} - D_{0.1}) \div D_{0.5} \qquad (3)$$

where $D_{0.9}$, $D_{0.5}$, and $D_{0.1}$ are the particle diameters determined respectively at the 90th, 50th, and 10th percentile of undersized particles.

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 322 nm after suitable dilution in a double-beam spectrophotometer (UV 1601 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.

Mechanism of Drug Release

The profile and kinetics of drug release are important because they correlate the in vitro and in vivo drug responses by comparing results of pharmacokinetics and dissolution profile patterns.[11] Different mathematical models may be applied for describing the kinetics of the drug-release process from microspheres, the most suited being the one which best fits the experimental results. These models best describe drug release from pharmaceutical systems resulting from a simple phenomenon, or when this phenomenon, by being the rate-limiting step, conditions all the other processes occurring in the system.

The kinetics of KT release from albumin microsphere formulations were determined by finding the best fit of the release data to Higuchi and Korsmeyer-Peppas plots.

Higuchi developed several theoretical models to study release of high and low water-soluble drugs incorporated in semisolid and/or solid matrices. According to this model, drug release was described as a square-root time-dependent diffusion process based on Fick's law. This relation can be used to describe drug dissolution from several types of modified-release pharmaceutical dosage forms.

$$Q = tK_H\sqrt{t} \tag{4}$$

where K_H is the Higuchi's rate constant, and Q_t is the amount of drug released at time t. If a plot of square root of time versus cumulative amount of drug released yields a straight line, and the slope is one or more than one, then the particular dosage form is considered to follow Higuchi kinetics of drug release.[12]

Under some experimental situations the release mechanism deviates from the Fick's equation, following an anomalous behavior (non-Fickian release). In these cases a more generic equation can be used. Korsmeyer et al developed a simple, semi-empirical model, relating exponentially the drug release to the elapsed time.[13]

$$Q/tQ_\alpha = Kt^n \tag{5}$$

where Q_t/Q_α is the fraction of drug released at time t; K is a constant comprising the structural and geometric characteristics of the microsphere; and n, the release exponent, is a parameter that depends on the release mechanism and is thus used to characterize it.[14]

Peppas used this n value in order to characterize different release mechanisms. If the n value is 0.5 or less, the release mechanism follows Fickian diffusion, and higher values $0.5 < n < 1$ for mass transfer follow a non-Fickian model (anomalous transport). The drug release follows zero-order drug release and case-II transport if the n value is 1. For the values of n higher than 1, the mechanism of drug release is regarded as super case-II transport. This model is used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena was involved. For the determination of the exponent n, the portion of the release curve where $Q_t/Q_\infty < 0.6$ should only be used. The n value could be obtained from the slope of a plot of log Q_t/Q_∞ versus log time.

Results and Discussion

Preparation of Microspheres

A typical emulsion cross-linking method was used to prepare the microspheres because BSA and KT are water soluble. Albumin and KT were dissolved in distilled water, and this solution acted as the aqueous phase. The method involved the formation of small droplets of aqueous albumin in immiscible liquid, light liquid paraffin. The water-in-oil emulsion was stabilized by a lipophilic surfactant, Span 80. Being a soluble polymer, albumin has to be chemically cross-linked to become insoluble at 37°C. Glutaraldehyde saturated toluene is used as a cross-linking agent to obtain rigid microspheres in this study. Gst was added after 15 minutes of stirring the system at 2500 rpm. Fifteen minutes was given for the droplet formation, which is a dynamic process that approaches a steady-state droplet size distribution within a period of several minutes depending on various parameters of the system. An important feature of this technique is that the albumin droplets are converted to swollen particle of the same size. The individuality of the initially formed droplets is maintained by performing the cross-linking reaction at constant stirring and in the presence of lipophilic surfactant Span 80. Initially formed albumin microspheres are hardened with the chemical cross-linking agent Gst. The cross-linking reaction was initiated after the droplet formation process was complete. Gst offers uniform distribution of the cross-linking agent in the oil phase. Due to nonmiscibility in the oil phase, the aqueous solution of glutaraldehyde may result in nonuniformly cross-linked product, which will lead to erratic drug entrapment and drug release kinetics. The excess oil was removed by washing with n hexane (75 mL) in order to prevent microsphere aggregation and changes in the morphological properties.[15] The percentage yield of the microspheres ranged from 71.10% (KTAL F8) to 92.21% (KTAL F15). Percentage yield and physical appearance are given in Table 2. The effect of different factors on the percentage yield of microspheres was not clear, possibly as a result of the improper recovery of microspheres from the filter paper after filtration.

Encapsulation Efficiency

The analysis of KT content and encapsulation efficiency within microspheres prepared using different process parameters are reported in Table 3.

Response surface linear model with F value 37.08 was selected as a model. ANOVA results indicated that drug:polymer ratio was the most significant factor influencing entrapment efficiency. Volume of glutaraldehyde was also seen to be significant, with entrapment efficiency increasing with an increase in volume. The cross-linking time was not found to be a significant factor ($P >$ F value $> .05$).

ANOVA (Table 4) for entrapment efficiency indicated that entrapment efficiency is best fitted to a response surface linear model rather than a quadratic or cubic model because entrapment efficiency changed in a linear fashion with change

Table 2. Percentage Yield and Physical Appearance Microspheres

Serial No.	Formulation Code	*Percentage Yield (%)	±SD	Physical Appearance
1	KTAL F1	83.6	2.0	Fine free flowing powder
2	KTAL F2	75.0	5.0	-do-
3	KTAL F3	88.8	5.0	-do-
4	KTAL F4	81.6	5.2	-do-
5	KTAL F5	74.7	8.9	-do-
6	KTAL F6	91.9	3.7	Aggregates and sticky powder
7	KTAL F7	85.8	2.8	Fine free flowing powder
8	KTAL F8	71.1	5.0	-do-
9	KTAL F9	84.6	6.5	-do-
10	KTAL F10	80.8	6.2	-do-
11	KTAL F11	78.8	5.0	-do-
12	KTAL F12	84.1	9.6	Aggregates and sticky powder
13	KTAL F13	83.3	7.6	Fine free flowing powder
14	KTAL F14	79.4	4.1	-do-
15	KTAL F15	92.2	3.3	-do-
16	KTAL F16	89.5	4.3	-do-
17	KTAL F17	82.4	5.3	-do-
18	KTAL F18	90.1	3.3	-do-
19	KTAL F19	85.2	6.1	-do-
20	KTAL F20	81.6	4.0	-do-
21	KTAL F21	86.1	10.1	-do-
22	KTAL F22	85.4	8.5	-do-
23	KTAL F23	78.7	13.7	-do-
24	KTAL F24	87.1	5.0	-do-
25	KTAL F25	86.5	7.9	-do-
26	KTAL F26	83.6	3.1	-do-
27	KTAL F27	86.5	3.6	-do-

*Average of 3 determinations. -do- indicates same as above.

Table 3. Design Matrix and Encapsulation Efficiency

Formulation Code	Factor A	Factor B	Factor C	Encapsulation Efficiency (%)
1	1.00	0.50	4.00	28.1
2	0.50	0.50	4.00	21.8
3	2.00	0.50	4.00	48.9
4	1.00	1.00	4.00	37.4
5	0.50	1.00	4.00	35.5
6	2.00	1.00	4.00	44.8
7	1.00	2.00	4.00	44.0
8	0.50	2.00	4.00	30.3
9	2.00	2.00	4.00	53.0
10	1.00	0.50	8.00	38.1
11	0.50	0.50	8.00	30.3
12	2.00	0.50	8.00	37.7
13	1.00	1.00	8.00	41.5
13	1.00	1.00	8.00	41.4
13	1.00	1.00	8.00	41.2
13	1.00	1.00	8.00	41.4
13	1.00	1.00	8.00	41.4
13	1.00	1.00	8.00	41.4
14	0.50	1.00	8.00	26.6
15	2.00	1.00	8.00	52.7
16	1.00	2.00	8.00	38.0
17	0.50	2.00	8.00	31.5
18	2.00	2.00	8.00	57.2
19	1.00	0.50	12.00	32.8
20	0.50	0.50	12.00	28.6
21	2.00	0.50	12.00	53.0
22	1.00	1.00	12.00	36.6
23	0.50	1.00	12.00	33.3
24	2.00	1.00	12.00	48.5
25	1.00	2.00	12.00	38.0
26	0.50	2.00	12.00	30.5
27	2.00	2.00	12.00	59.4

in the evaluated factors. The polynomial equation obtained is as follows.[16]

$$\text{Entrapment Efficiency } (\%) = 17.7911 + 13.41970 \times A + 4.03280 \times B + 0.23502 \times C \quad (6)$$

The model F value of 37.08 with probability $P > F$ of .0001 implies that this model is significant with only a 0.01% chance that this F value could have occurred due to noise. The Pred R-Squared of 0.7357 is in reasonable agreement with the Adj R-Squared of 0.7773. Precision is a measure of the signal-to-noise ratio, and a value greater than 4 is required. The probability P value is used to quantify this probability and is a very good indicator of significance.[17]

Normal plot of residuals clearly showed that the variables influencing entrapment efficiency lie far away from the center (Figure 1). The perturbation plot is shown in Figure 2. The statistical differences were assessed using ANOVA to prove these results.

ANOVA results indicated drug:polymer ratio is the most important factor influencing entrapment efficiency. Also significant is the volume of glutaraldehyde.

A 3-level full-factorial design with axial and center points was used. Thirty-two formulations were prepared. Actual

Table 4. ANOVA for Response Surface Linear Model Analysis of Variance (Partial sum of squares - Type III)

Source	Sum of Squares	Mean Square	F Value	Prob >
Model	2113.29	704.43	37.08	<.0001
D:P	1911.80	1911.80	100.62	<.0001
Volume of glutaraldehyde	172.65	172.65	9.09	.0054
Cross-linking time	15.91	15.91	0.84	.3680

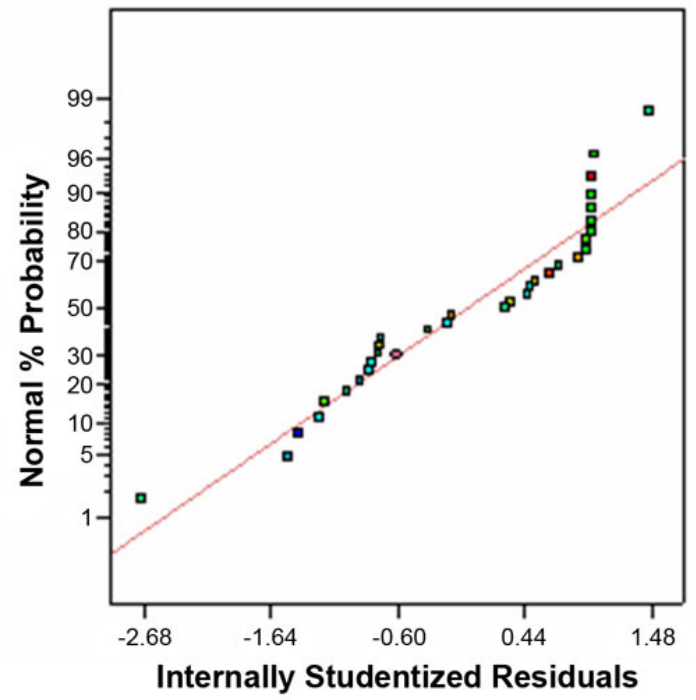

Figure 1. Normal plot of residuals.

fitting of the model was computed using the statistical software. An interaction is said to occur when the effect of one factor on a particular response varies with change in another factor. But this did not happen with the selected model. The perturbation plot helps to compare the effect of all the factors at a particular point in the design space. The response was plotted by changing only one factor over its range, while holding the other factors constant. A steep slope or curvature in a factor shows that the response is sensitive to that factor.

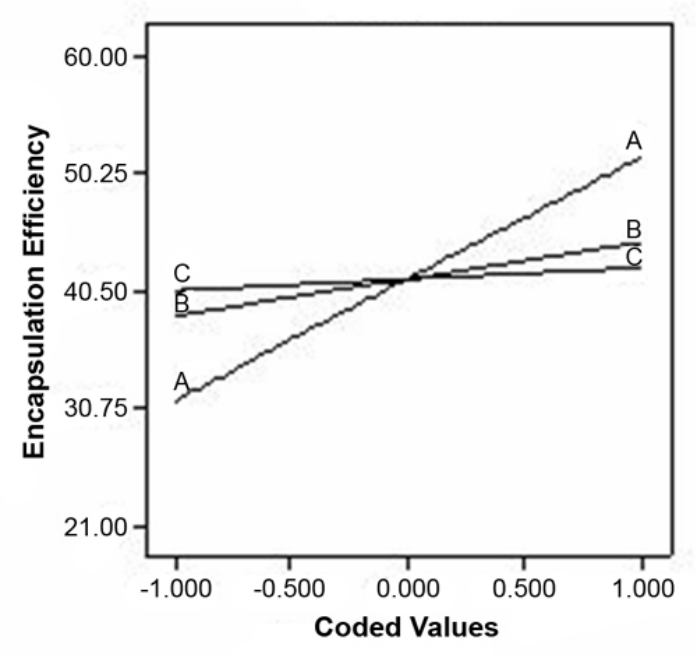

Figure 2. Perturbation plot: (A) drug:polymer ratio, 1:1; (B) volume of glutaraldehyde, 1 mL; (C) duration of cross-linking, 8 hours.

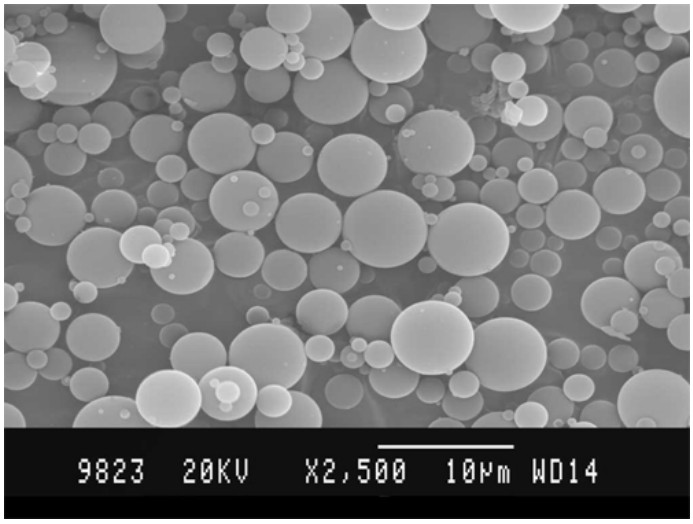

Figure 3. Scanning electron microscopy photomicrograph of KTAL microspheres.

From this plot it can be assumed that as the drug:polymer ratio and volume of glutaraldehyde increases, there is a significant increase in the encapsulation efficiency.

The increase in encapsulation efficiency with increase in drug:polymer ratio may be owing to the greater availability of polymer to coat the drug. Observations support that an increase in glutaraldehyde amount had helped in satisfactory cross-linking of microspheres, thereby avoiding the leaching out of drug from the individual microspheres. Based on the encapsulation efficiency, 9 formulations, KTAL F3, KTAL F4, KTAL F9, KTAL F10, KTAL F15, KTAL F18, KTAL F21, KTAL F24, and KTAL F27, were selected for further study.

Surface Morphology and Characteristics

Figure 3 shows the morphological characteristics of KTAL F3 microspheres. The SEM photomicrographs of the microspheres reveal that they are spherical, nonporous, and

Table 5. $D_{0.9}$, $D_{0.5}$, $D_{0.1}$, and Polydispersity of the Selected Formulations

Formulation Code	$D_{0.9}$ (µm)	$D_{0.5}$ (µm)	$D_{0.1}$ (µm)	Polydispersity
KTAL F3	11.7	9.2	7.1	0.49
KTAL F4	39.6	16.5	4.8	2.10
KTAL F9	38.5	22.5	8.0	1.35
KTAL F10	24.9	13.9	4.9	1.42
KTAL F15	38.6	16.6	3.4	2.11
KTAL F18	33.6	14.7	8.1	1.73
KTAL F21	37.5	23.1	10.9	1.14
KTAL F24	30.8	19.4	4.6	1.34
KTAL F27	31.5	15.1	4.4	1.79

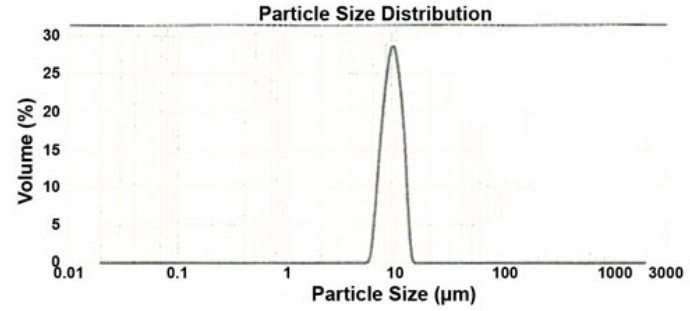

Figure 4. Classical size distribution curve of KTAL F3.

uniform with a smooth surface. The particles appeared to be aggregate in nature without evidence of any collapsed particles. Occasionally, microspheres obtained from natural polymers are not perfectly spherical because of the variations in molecular weight and other properties of the polymer, but we obtained microspheres with a uniformly smooth surface, without any deformed or rough surfaces. This result may be because of the low viscosity of light liquid paraffin, which was the external phase. The microspheres formed may not have experienced much resistance from the dispersion medium due to the low viscosity.

Particle Size Analysis

Size analysis of pharmaceutical products and their components is highly dependent on variables related to the particles themselves, the method of sampling, the technique of analysis, and the way in which the data are expressed. The dosage form and route of administration may necessitate the use of particles with unique characteristics especially in the case of parenteral preparations.

In the present study, the particle size of selected formulations was determined using a Malvern Mastersizer. $D_{0.9}$, $D_{0.5}$, $D_{0.1}$, and polydispersity are reported in Table 5.

The prepared microspheres revealed a unimodal size distribution. A classical size distribution curve of KTAL F3 is presented in Figure 4. The maximum particle size observed was below 40 µm. Comparatively narrow size distribution may be because of the lower viscosity of the external phase, which offered less resistance to the spheres formed. The maximum particle size observed was below 40 µm. The particle size distribution observed is similar to that required for intramuscularly administered products.[18] Sahin et al describe several different factors that can affect the particle size distribution including concentration of the polymer, glutaraldehyde concentration, and cross-linking time.[15]

Release Studies

Figure 5 shows the in vitro release behavior of KTAL microspheres. The standard deviation at each point, measured in triplicate, was less than 8%.

All selected microspheres except KTAL F4 and KTAL F10 exhibited a prolonged release for almost 24 hours. F4 and F10 released 100% drug in the 3rd and 4th hour, respectively.

The release pattern observed was a biphasic, characterized by an initial burst effect followed by slow release. Drug: polymer ratio had a remarkable influence on the drug release; higher polymer ratios retarded the drug release. The immediate release of drug from F4 and F10 may be due to the low proportion of the polymer (D:P is 1:1) and reduced amount of glutaraldehyde (0.5-1 mL) for cross-linking. As the glutaraldehyde concentration increases, the extent of denaturation increases, and this makes the albumin microspheres insoluble. This will lead to slower release of the drug from microspheres. Around 30% of the drug was found to be released in the first 30 minutes. This result could be due to the loosely bound or surface-embedded drug. The subsequent slow release may be because of the release medium

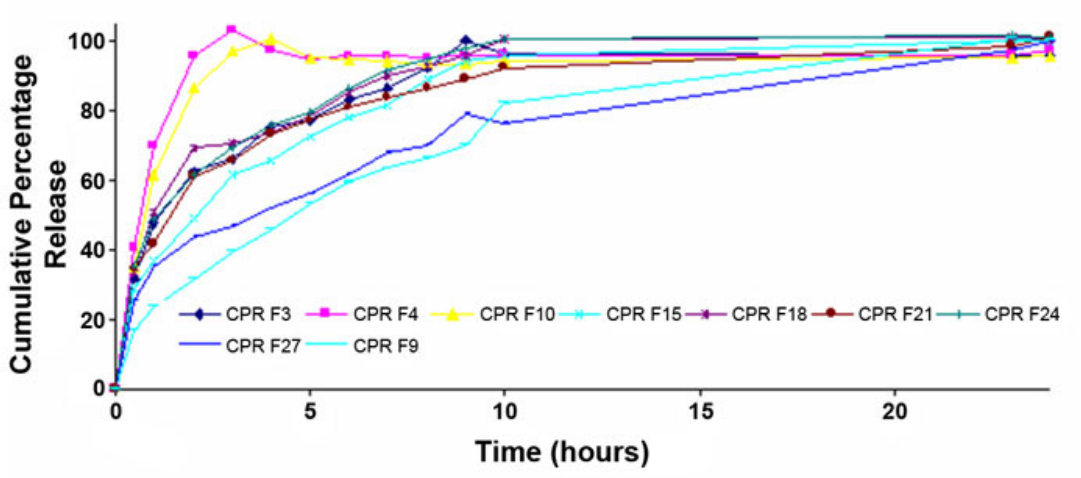

Figure 5. In vitro release behavior of KTAL microspheres.

Table 6. Values of r^2, k, and n for Selected Formulations

	Higuchi			Korsmeyer-Peppas			
Formulation	r^2	y	k	r^2	y	n	Mechanism of Drug Release
F3	0.964	$1.51x + 0.52$	1.51	0.979	$0.49x + 0.46$	0.49	Fickian release
F4	0.980	$3.13x + 0.04$	3.13	0.985	$0.79x + 0.65$	0.79	Non-Fickian release
F9	0.976	$1.05x + 0.20$	1.05	0.934	$0.51x + 0.21$	0.51	Diffusion/Fickian
F10	0.972	$2.67x + 0.11$	2.67	0.983	$0.74x + 0.59$	0.74	Non-Fickian release
F15	0.879	$1.13x + 0.83$	1.13	0.958	$0.50x + 0.39$	0.50	Fickian release
F18	0.805	$1.06x + 1.33$	1.06	0.955	$0.42x + 0.50$	0.42	Fickian release
F21	0.819	$0.91x + 1.36$	0.91	0.981	$0.41x + 0.46$	0.41	Fickian release
F24	0.821	$1.09x + 1.27$	1.09	0.959	$0.44x + 0.49$	0.44	Fickian release
F27	0.963	$0.94x + 0.65$	0.94	0.950	$0.44x + 0.31$	0.44	Diffusion/Fickian

being diffused into the polymer matrix, whereby drug may have diffused out of the microspheres. All these results indicate that release of KT from albumin microspheres can be controlled by varying the drug:polymer ratio and the volume of the cross-linking agent.

Ideally, controlled drug delivery systems should deliver the drug at a controlled rate over a desired duration. The primary objectives of the controlled drug delivery systems are to ensure safety and to improve efficacy of drugs, as well as to improve patient compliance. The drug release mechanism from controlled release devices is very complex, and not yet completely understood. Although some processes may be classified as either purely diffusional or purely erosion controlled, many others can only be interpreted as being governed by both.

Mechanism of Drug Release

The release data were analyzed on the basis of Korsmeyer-Peppas equation and Higuchi kinetics. The release rates k and n of each model were calculated by linear regression analysis using Microsoft Excel 2003 software. Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit. The r^2, k and n values are given in Table 6.

On calculating and comparing r^2 (0.9761 and 0.9636) values for Higuchi and Peppas kinetic models, F9 and F27 gave good fit to the Higuchi model. According to this model, the drug release from these microspheres may be controlled by micropore diffusion. The remaining formulations were best fitted into the Korsmeyer-Peppas model. F4 and F10 showed a non Fickian or anomalous release and formulations F3, F15, F18, F21, and F24 exhibited Fickian release. Fickian drug release is characterized by a linear dependence of the released drug with the square root of time that is concentration dependent. The fundamental of diffusion is based on Fick's laws, which describe the macroscopic transport of molecules by a concentration gradient. But in a non-Fickian case, the drug release varies with time t according to the power law, which has been already described. Formulations F4 and F10 showed anomalous release due to the polymer erosion and less proportion of polymer in the formulation (D:P = 1:1). But other formulations exhibited a Fickian or diffusion mechanism of drug release when the drug:polymer ratio was increased (1:2). This result may be caused by the diffusion of the release medium into the microspheres, thereby solubilizing the drug and releasing the drug slowly from the microspheres.

CONCLUSION

From the experimental results obtained with respect to particle size and prolonged drug release, it may be concluded that the developed albumin microspheres could be useful for once-a-day IM administration of ketorolac tromethamine. Further, pharmacokinetic and pharmacodynamic studies are required to confirm the application of these microspheres for IM administration.

ACKNOWLEDGMENT

The study was supported by the Indian Council of Medical Research, New Delhi, India, who provided a Senior Research fellowship.

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