Research Article

Evaluation of Ionotropic Cross-Linked Chitosan/Gelatin B Microspheres of Tramadol Hydrochloride

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Abstract. Microspheres of tramadol hydrochloride (TM) for oral delivery were prepared by complex coacervation method without the use of chemical cross-linking agents such as glutaraldehyde to avoid the toxic reactions and other undesirable effects of the chemical cross-linking agents. Alternatively, ionotropic gelation was employed by using sodium-tripolyphosphate as cross-linking agent. Chitosan and gelatin B were used as polymer and copolymer, respectively. All the prepared microspheres were subjected to various physicochemical studies, such as drug–polymer compatibility by thin layer chromatography (TLC) and Fourier transform infrared (FTIR) spectroscopy, surface morphology by scanning electron microscopy, frequency distribution, drug entrapment efficiency, *in vitro* drug release characteristics and release kinetics. The physical state of drug in the microspheres was determined by differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). TLC and FTIR studies indicated no drug–polymer incompatibility. All the microspheres showed initial burst release followed by a fickian diffusion mechanism. DSC and XRD analysis indicated that the TM trapped in the microspheres existed in an amorphous or disordered-crystalline status in the polymer matrix. From the preliminary trials, it was observed that it may be possible to formulate TM microspheres by using biodegradable natural polymers such as chitosan and gelatin B to overcome the drawbacks of TM and to increase the patient compliance.

KEY WORDS: chitosan; complex coacervation; gelatin B; microspheres; tramadol hydrochloride.

INTRODUCTION

The present study reports a novel attempt to prepare complex coacervates of chitosan and gelatin B as carriers for the widely used nonsteroidal anti-inflammatory drug tramadol hydrochloride (TM). It is a centrally acting opioid analgesic, used in severe acute or chronic pains. TM is an aminocyclohexanol derivative or 4-phenyl piperidine analog of codeine. Its analgesic effect is mediated through norepinephrine reuptake inhibition (1). The mean elimination half-life is ~6 h and requires dosing every 6 h in order to maintain optimal relief of chronic pain (2). Consequently, once-daily extended-release tablets have been formulated (tramadol ER). Long-term treatment with sustained-release TM once daily is generally safe in patients with osteoarthritis or refractory low back pain and is well tolerated (3).

TM offers several therapeutic advantages over other analgesics, such as good oral bioavailability and long elimination half-life (5–7 h). Despite the long elimination half-life, TM is prescribed three to four times a day. Frequent dosing schedule often leads to decreased patient compliance, increased incidence of side effects, and tolerance development, especially, in long-term use in conditions like arthritis, osteoarthritis, arthralgia, postoperative surgical pains, etc. It seems that there is a strong clinical need and market potential for a delivery system that can deliver TM in a controlled manner (4,5). TM's apparently negligible effect on respiration, as demonstrated in studies in adults and children, suggests that TM may offer a distinct advantage over typical opioid analgesics for the relief of postoperative pain in children (6). To reduce the frequency of administration and to improve patient compliance, a sustained-release formulation of TM is desirable.

Chitosan or β(1,4) 2-amino-2-deoxy-D-glucose is a hydrophilic biopolymer obtained by hydrolysis of the amino acetyl groups of chitin, a polysaccharide found in a wide variety of crustaceans, insects, and fungi. Chitosan, with excellent biodegradable and biocompatible characteristics, is a naturally occurring polysaccharide. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been examined extensively used in the pharmaceutical industry for its potential in the development of drug delivery system.

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Chitosan has been extensively used by many researchers for the encapsulation and controlled delivery of various drugs (7–9).

The use of complexation between oppositely charged macromolecules to prepare chitosan beads or microspheres as a controlled release formulation has attracted much attention, because this process is very simple and mild. As coacervation can be induced in systems containing both cationic and anionic hydrophilic colloids, complex coacervation is likely to occur between chitosan, a water soluble cationic poly-saccharide, and type B gelatin, a protein which is negatively charged at pH values above its isoelectric point (10–13). Recently, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, is applied to avoid possible toxicity of reagents and other undesirable effects. Sodium-tripolyphosphate (Na-TPP) is a poly anion and can interact with cationic chitosan by electrostatic forces (14,15). A chitosan complex coacervate was aimed to prepare by using Na-TPP as cross-linking agent.

In this present study, preparation and evaluation of microspheres of TM with biodegradable natural polymers chitosan and gelatin B as carriers by using physical cross-linking to avoid toxicity of chemical cross-linking agents has been attempted to obtain a suitable oral controlled drug delivery which can overcome the disadvantages of the selected drug.

MATERIALS AND METHODS

Chemicals

TM was obtained from (Sun Pharmaceutical Industries Ltd., Maharashtra, India) as a gift sample, chitosan with a degree of deacetylation of >85% and viscosity of 500 cps at 1% (*w/v*) in 1% (*v/v*) aqueous acetic acid at 20°C was supplied from (Central Institute of Fisheries and Technology, Cochin, India) as a gift sample and was used as received. Type B gelatin, bloom strength 225 received from (Sigma Chemical Company, St. Louis, MO), Na-TPP from (Fluka Chemical Company, GmbH, Switzerland), light and heavy liquid paraffins, Tween 80, acetone, glacial acetic acid, methanol, and other chemicals were from S.D. Fine Chem. Limited (Mumbai, India).

Preparation of TM Microspheres

The TM microspheres were prepared by complex coacervation technique by using chitosan/gelatin B mixture as coating material. Chitosan and gelatin were dissolved in dilute acetic acid solution (1% *v/v*) together at concentrations of 1–4% *w/v* and adjusted to a certain solution pH (usually 5.0). TM (100 mg) was dissolved in the above polymeric mixture. The drug in polymeric mixture was emulsified in 200 ml of liquid paraffin (1:1 mixture of light and heavy liquid paraffin) at 40°C containing 1 ml Tween 80 (2% *w/v*). The emulsification time was allowed for 10 min under mechanical stirring (500 rpm). The *w/o* emulsion was cooled to 4°C to induce coagulation of gelatin. Then, 50 ml Na-TPP (1% *w/v*) with pH in the range 4–5 at 4°C was added drop wise. Stirring was continued for 15–60 min to obtain cross-linked micro-spheres. Microspheres were collected by centrifugation and washed with water several times then with acetone to remove

water and dried at room temperature under vacuum. The prepared microspheres were stored in desiccator for further studies. TM-loaded microspheres with different polymer compositions (1:1, 1:2, 1:3, and 1:4) were named as TM1, TM2, TM3, and TM4, respectively.

Compatibility Studies

Chemical interaction between the drug and the polymeric material, if any, during the preparation of the microspheres was studied by using thin layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR).

Thin Layer Chromatography

Thin Layer Chromatography was carried out in TLC chamber. The sample solutions of pure drug and prepared microspheres were prepared by dissolving in methanol and applied to silica gel G plates. The plates were then developed in the following solvents systems.

Solvent system 1: Chloroform/methanol/acetic acid 9:2:0.1 (%*v/v/v*)
Solvent system 2: Chloroform/toluene/ethanol 9:8:1 (%*v/v/v*)

The R_f value of the pure drug as well as prepared microspheres were determined by placing the plates in an iodine chamber and the R_f value of pure drug was compared with the R_f value of prepared microspheres.

Fourier Transform Infrared Spectroscopy

FTIR spectrum of the drug, drug-loaded microspheres, blank microspheres, and physical mixture of drug and empty microspheres were recorded using a FTIR (model 4100 type A, Perkin-Elmer, Norwak, CT, USA) spectrometer using KBr pellets (400–4,000^{-1}) with a scanning speed of 2 mm/s with normal slit.
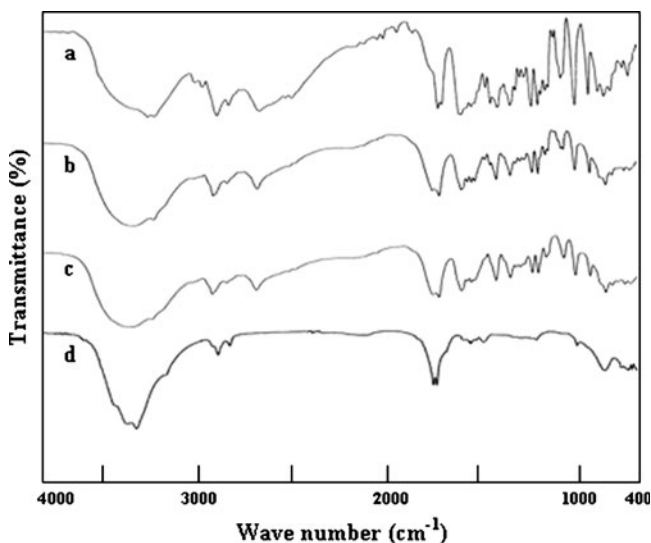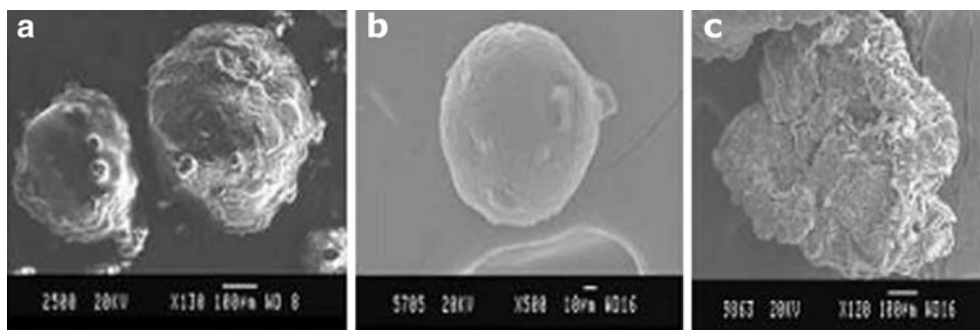

Fig. 1. FTIR Spectrum. **a** Tramadol hydrochloride. **b** Tramadol hydrochloride-loaded microspheres. **c** Physical mixture of tramadol hydrochloride and blank microspheres. **d** Blank microspheres

Fig. 2. SEM of tramadol hydrochloride and its microspheres. **a** Tramadol hydrochloride Microspheres prepared with 1:1 drug/polymer ratio. **b** Tramadol hydrochloride Microspheres prepared with 1:4 drug/polymer ratio. **c** After *in vitro* release studies

Scanning Electron Microscopy

The shape and surface morphology of the TM-loaded microspheres were studied using (Jeol, JSM-840A scanning electron microscope, Japan). The gold-coated (thickness 200A°; Jeol, JFC-1100E sputter coater, Japan) microspheres were subjected to secondary imaging technique at 15° tilt, 15 mm working distance, and 20 kV accelerating voltage.

Frequency Distribution Analysis

Samples of microspheres were analyzed for frequency distribution with calibrated optical microscope fitted with a stage and an ocular micrometer. Small quantities of microspheres were spread on a clean glass slide, and the average size of 200 particles, frequency distribution, and precision and accuracy of the distribution were determined in each batch using the calibration factor.

Determination of Percentage Drug Entrapment

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula;

$$PDE = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100$$

Theoretical Drug Loading

Theoretical drug loading was determined by calculation assuming that the entire drug present in the polymer solution used gets entrapped in microspheres, and no loss occurs at any stage of preparation of microspheres (16).

Practical Drug Loading

Practical drug loading was analyzed as follows: 20 mg of microspheres were added to 100 ml of glacial acetic acid (1% *v/v*) and methanol in the ratio of 3:2 and occasionally shaken for 30 min. The solution was centrifuged and 1 ml of the clear supernatant was diluted to 10 ml with 0.1 N HCl, the supernatant liquid was filtered through Watt Mann filter paper and analyzed for TM by high-performance thin layer chromatography (HPTLC) (17). The quantitative determina-

tion of TM in microspheres was carried out by using a Camag HPTLC system with Win CATS 4 software; Linomat 5 sample applicator and scanner were used for the analysis and interpretation of data. The experiment was performed on a silica gel G60 F254. HPTLC plates (20×10) using mobile phase comprised of chloroform/methanol/acetic acid 9:2:0.1 (% *v/v/v*).The plates were activated by at 1,100 for 30 min prior to chromatography. Samples were applied as 6 mm bands at 10-mm interval under a stream of inert gas. Ascending development to distance of 76 mm was performed in saturated 20×10 twin trough TLC developing chamber for 30 min at room temperature. The plate was scanned and quantified at 270 nm using slit dimension of 5×0.45 mm at a scanning speed of 20 mm/s. The peak area was noted after development, and the amount present in microspheres was calculated using respective standard calibration curve.

In Vitro Drug Release Studies

Microspheres equivalent to 200 mg TM were subjected to *in vitro* drug release studies in simulated intestinal fluids to assess their ability in providing the desired controlled drug delivery. Drug release studies were carried out using USP XXIII basket dissolution rate test apparatus (100 rpm, 37 ± 1°C) for 12 h in 7.4 pH phosphate-buffered medium (900 ml). At different time intervals, 5 ml of the sample was withdrawn and replaced with same amount of pH 7.4 phosphate buffer. The
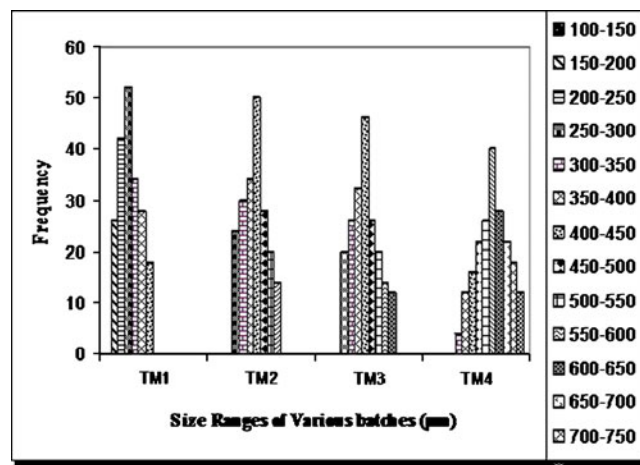
Fig. 3. Frequency distribution of tramadol hydrochloride microspheres

Table I. Particle Size, Drug Entrapment, and Entrapment Efficiency of Tramadol Hydrochloride Microspheres

Formulation	Mean particle size (μm) ± SEM	% Yield	(%) Drug entrapment	Drug entrapment efficiency (%)
TM1	289.95±7.61	85	14.48	24.61
TM2	413.70±8.72	69	16.02	33.00
TM3	436.05±10.28	65	33.24	85.76
TM4	574.80±11.50	54	36.76	99.23

sample was analyzed for TM directly or often appropriate dilution with the pH 7.4 phosphate buffer spectrophotometrically at 271 nm using a UV/VIS spectrometer against a reagent blank. The *in vitro* release pattern of the selected best batch (TM3) of TM microspheres was compared with the marketed product using similarity factors *f*2. The changes in the surface integrity of microspheres after *in-vitro* drug release studies were observed by using scanning electron microscopy (SEM).

Kinetics of Drug Release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero-order (Q v/s t), first-order (log (Q_0–Q) v/s t), Higuchi's square root of time (Q v/s $t1/2$) and Korsemeyer peppas double log plot (log Q v/s log t), respectively, where Q is the cumulative percentage of drug released at time t and (Q_0–Q) is the cumulative percentage of drug remaining after time t. The release kinetics of selected best batch (TM3) was compared with the marketed product.

Differential Scanning Colorimetry

The physical state of drug in the microspheres was analyzed by differential scanning calorimeter (Mettler-Toledo star 822^e system, Switzerland). Sample was sealed in a volatile type Aluminum pan. The thermo grams of the samples were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25–250°C, respectively. Nitrogen was the sweeping gas and Indium was used as the standard reference material to calibrate the temperature and energy scales of the differential scanning colorimetry (DSC) instrument.

X-Ray Diffractometry

X-ray diffractometry of the TM microspheres were performed by a diffractometer using model (Joel JDX-8030, Japan) equipped with a graphite crystal monochromator (Cu-Kα) radiations to observe the physical state of drug in the microspheres.

RESULTS AND DISCUSSION

Formulation Optimization of Tramadol Hydrochloride Microspheres

The formulation conditions for the preparation of complex coacervates were first optimized. The electrostatic interaction between Na-TPP (anion) and Chitosan (cation) may exist only at certain pH region (1.9–7.5). In this study,

the pH of chitosan or chitosan/gelatin mixture and the cross-linker solutions were usually adjusted to 4–5. This is valuable for the selection of the preparation conditions of ionotropic gelation of chitosan microspheres. It can be seen that the solution pH may play an important role on the chitosan microsphere formation (12,18).

The important fact in the preparation was the introduction of gelatin B in the chitosan solution. At first, the chitosan and gelatin mixture solution was prepared at a temperature higher than the gelatin gelation point (25°C) and emulsified in an oil phase at the same temperature. Then, the formed *w/o* emulsion was cooled down to a temperature below the gelatin gelation point (4°C), so the chitosan/gelatin micro-droplets were coagulated, then the ionic cross-linking process takes place under coagulation conditions, which is beneficial in keeping the spherical shape of the formed microspheres (15).

Ice bath temperature in method was found to be essential for improving the yield and easing the solidification of the micro coacervates, probably by increasing the effectiveness of the coacervation process. Also, variation in the pH from the optimum value of 4–5 on either side yielded an aggregated fluffy mass.

Compatibility Studies

Chemical interaction between drug and the polymeric material, if any, during the preparation of the microspheres was studied by using a TLC and FTIR. The comparable R_f values of pure drug and microencapsulated drug indicated the compatibility of drug with polymer and other excipients used in the preparation of TM microspheres (19). No difference in the IR patterns of a physical mixture of the drug and blank microspheres, and drug-loaded microspheres was observed (Fig. 1). Therefore, the FTIR studies ruled out the possibility
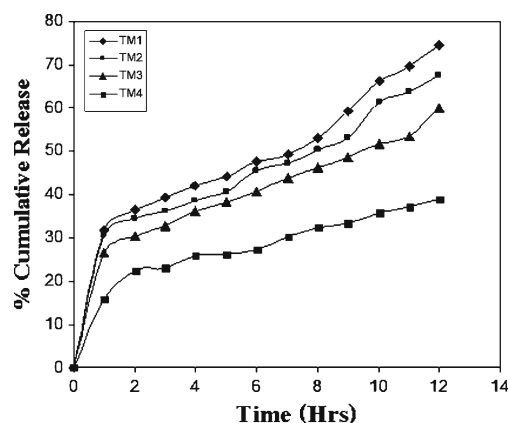
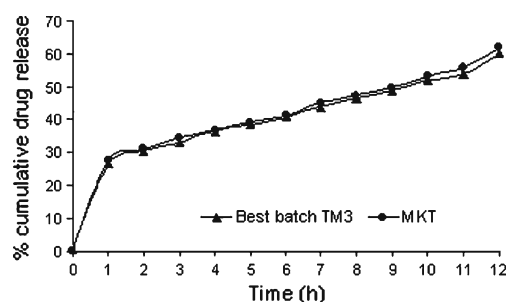**Fig. 4.** *In vitro* release of tramadol hydrochloride microspheres

Fig. 5. Comparison of *in vitro* release of best batch TM3 and marketed formulation (MKT)

Morphological Characteristics (SEM)

The surface morphology of the TM and TM-loaded microspheres were studied by scanning electron microscopy (Fig. 2). Surface smoothness of microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. At lower polymer concentration (1% *w/v*), rough and wrinkled surface of microspheres was obtained (Fig. 2a), and at higher polymer concentration (4%), the microspheres with smooth surface was obtained (Fig. 2b).

Particle Size Distribution

The results of accuracy and precision of frequency distribution studies and histograms showed the normal frequency distribution of microspheres (Fig. 3). As the drug to polymer ratio was increased, the mean particle size (MPS) of TM microspheres was also increased (Table I).The significant increase may be because of the increase in the viscosity of the droplets (due to the increase in concentration of polymer solution). This increase is high enough to result in difficult dispersion and subdivision of droplets reported (21). Increase in MPS due to increased viscosity of the polymer solution has also been reported (22, 23). A surfactant (Tween 80) was found to play an important role in controlling the particle size of the micro-coacervates. As the Tween concentration increased from 0.5% to 2.0% *w/v*, the particle size reduced. However, further increase in the Tween concentration produced bigger particles. The presence of Tween 80 was found to be essential for reducing aggregation of the microspheres.

Table II. Diffusion Exponent (*n*) of Peppas Model and Regression Coefficient (r^2) of Tramadol Hydrochloride Release Data from Microspheres According to Different Kinetic Models

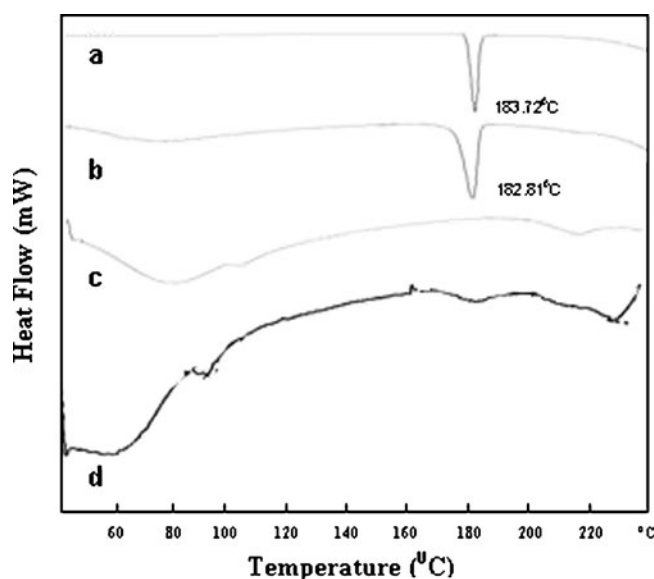| Formulation	Peppas Model (*n*)	Zero Order	First Order	Higuchi
TM1	0.338	0.974	0.925	0.921
TM2	0.315	0.978	0.944	0.924
TM3	0.322	0.992	0.964	0.969
TM4	0.333	0.969	0.971	0.975

Fig. 6. DSC thermograms. **a** Tramadol hydrochloride. **b** Physical mixture of tramadol hydrochloride and blank microspheres. **c** Tramadol hydrochloride-loaded microspheres. **d** Blank microspheres

of any drug polymer interaction during the preparation of microspheres (20).

Drug Entrapment Efficiency

The drug loading efficiency of TM microspheres was determined by HPTLC method. A maximum of 99% of drug entrapment efficiency was obtained by method employed. By increasing the polymer concentration, the entrapment efficiency was increased (Table I).

In Vitro Drug Release Studies

The *in vitro* release of TM microspheres was studied in the pH 7.4 phosphate-buffered medium. It was observed that
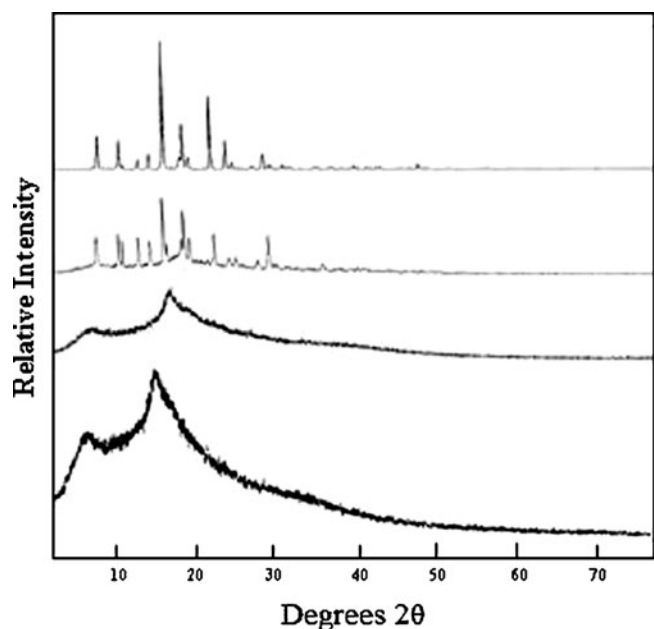

Fig. 7. XRD. **a** Tramadol hydrochloride. **b** Physical mixture of tramadol hydrochloride and blank microspheres. **c** Tramadol hydrochloride-loaded microspheres. **d** Blank microspheres

the rate of release decreased as the concentration of the carrier was increased. This may be due to low permeability of polymer to the drug. The *in vitro* release profiles are shown in Fig. 4. The data obtained were fitted to zero-order, first-order, and Higuchi square root of time and Korsmeyer–Peppas equations to understand the mechanism of drug release from the microspheres (24). The *in vitro* release pattern of the selected best batch (TM3) of TM microspheres was compared with the marketed product (Fig. 5), using similarity factors, *f*2, (25).The similarity factor (*f*2) is a logarithmic reciprocal square root transformation of one plus the mean squared (the average sum of squares) differences of drug percent dissolved between the test and the reference products. Drug Evaluation and Research (FDA) and the Evaluation of Medicinal Products (EMEA) suggest that two dissolution profiles are declared similar if *f*2 is between 50 and 100. The similarity factor fits the result between 0 and 100. It is 100 when the test and reference profiles are identical and approaches 0 as the dissimilarity increases. An *f*2 above 50 (77.51) indicates that the two profiles are similar.

Kinetics of Drug Release

The slopes and the regression coefficient of determinations (r^2) are listed in (Table II). The coefficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. Additional evidence for the diffusion controlled mechanism was obtained by fitting the Korsmeyer–Peppas equation to the release data. The diffusion exponent *n* value was found to be less than 0.5 for different drug–polymer compositions, indicating Fickian diffusion of drug through microspheres. The release kinetics of the selected best batch (TM3) was compared with the marketed product. The marketed product showed the zero order release kinetics ($r^2=0.9923$) followed by Fickian diffusion ($n=0.3235$). The changes in the surface integrity of microspheres during *in vitro* drug release studies were confirmed by SEM (Fig. 2c).

Differential Scanning Calorimetry

In order to confirm the physical state of the drug in the microspheres, DSC of the drug alone, physical mixture of drug and blank microspheres, and drug-loaded microspheres were carried out (Fig. 6). The DSC trace of drug showed a sharp endothermic peak at 183.72°C, its melting point. The physical mixture of drug and blank microspheres showed the endothermic peak at 182.81°C as the individual component, indicating that there was no interaction between the drug and the polymer in the solid state. The absence of endothermic peak of the drug at 183.72°C in the DSC of the drug-loaded microspheres suggests that the drug existed in an amorphous or disordered crystalline phase as a molecular dispersion in polymeric matrix (26,27).

X-Ray Diffractometry

In order to confirm the physical state of the drug in the microspheres, powder X-ray diffraction studies of the drug alone, physical mixture of drug and blank microspheres, and drug-loaded microspheres were carried out (20). X-ray diffractograms (Fig. 7) of the samples described above showed that the drug is still present in its lattice structure in the physical mixture where as it is completely amorphous inside the microspheres. This may be due to the conditions used to prepare the microspheres lead to cause complete drug amorphization.

CONCLUSION

The goal of present work was to provide a therapeutic amount of TM in the body and also to achieve and maintain the desired TM concentration. All the formulations were subjected for evaluation. Results of FTIR, SEM, particle size and size distribution, % yield, drug content and entrapment efficiency, *in vitro* dissolution and release kinetics, DSC and X-ray diffractometry (XRD) have shown satisfactory results. The microparticulate drug delivery system proposed in this work based on chitosan and gelatin B, natural biodegradable polymers, seems to hold promise for oral administration of TM. The method of preparation of TM microspheres used in this study was found to be simple and reproducible. Chitosan and gelatin B, which are used as carrier, are easily available and biocompatible. From the preliminary trials, it may be concluded that drug-loaded microspheres are a suitable delivery system for TM.

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