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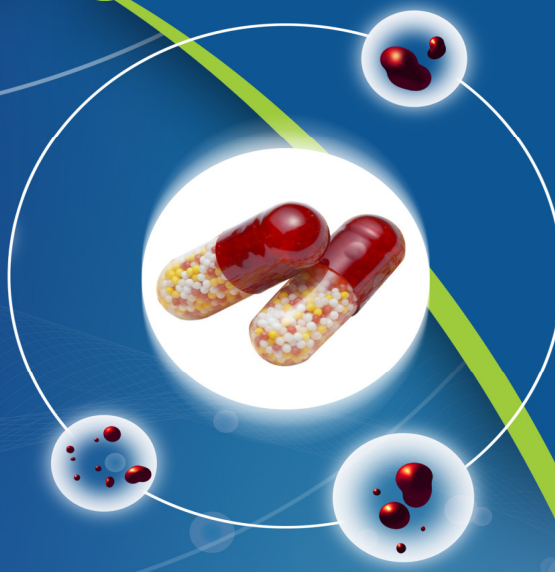

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Research Article

NA-TPP CROSS-LINKED CHITOSAN MICROSPHERES FOR CONTROLLED RELEASE OF TRAMADOL HYDROCHLORIDE

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ABSTRACT

In this study preparation and evaluation of Tramadol Hydrochloride (TM) microsphere prepared by emulsion crosslinking method with biodegradable polymer as chitosan. Chitosan was carrier by using physical crosslinking with Sodium Tripolyphosphate (Na-TPP) to avoid toxicity of chemical cross–linking agent (glutaraldehyde) and other undesirable effects.Prepared microspheres were subjected to variousphysico-chemical studies, such as drug-polymer compatibility by FourierTransform Infrared Spectroscopy (FTIR) and Differential scanning colorimetry (DSC), surface morphology by scanning electron microscopy (SEM), frequencydistribution, encapsulation efficiency, in-vitro drugrelease characteristics and release kinetics. FTIR studies reveled that there is no drug-polymer incompatibility. Surfacesmoothness of microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. As the drugto polymer ratio was increased, the mean particle size (MPS) of TM microspheres was also increased. A maximum of 87% of drug entrapment efficiency was obtained by the method employed. All the microspheres showed initial burst releasefollowed by a Fickian diffusion mechanism. It is possible to design a controlled drug delivery system for the prolongedrelease of TM, improving therapy by possible reduction of time intervals between administrations.

Keywords: *Tramadol Hydrochloride, Microspheres, Emulsification method, Chitosan.*

INTRODUCTION:

The present study reports a novel attempt to prepare chitosan microsphere for widely used Nonsteroidal anti-inflammatory drug Tramadol Hydrochloride (TM). TMa centrally acting opioid analgesic is used in severeacute orchronic pains [1].

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Tramadol is an aminocyclohexanol derivative or 4-phenylpiperidine analogue of codeine. Its analgesic effect is mediated through norepinephrine reuptake inhibition [4]. Its mean elimination half-life is ~ 6 hrs and requires dosing every 6 hrs in order to maintain optimal relief of chronic pain [5]. It 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 3–4 times a day [2]. Frequent dosing schedule often leads to decreased patient compliance, increased incidence of side effects and tolerance development, especially, in long-term use [3] 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. [6,7]. Chitosan, (1–4)-2-amino-2-deoxy-B-D-glucan, is a linear cationic polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine and can be derived by partial deacetylation of chitin from crustacean shells. The term chitosanis used to describe a series of chitosan polymers with different molecular weights (50-2000 kd), viscosity (1% chitosanin 1% acetic acid, G2000 mPa), and degree of deacetylation(40%-98%). Chitosan salts are soluble in water, with the solubility being dependent on the degree of deacetylation and thereby on the pH of the buffer. Chitosans with a relatively low degree of deacetylation (40%) have been found to be soluble up to pH 9, whereas chitosans with a degree of deacetylation of ~85% have been found to be soluble up tocpH 6.5. At acidic pH, the amine groups of chitosan are protonated, acquire positive charge, and coagulate upon addition of negatively charged molecules.**[8]** With increasing degree of deacetylation, the viscosity of chitosan increases because of the different confirmations of the molecule. At a high degree it is highly charged and has an extended confirmation with more flexible chains, whereas at a lower degree of deacetylation the chitosan molecule adopts a rod- or coillike shape because of low charges [9,10]. 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. Na-TPP is a poly anion, and can interact with cationic Chitosan by electrostatic forces [11,12]. Hence, microspheres of TM for oral delivery were developed with the aim to improve patient compliance and to obtain improved therapeutic efficacy in the treatment of postoperative pain and migraine.

MATERIALS AND METHODS:

Materials:

Tramadol Hydrochloride was obtained from (SunPharmaceutical Industries Ltd., Maharashtra, India) as a gift sample, Chitosan with a degree of deacetylation of > 85%and viscosity of 500cps at 1%(w/v) in 1 % (v/v) aqueous acetic acid at 200C was supplied from (Central Institute of Fisheries and Technology, Cochin, India) as a gift sample and was used as received. Sodium tri polyphosphate (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 India (S.D. Fine Chem. Limited, Mumbai, India).

Preparation of Tramadol Hydrochloride microspheres:

The TM microspheres were prepared by emulsion crosslinking method using Na-TPP as crosslinking agent. Chitosan was dissolved in dilute acetic acid solution (1% v/v) at the concentration of 1-4% w/v and adjusted to certain solution pH usually pH 5.0.TM (100 mg) was dissolved in the above polymeric solution. The dispersed phase was then add drop-wise through a disposable syringe to a continuous phase 200 ml of liquid paraffin (1:1 mixture of light and heavy liquid paraffin) containing 2 ml of tween 80 (2% w/v) to form a water in oil emulsion. Stirring was continued for 2 hrs under mechanic stirring at 100 RPM. Then 50 ml ofNa-TPP (1% w/v) with pH in the range 4-5 was added drop wise. Stirring was continued for 60 min to obtain crosslinked microspheres. microspheres were collected by centrifugation and washed with double distilled 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 asTC1, TC2, TC3 and TC4 respectively.

Fourier Transform Infrared Spectroscopy (FTIR):

Infrared (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 (400-4000-1) with a scanning speed of 2 mm/sec with normal slit.

Scanning Electron Microscopy (SEM):

The shape and surface morphology of the TM loaded microspheres were studied using (Jeol, JSM-840A scanning electron microscope, Japan). The gold coated (thickness200A0; Jeol, JFC-1100E sputter coater, Japan) microspheres were subjected to secondary imaging technique at 150 tilt,15mm working distance and 20Kv 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 MS were spread on a clean glass slide and the average size of200 particles and frequency distribution were determined in each batch using the calibration factor.

Determination of Percentage Drug Entrapment (PDE):

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 [13].

Practical drug loading:

Practical drug loading was analyzed as follows. 20mg of microspheres were added to100ml of glacial acetic acid (1%v/v) and methanol in the ratio of 3:2 and occasionally shaken for 30min. The solution was centrifuged and 1ml of the clear supernatant was diluted to 10ml with 0.1N HCl, the supernatant liquid was filtered through Watt Mann filter paper and analyzed for TM by UV Spectroscopy at 269 nm [14].

***In vitro* drug release studies:**

Microspheres equivalent to 200 mg TM were subjected to *in-vitro* drug release studies 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 ± 10C). 900 ml of 1.2 pH was used as dissolution media for 2 h followed by 10 h study in 7.4 pH phosphate buffer. At different time intervals, 5 ml of the sample was withdrawn and replaced with same amount of fresh media.. The sample was analyzed for TM spectrophotometrically at 271nm using a UV/ VIS spectrometer against a reagent blank. The in-vitro release pattern of the selected best batch of TM microspheres was compared with the marketed SR product.

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 (Q0-Q) v/s t), Higuchi's square root of time (Q v/s t1/2) and

Korsemeyerpeppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0-Q) is the cumulative percentage of drug remaining after time t. The release kinetics of selected best batch was compared with the marketed SR product.

Differential Scanning Calorimetry (DSC) [16]

The physical state of drug in the microspheres was analyzed by Differential Scanning Calorimeter (Mettler-Toledo star 822e system, Switzerland). The DSC thermograms of drug, physical mixture of drug and blank microspheres, drug loaded microspheres and blank microspheres were recorded. Sample was sealed in a volatile type Aluminum pan. The thermo grams of the samples were obtained at a scanning rate of 100C/min conducted over a temperature range of 25 to 2500C, respectively. Nitrogen was the sweeping gas and Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument.

X-ray powder Difftactometry (X-RD) [17]

X-ray powder diffractometry was carried out to investigate the effect of microencapsulation process on crystallinity of drug. Powder X-RD patterns were recorded on X -RD (Model no-PW3040 Xpert MPDsystem) using Philips Analytical X-Ray B.V. The scanning rate employed was 20 min -1, over the 14 to88 diffraction angle (02θ) range. The X-RD patterns of drug, polymer, blank microspheres and drug loaded microspheres were recorded.

RESULTS AND DISCUSSION:

Preparation of Microspheres:

TM microspheres were prepared by emulsion crosslinking method without the use of chemical cross linking agent: to avoid the toxic and undesirable effects such as loss of protein bioactivity, neurotoxicity of chemical crosslinking agents usually glutaraldehyde. It can be seen that solution pH may also play an important role on the chitosan microspheres formation. in this study pH of chitosan and crosslinker solution were adjusted to 4-5.Out of the pH region were Na-TPP interact with chitosan and no microspheres were formed. TM loaded microspheres with different polymer compositions (1:1, 1:2, 1:3 and 1:4) were named asTC1, TC2, TC3 and TC4 respectively.

Compatibility Study:

Chemical interaction between drug and polymeric material, if any, during the preparation of microspheres was studied by using FTIR. No difference in IR patterns of physical mixture of the drug and blank microspheres, and drug loaded microspheres was observed. Therefore, the FTIR studies ruled out the possibility of any drug polymer interaction during the preparation of microspheres [15].

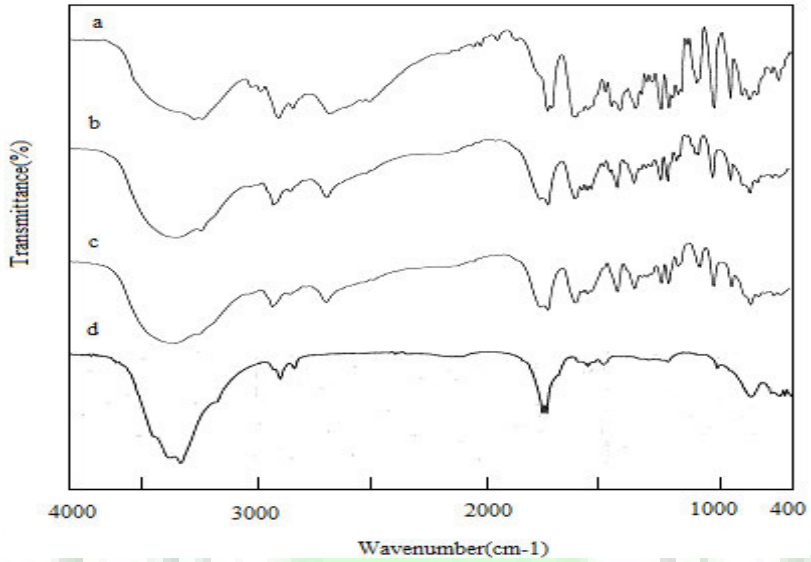

Fig. 1. Fourier Transform Infrared (FTIR) Spectrum

(a) Tramadol Hydrochloride drug, (b) Tramadol Hydrochloride loaded microspheres (c) Physical mixture of Tramadol Hydrochloride and blank microspheres (d) Blank microspheres.

Scanning Electron Microscopy:

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 and at higher polymer concentration (4%)the microspheres with smooth surface was obtained.

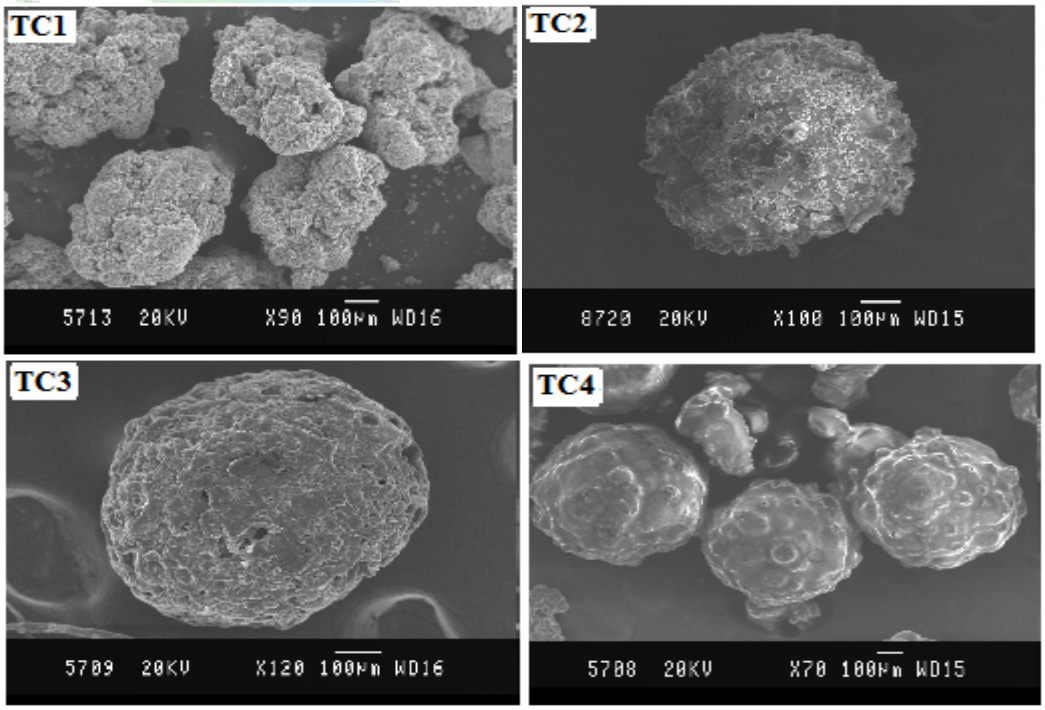

Fig. 2. Scanning Electron Micrographs (SEM) of Tramadol Hydrochloride Microspheres.

Frequency distribution analysis:

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 1).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 reported18, 19. A surfactant (tween 80) was found to play an important role in controlling the particle size of the microspheres. As the tween concentration increased from 0.5-2.0% 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.

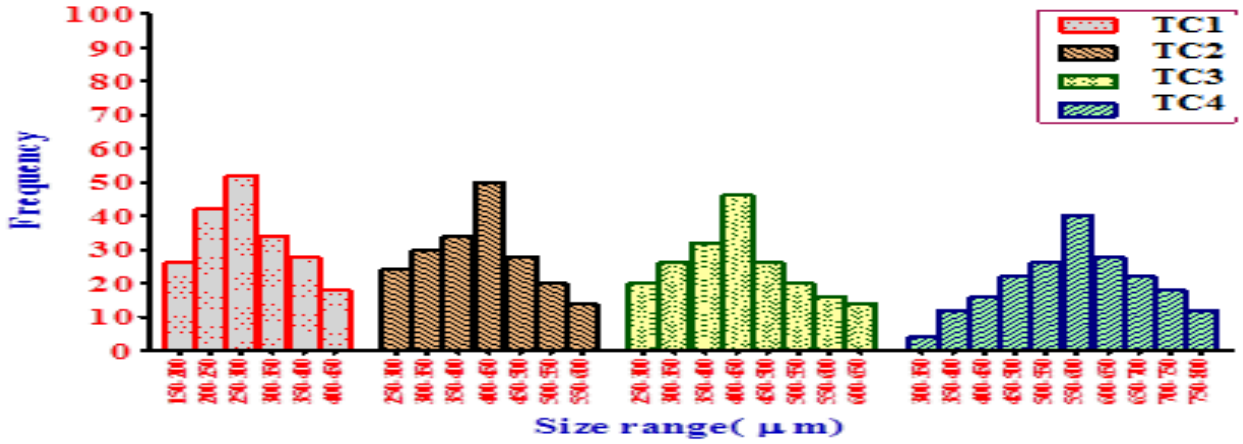

Figure 3: Frequency distribution of Tramadol Hydrochloride microspheres.

Table 1: Particle size and Drug entrapment of TM microspheres Formulation

Formulation	Mean particle size (µm)	% drug Entrapment
TC1	259.35±8.21	35.82
TC2	342.90±9.84	35.11
TC3	390.75±10.88	34.68
TC4	481.95±11.70	33.43

Drug entrapment efficiency:

Entrapment efficiency increase with increase in the polymer conc. From the results as in Table 1 it can be inferred that there is a proper distribution of TM in the microspheres and the deviation is within the acceptable limits.

The percentage entrapment efficiency was found to be in the range of 33.43% to 35.82%. It was further observed that the drug entrapment was proportional to the TM: polymer ratio and size of the TM microspheres.

In vitro drug release study:

The in-vitro drug release characteristics were studied in 900 ml of pH 1.2 was used for 2 h followed by 10 h study in pH 7.4 phosphate buffer using USP XXIII basket dissolution apparatus. The theoretical release profile calculation is important to evaluate the formulation with respect to release rates and to ascertain whether it releases the drug in predetermined manner. Dissolution profile of microspheres i.e. TC1, TC2, TC3 and TC4 shows 81.44±1.10%, 74.15±1.63%, 67.74±0.93% and 44.61±0.97% drug release at the end of 12 h respectively as shown in Figure 4.It is reasonable to conclude that the release profiles of TM from the microspheres showed

two distinct phases. An initial burst release phase occurs in the first hour, followed by a gradual release phase. It was observed that 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 pattern of the selected best batch (TC3) of TM microspheres was compared with the marketed SR product shown in (Fig. 5).Comparison of release pattern of batch TC3with marketed SR product, showed the similar release pattern of marketed SR product.

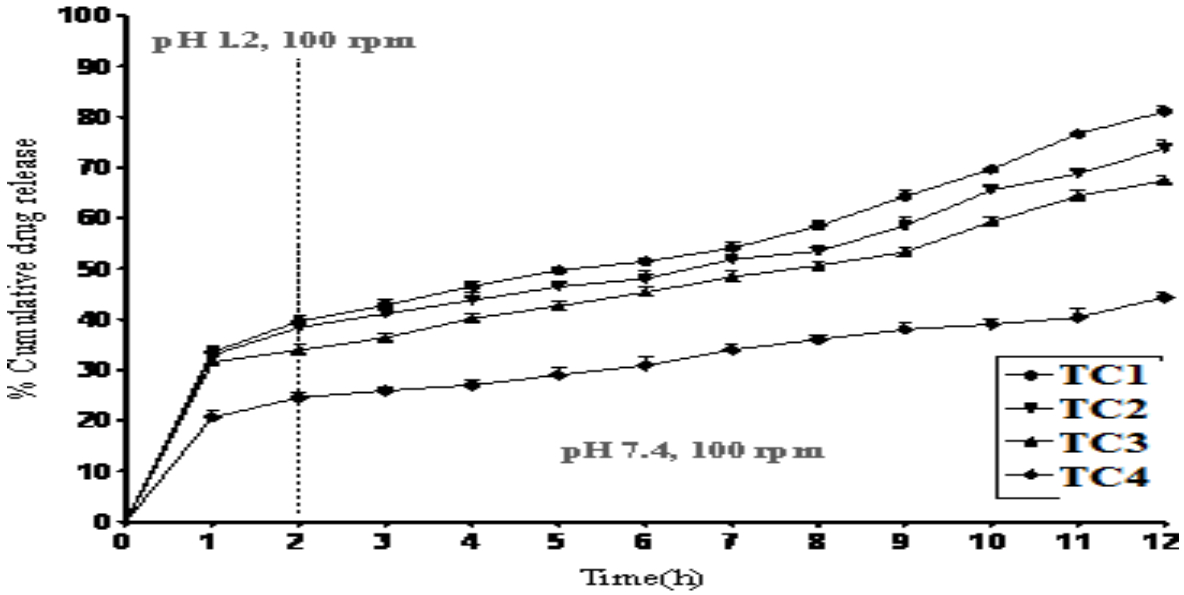

Fig. 4. In - vitro release of Tramadol Hydrochloride microspheres.

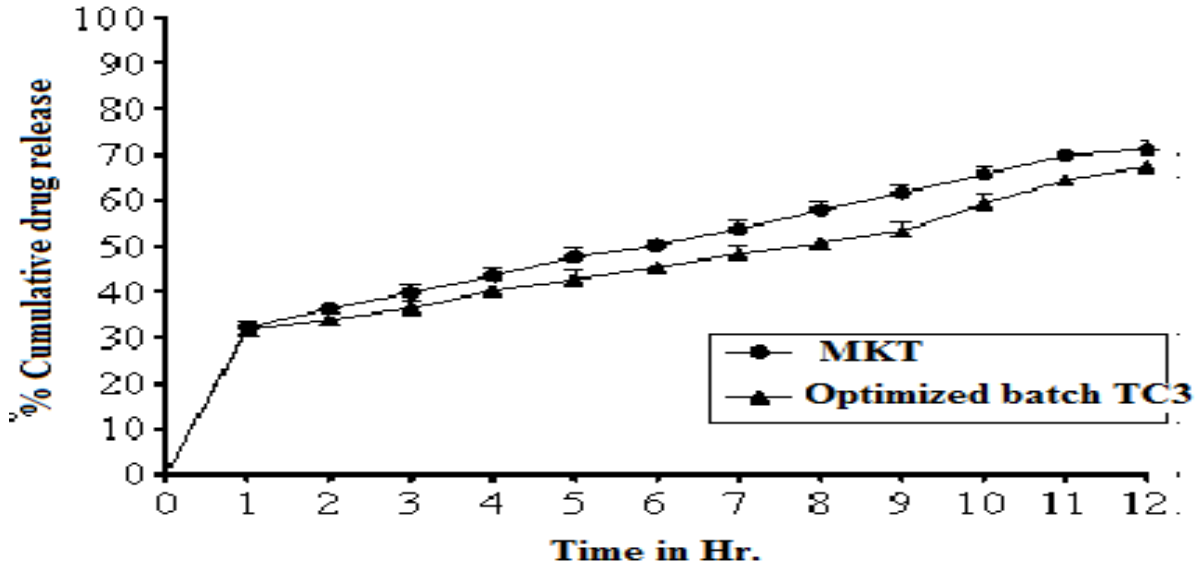

Figure 5: Comparision of In vitro release of best batch TC3 and marketed SR product (MKT)

Kinetics of drug release:

The data obtained in In-vitro release study were fitted to zero order, first order, Higuchi square root of time and Korsemeyer-Peppas equations to understand the mechanism of drug release from the microspheres [20]. The slopes and the regression co-efficient of determinations (r2) are listed in Table 2. The co-efficient 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 0.5for different drug polymer compositions, indicating Fickian diffusion of drug through microspheres. Thus all microspheres showed initial burst release followed by Fickian diffusion. The release kinetics of the selected best batch (TC3) was compared with the marketed SR product. The marketed product showed the zero order release kinetics (r2=0.9978) followed by Fickian diffusion (n=0.3364).

Table 2: Diffusion exponent (n) of Peppas model and Regression co-efficient (r2) of M release data from microspheres according todifferent kinetic models.

Formulation code	Zero order (r^2)±SD	First order (r^2)±SD	Higuchi kinetics (r^2)±SD	Peppas Equation	
				(n)	(r^2)±SD
TC1	0.983±0.004	0.917±0.003	0.942±0.005	0.345	0.925±0.008
TC2	0.975±0.003	0.890±0.002	0.928±0.006	0.308	0.905±0.003
TC3	0.985±0.004	0.952±0.004	0.938±0.004	0.314	0.908±0.002
TC4	0.989±0.005	0.988±0.004	0.970±0.002	0.301	0.947±0.003
Marketed Preparation	0.990±0.003	0.968±0.002	0.959±0.003	0.313	0.940±0.005

SD=Standard deviation (n=3)

Differential Scanning corimetry

In order to confirm the physical state of the TM in the microspheres, DSC of the TM alone, physical mixture of TM and blank microspheres, and TM loaded microspheres were carried out (Figure 6).The DSC trace of drug showed a sharp endothermic peak at 183.720 ºC, its melting point. The physical mixture of TM and blank microspheres showed the endothermic peak at 182.810 ºC as the individual component, indicating that there was no interaction between the TM and the polymer in the solid state and the drug still present in its lattice structure in the physical mixture. The absence of endothermic peak of the TM at 183.720C in the DSC of the TM loaded microspheres suggests that the drug existed in an amorphous or disordered crystalline phase as a molecular dispersion in polymeric matrix.

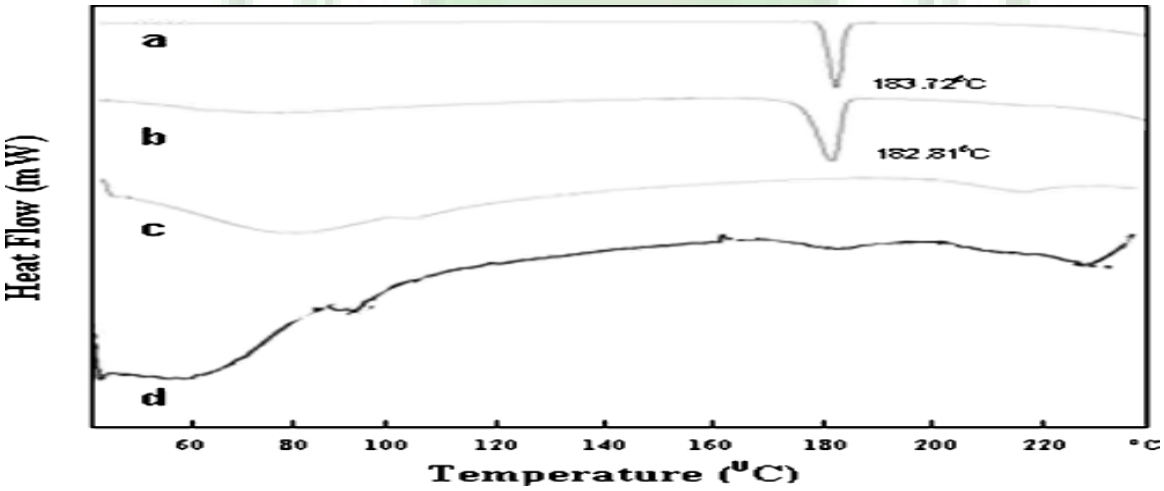

Figure 6: DSC thermograms. a Tramadol hydrochloride. b Physical mixture of tramadol hydrochloride and blank microspheres. c Tramadol hydrochloride-loaded microspheres. d Blank microspheres

X-ray Diffractometry (XRD)

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

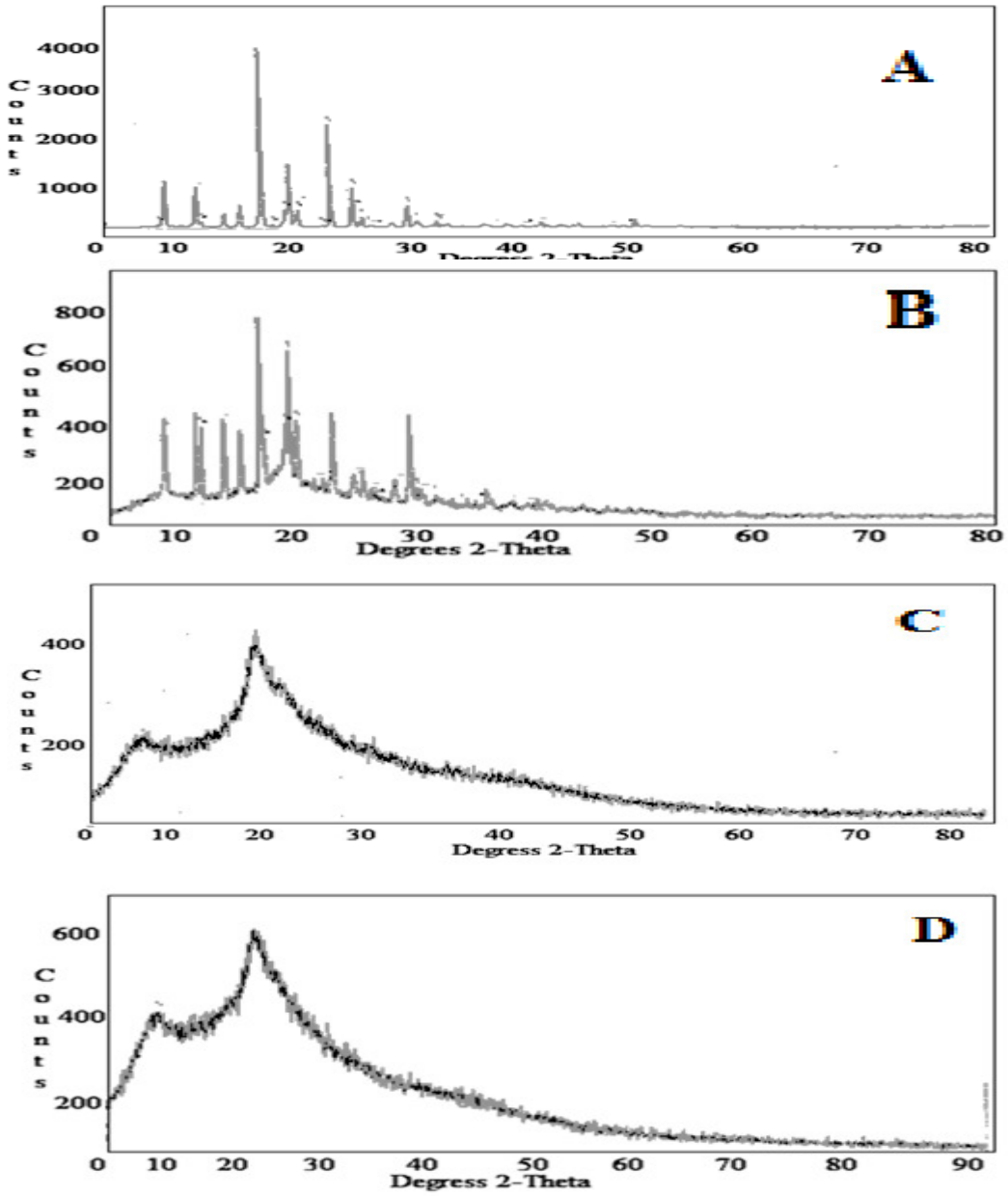

Figure 7 XRD thermograms of (A) TMH, (B) Physical mixture of TMH and blank microspheres, (C) TMH microspheres and (D) Blank microspheres.

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