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ORIGINAL RESEARCH ARTICLE

Preparation And Charactrisation Of Tramadol Microspheres For Post-Operative Pain

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ABSTRACT

Tramadol is a centrally acting opioid analgesic mainly used for post-operative pain with the absence of clinical relevant cardiovascular or respiratory side effects. It has a short half-life of 3-5hrs. Due to their short half-life they need to be administered frequently. For the sustained delivery of Tramadol, microspheres containing Polycaprolactone (PCL) and Polyvinylpyrrolidone (PVP) in different ratios were prepared by o/o solvent evaporation method using Span 20 and span 80 as surfactants. Encapsulation efficiency and drug loading of microspheres increased with decrease in concentration of emulsifying agent. The encapsulation efficiency and drug loading of the microspheres increased with increase in PVP concentration due to affinity between the drug and the hydrophilic polymer. The particle size increases with increase in the HLB values of the surfactants used. SEM (Scanning electron microscope) revealed microspheres were discrete, spherical. FTIR of pure and encapsulated drug in all formulation showed no significant difference in characteristic peaks, suggesting stability of tramadol during encapsulation process. Rapid drug release was observed in microspheres with higher concentration of PVP (polyvinylpyrrolidone), PVP acts as channeling agent.

Key words: Tramadol Hydrochloride, Polycaprolactone, Encapsulation Efficiency, Drug release Kinetics

INTRODUCTION

Tramadol is a synthetic codeine analog that is a central analgesic and a weak μ-opioid receptor agonist. Part of its analgesic effect is produced by inhibition of uptake of serotonin and norepinephrine[1]. It has used in the treatment of post-surgical pain, obstetric pain, cancer pain, and chronic pain of mechanical and neurogenic origin. The absence of clinical relevant cardiovascular or respiratory side effects explains its use for post operative pain than other opioids [2]. The half life of the drug is 5 hrs and dose is 50 -100 mg every 4 to 6 hrs [1]. The major adverse effect of Tramadol is headache and nausea. Immediate release formulations of Tramadol show the incidence of headache and nausea in 29% and 21%, respectively, whereas sustained release formulations shows only 18% and 11% respectively [3]. So, a sustained release formulation of Tramadol is desirable for pain management with lesser side effects.

In the present study, a sustained release formulation of Tramadol is developed using Polycaprolactone (PCL) and Polyvinyl pyrrolidone (PVP). PCL is a biodegradable, semi-crystalline polymer having a low glass transition temperature (~60 °C). A number of drugs have been encapsulated using PCL. Due to its crystallinity and hydrophobicity, degradation of PCL is very slow, rendering it suitable for long-term delivery [4]. PVP is a water soluble polymer. It is blended in different ratios with PCL. As the drug is water soluble, the microspheres were prepared by oil/oil solvent evaporation method, using dichloromethane as inner phase and liquid paraffin as external phase. Span 20 and span 80 are used as surfactants.

MATERIALS AND METHODS:

Polycaprolactone (Sigma Aldrich), Polyvinylpyrrolidone (Sigma Aldrich), Tramadol Hydrochloride (Caplin point), Dichloromethane, Span-80 (Merck), Span-20 (Merck). All the

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reagents and solvents used were of analytical grade.

Preparation of Microspheres

Microspheres were prepared using a solvent evaporation method. An oil-in-oil system for microspheres was adopted. Polycaprolactone and Polyvinylpyrrolidone in the ratios specified in table 1 were dissolved in 20ml of dichloromethane. 100mg of Tramadol dissolved in the polymeric solution. The above solution was added drop wise to 200ml liquid paraffin (light) containing surfactant as specified in (**Table 1**) under vigorous stirring. It was sonicated to decrease the particle size. The emulsion was stirred at 50°C for 1 h or at 35°C for 2 h during evaporation of dichloromethane. The microspheres were washed three to four times with hexane for complete removal of oil and hardening of microspheres [5].

Table 1: Formulation and processing conditions of Polycaprolactone microspheres

S.N	Formulation code	Surfactant		PCL concentration (%w/v)	PVP concentration (%w/v)
		Span 20 (%w/v)	Span 80 (%w/v)		
1.	FT1	2% Span 20	-	80	20
2.	FT2			85	15
3.	FT3			90	10
4.	FT4			95	5
5.	FT5	-	2% Span 80	80	20
6.	FT6			85	15
7.	FT7			90	10
8.	FT8			95	5
9.	FT9	1% span 20	1% Span 80	80	20
10.	FT10			85	15
11.	FT11			90	10
12.	FT12			95	5

CHARACTERISATION OF MICROSPHERES

Particle Size Determination

Particle size and particle size distribution for the microspheres prepared at different drug and polymer concentration were measured by a laser light scattering analyser (Microtrac Inc). The prepared microspheres were volume mean diameter and particle size distribution were analysed using the software Microtrac FLEX.

Product yield

Product yield of the microspheres was calculated by the following equation to determine the efficiency of the process [6, 7].

$$\text{Product yield (\%)} = \frac{M_1}{M_0} \times 100$$

Where, M_0 = Initial weight of drug and polymer

M_1 = Weight of microspheres

Particle Shape and Surface Morphology by Scanning Electron Microscopy

Morphology of the Microspheres of the best formulation was studied using scanning electron microscope (HITACHI, S-3400). The microspheres were sprinkled on to one side of the adhesive stub. The excess microspheres were removed and the stub sputter was coated with gold using a vaccum evaporator to render them electrically conductive. The coated microspheres were viewed at 20 kV to disclose the surface quality.

Drug loading and Encapsulation efficiency

The tramadol content of biodegradable microspheres was determined for all the formulations by dissolving 50mg of microspheres in 1ml of dichloromethane (DCM) in a test tube and vortexed for 5 min. Then, 10ml of water was added and vortexed for 10min to evaporate all DCM, to precipitate polycaprolactone (insoluble in water) and to extract the tramadol into water. The resulting solution was centrifuged for 10 min at 3000 rpm to settle down the precipitated polymer. The supernatant solution was taken and the absorbance was measured at 271nm by UV-Visible spectrophotometery and equivalent concentration was determined using the calibration curve prepared using the same proportion of solvents. The percentage drug loading (% drug loading) and percentage encapsulation efficiency (%EE) of the blend microspheres were calculated using the following formula. All the experiments were carried out in triplicate [8].

Drug loading is determined by this formula:

$$\text{Drug loading} = \frac{\text{Mass of drug in microspheres}}{\text{Mass of microspheres}} \times 100$$

Percentage encapsulation efficiency was calculated as follows:

$$\text{Encapsulation efficiency \%} = \frac{\text{Entrapped amount of drug per g microsphere}}{\text{Theoretical amount of drug per g microsphere}} \times 100$$

***Invitro* Release Study**

The *invitro* dissolution study was carried out in a system composed of a glass tube in which a cellophane membrane (soaked overnight in phosphate buffer, pH 7.4) was stretched and securely fastened with a rubber band. 100mg of microspheres was suspended in 5ml of phosphate buffer, pH 7.4, was placed in the former tube (donor phase). This was then hung vertically in a beaker (500ml capacity) containing 500ml of phosphate buffer, pH 7.4 (acceptor phase). The buffer in the acceptor phase is stirred by a magnetic stirrer. At predetermined time intervals, 5ml of the solution were removed from the acceptor phase and absorbance was measured at 271 nm using UV-Visible spectrophotometery. The volume of the acceptor medium removed at each time point was replaced by the same quantity of fresh medium. The experiments were carried out in triplicate [9].

Drug Excipent Interaction & Polymorphism Studies

Fourier Transform Infrared Spectroscopy

Drug-polymer interactions were studied by ABB MB3000 FT-IR Spectrophotometer. The spectra were recorded for pure drug, polymer and drug loaded microsphere of the best formulation. The samples were prepared as KBr discs. The scanning range was 500-4000 cm^{-1}.

Differential Scanning Calorimetry (DSC) Analysis

DSC furnishes information on the physical state of the active substance in the carrier and on the degree of crystallinity of the components and possible alternations undergone by the constituents of the formulation. Thermal analysis of the microspheres and the pure drug were performed with a differential scanning calorimeter (TA Instruments, model Q100 MDSC). Samples of 2.5-12 aluminium containers and heated, at a constant rate (10̊ C min^{-1}), from 20 to 400C under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Preparation of microspheres

The Emulsification solvent evaporation method is the most common technique used for preparation of PCL microspheres since it is simple requires less organic solvent and is easy to scale up compared to other methods.

In the first step, PCL and PVP were dispersed in DCM and after that drug; tramadol was dispersed in the polymeric solution. In the second step, the polymeric solution was emulsified into external oil phase to form an O/O emulsion. Span 80 and 20 were used as surfactants. Solvent removal was accomplished by evaporation at room temperature assisted by continuous stirring. As DCM evaporates during solvent evaporation process, usually it results in the formation of compact monolithic matrix type microspheres. To encapsulate a water soluble drug it is preferable to use oil for the continuous phase [8]. Liquid paraffin was chosen as continuous phase since tramadol, PCL, and PVP are not soluble in oil. After complete removal of solvent by extraction and evaporation the solid microspheres were separated from liquid paraffin by centrifugation at 6000 rpm for 15 min. The supernatant was removed and the microspheres were washed three times with n-hexane for complete removal of oil and hardening of microspheres.

In the present study, PCL microspheres of tramadol were prepared using 200 ml of continuous phase. And PCL and PVP of varying concentrations as denoted in the table 2 were prepared with span 80, span 20 and mixture of span 80 and 20.

Effect of PCL and PVP concentration on entrapment efficiency

The microspheres were prepared in the concentration specified in (**Table 2**). From the results given in table 2 encapsulation efficiency of microspheres increased with increase in the concentration of PVP. The probable reason is the loss of hydrophilic polymer from dispersed phase into aqueous continuous phase due to being its more solubility in water [8]. As a result, formulation containing higher concentration of PVP has more encapsulation efficiency and by decreasing PCL/PVP ratio encapsulation efficiency could be enhanced.

Effect of surfactants on particle size and entrapment efficiency

The effect of surfactants like span 80 and span 20 on particle sizes and entrapment efficiency of tramadol loaded PCL microspheres were studied. The microspheres prepared using span 80 as surfactant shows an average mean diameter in the

range 80µm to 160 µm; the microspheres prepared using span 20 as surfactant showed shows an average mean diameter in the range 200 µm to 500 µm and the microspheres prepared using mixture of span 80 and span 20 as surfactant shows an average mean diameter in the range 89 µm to 320 µm. Thus the microspheres prepared using span 80 is smaller than that of span 20. And the mixture of surfactants showed further smaller microspheres. This is due to the fact that, the particle size increases with increase in HLB values of the surfactant used [11]. Span 80 has a HLB value of 4.3 and span 20 has an HLB Value of 8.6.

Since the microspheres prepared using span 20 is larger in size comparatively, they have greater encapsulation efficiency. This is due to the fact that, entrapment efficiency increases with increase in particle size [8].

Table 2: Effect of concentration of Polycaprolactone, Polyvinylpyrrolidone on particle size and entrapment efficiency

Formulation	Average particle size (µm)	Tramadol loading (µg)	Entrapment efficiency (%)	Poly-dispersity	Percentage yield
FT1	212.4	7.203	79.23± 1.813	2.048	55.11
FT2	506.6	6.550	66.10 ± 1.025	1.01	75.86
FT3	303.7	5.583	61.41 ± 0.975	2.09	90.12
FT4	375.0	5.020	50.66 ± 1.857	1.554	94.83
FT5	80.05	6.860	75.45 ± 1.572	1.142	69.99
FT6	136.9	6.193	62.50 ± 1.857	1.072	74.28
FT7	166.3	5.220	57.41 ± 0.810	0.753	93.52
FT8	104.0	4.083	41.20 ± 1.009	1.179	93.51
FT9	89.44	5.950	65.44 ± 1.781	1.488	73.82
FT10	314.8	5.370	54.19 ± 1.248	1.115	73.26
FT11	102.5	5.103	51.50 ± 1.137	0.892	86.30
FT12	147.8	3.990	40.29 ± 1.015	1.387	96.40

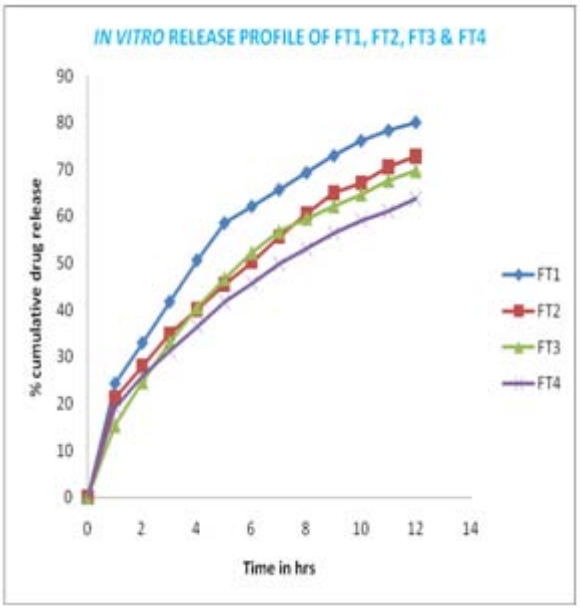
Invitro drug Release

The release profiles of tramadol from PCL microspheres in phosphate buffer solution pH 7.4 at 37˚C were found. The cumulative percentage drug releases from the formulations were given in the figure 1.

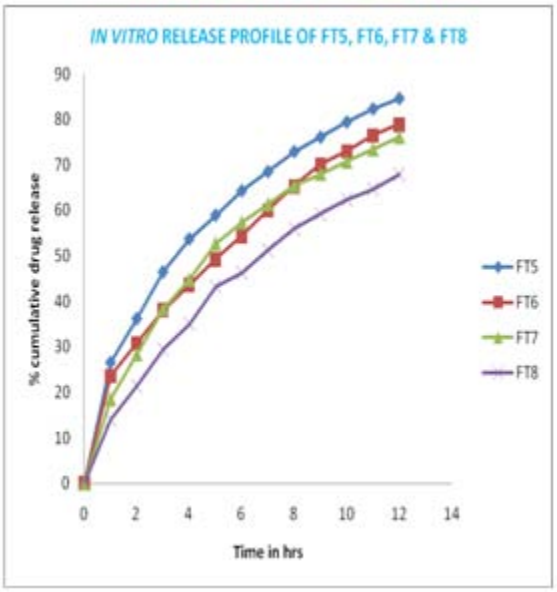
From the results it is evident that, microspheres showed a sustained release pattern up to 12 hrs with almost 30-40% release in first two hours. This is due to initial burst release. This is either due to loosely associated tramadol with the surface of the microspheres, which releases once they come in contact with the dissolution medium. Formulation showed sustained release of drug for 12hrs. The drug release from the formulations, FT1, FT2, FT5, FT6, FT9 and FT10 was almost 80% to 90 %. The fast release was observed from formulation with higher concentration of PVP. Due to high amount of PVP the drug release from the microspheres has been enhanced, which is due to hydrophilic nature of PVP. PVP helps in the hydration of microspheres by creating channels. Thus due to porous nature of the microspheres, there is enhanced penetration of dissolution medium into the polymeric matrix.

The formulations FT3, FT4, FT7, FT8, FT11 and FT12 released only 50 % to 60% in the 12 hours. This is due to less amount of PVP concentration.

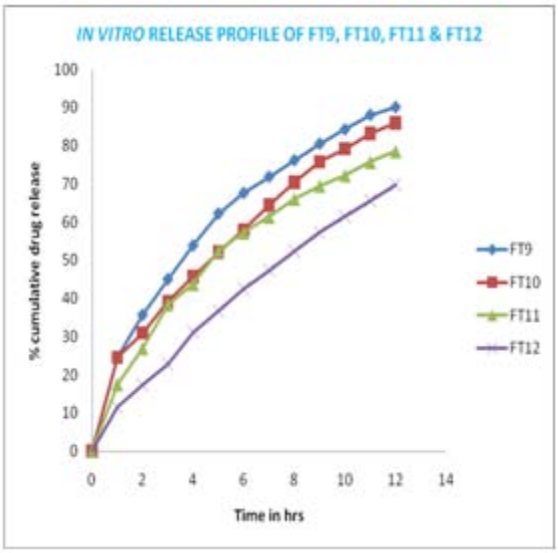
Figure 1: Dissolution profiles of prepared microspheres (Fig 1.1: *Invitro* Release Profile of FT1,FT2,FT3 & FT4)



(Fig 1.2: *Invitro* Release Profile of FT5, FT6, FT7 & FT8)



(Fig 1.3: *Invitro* Release Profile of FT9, FT10, FT11 & FT12)



Selection of Best Formulation

From the above results, it was found that formulations FT5, FT9, FT11 and FT12 have the smaller particle size range between 1-250 µm. But among the four formulations FT1 has lesser polydispersity index than the other three formulations. Also, FT5 has higher entrapment efficiency of 81% than the other three formulations. Based on the above said factors, formulation FT5 was been selected as the best formulation.

Scanning Electron Microscopy Images

Scanning electron microscopy is an excellent tool for physical observation of morphological features of the microspheres. Morphology of the Microspheres of the best formulation was studied using scanning electron microscope (HITACHI, S-3400). SEM image of the empty microsphere and FT5 is shown in figure 2 and 3. The microspheres were smooth and spherical in shape. The empty microspheres showed smooth surface. The surfaces of the drug loaded microspheres were rough comparatively which is due to higher concentration of drug in the microspheres as compared to the blank microspheres.

Figure 2: SEM Image of Empty Microspheres

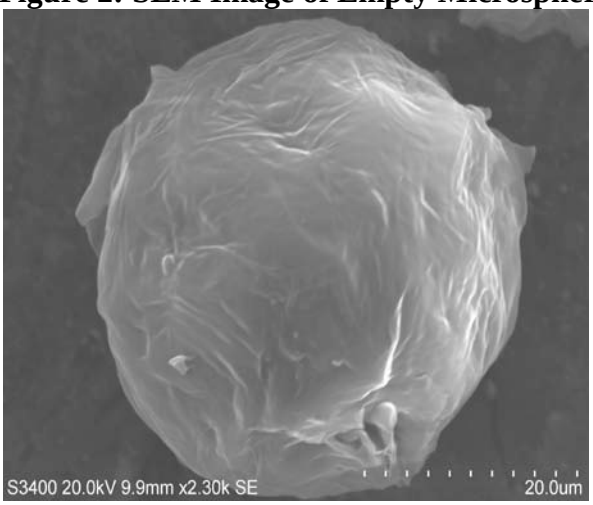
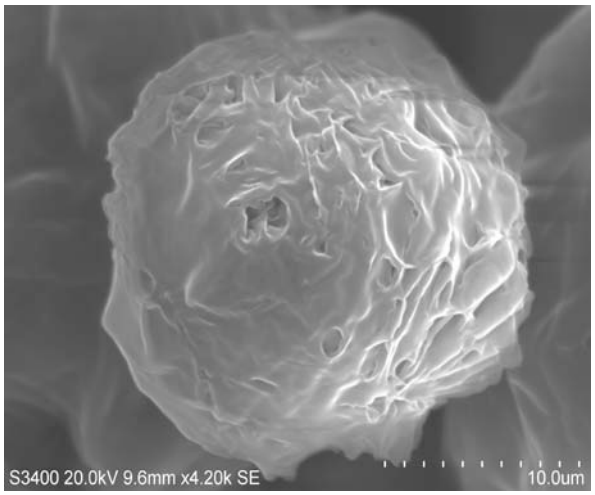


Figure 3: SEM Image of Formulation FT5



Drug-Excipient Interaction Studies

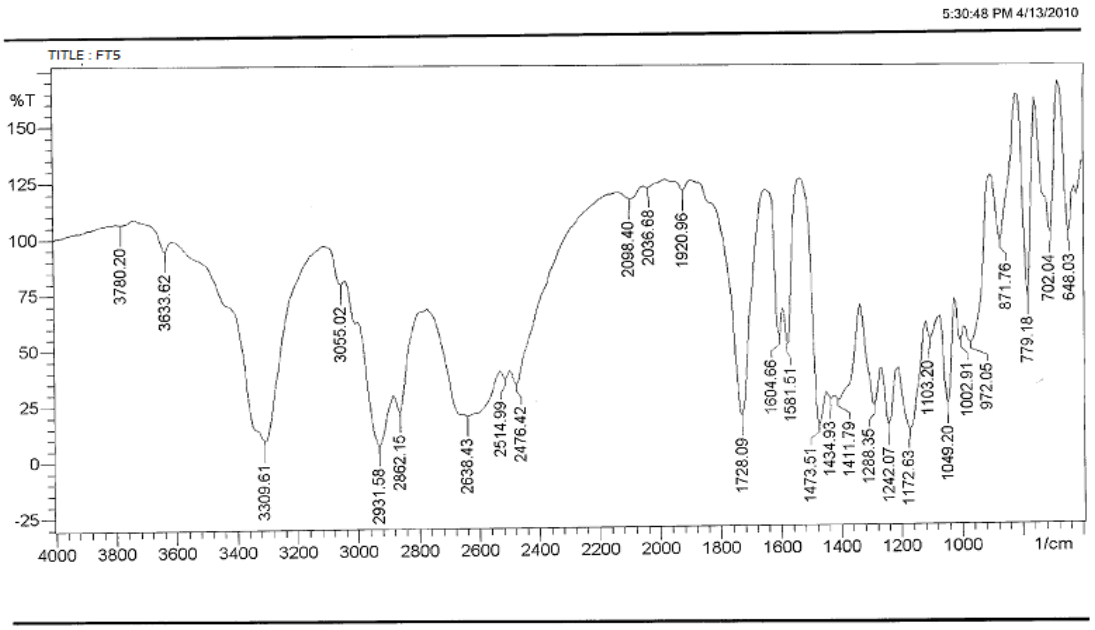
FT-IR ANALYSIS

FTIR spectra obtained could confirm the chemical stability of tramadol in the microspheres. FTIR spectra of tramadol loaded microspheres was shown in the figure 4.

Tramadol shows characteristic aromatic CH stretching vibration at about 3000 $cm^{-1,}$ OH shoulders at about 3300 cm-1, aliphatic CH stretching vibration at about 1600 cm-1. It is evident that only slight in some of the groups characteristics of the drug took place with

overlapping and broadening of similar peaks. No new bands were detected in the spectra of microspheres, indicating no interaction between tramadol, PCL and PVP.

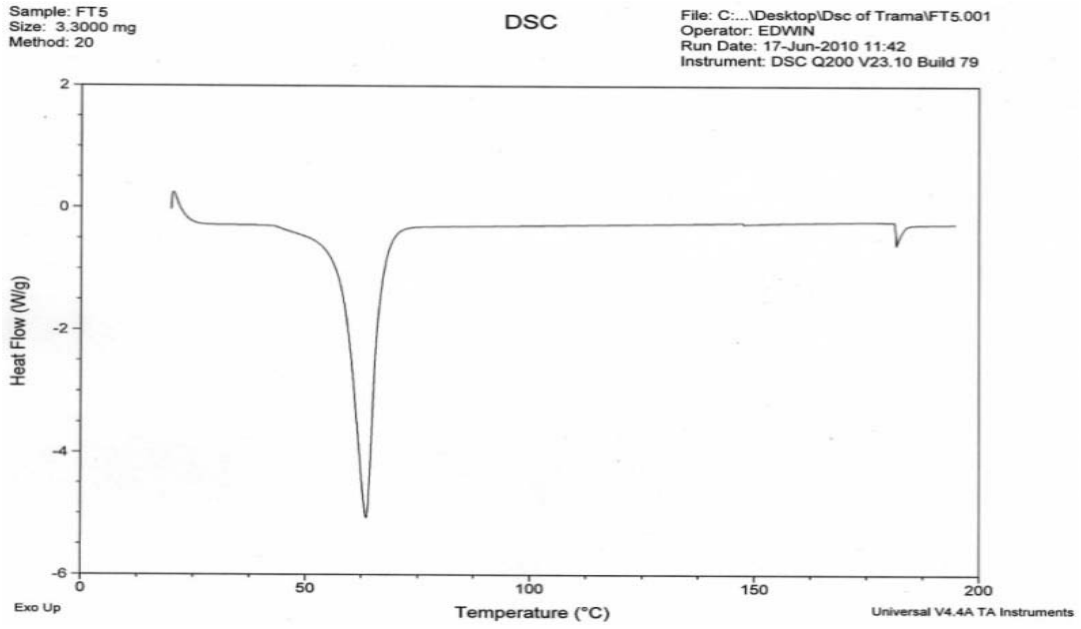
Figure 4 Fourier Transform Infrared spectra of Microsphere FT5



DSC ANALYSIS

DSC studies were performed to understand the nature of the encapsulated drug in the matrix. The physical state of the dug in the polymer matrix would influence its release characteristics. **DSC** thermogram of drug loaded microsphere is shown in figure 6. Tramadol shows a broad peak at 181˚C, PCL shows a sharp peak at 60˚C and PVP shows a broad peak at 110˚C which indicates their respective melting points. In the thermogram of empty microspheres, a sharp peak at 60C was observed. In the drug loaded microspheres, sharp peaks at 60C and a mild peak at 181˚C was observed. This suggests that the drug is dispersed in the amorphous in the PCL matrix.

Figure 5 Thermal analysis (differential scanning calorimetry) graph of Microsphere FT5.



CONCLUSION

In the present study, a sustained release formulation of tramadol was prepared using PCL and PVP. The encapsulation efficiency and drug loading of the microspheres increased with increase in PVP concentration due to affinity between the drug and the hydrophilic polymer. The particle size was measured. The particle size increases with increase in the HLB values of the

surfactants used. PVP has remarkable effect on release of tramadol. The drug release increases with increase in PVP concentration. This is due to the formation of pores and thus enhancing penetration of the dissolution medium.

From the above studies, FT5 is selected as the best formulation since it has higher entrapment efficiency, low Polydispersity, and releases up to 84% of the drug in 12 hrs.

SEM analysis of this formulation reveals that the microspheres are spherical. The surface of the drug loaded microspheres is rough. This is due to the presence of high concentration drug. The drug release kinetics fits best with Higuchi's model and release was found to be non-Fickian. The FT-IR studies indicated that there is no interaction between the polymer and the drug used. DSC studies reveal that the drug present in the microspheres is amorphous in nature. Thus, Polycaprolactone microspheres offer a suitable and practical approach to obtain sustained release of tramadol HCl with enhanced bioavailability and reduced dosing frequency for the management of Post operative pain.

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