Drug Release Kinetics and Stability Studies of Tablets of Tramadol HCl Microspheres

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Summary: The objectives of study were the development of new formulations of Tramadol HCl (TmH) microspheres and their evaluation primarily for kinetics and stability. Microspheres of different polymer concentration M_1 (1:1), M_2 (1:2) and M_3 (1:3) were developed and compressed into tablets i.e., T_1, T_2 and T_3, respectively. Zero order, First order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas kinetic models were applied to assess the mechanism and pattern of drug release. Higuchi model was found to be the best among all models. The chemical and physical stability of TmH formulation was studied using FTIR, Thermal analysis, X-ray diffraction and dissolution tests. In-vitro analysis showed that tablets of ratio T_2 released the drug over 12hrs and the release profile was comparable with that of reference tablet, Tramal® SR. The effect of different storage temperatures on the physicochemical stability of T_2 was insignificant ($p > 0.05$).

Introduction

TmH (Fig. 1) is a synthetic, centrally acting analgesic having bitter taste and is currently approved for use in many countries. It acts as opiate agonists, through selective binding to the μ-opioid receptor and weak inhibition of norepinephrine and serotonin uptake [1, 2]. TmH is available as drops, capsules and sustained-release formulations for oral use; suppositories for rectal use and solution for intramuscular, intravenous and subcutaneous injection. After oral administration, TmH is quickly and almost completely absorbed. SR tablets release TmH over a period of 12 hours, reach peak concentrations after 4.9 hours and have a bioavailability of 87-95 % [3]. TmH is rapidly distributed in the body and its plasma protein binding is about 20%. The elimination half-life is about 6.1 hours. TmH metabolized by cytochrome P-450 (2D6). TmH and its metabolites are mainly excreted through kidneys [4].

Ethyl cellulose (EC) is an inert hydrophobic polymer and is essentially stable, non-toxic, odorless, tasteless, colorless and soluble in a wide range of organic solvents. It an ethyl ether of cellulose is a long chain polymer of β-anhydro-glucose units joined together by acetal linkages [5]. It is widely used in matrix and coating systems. Because of better properties EC was used in this study.

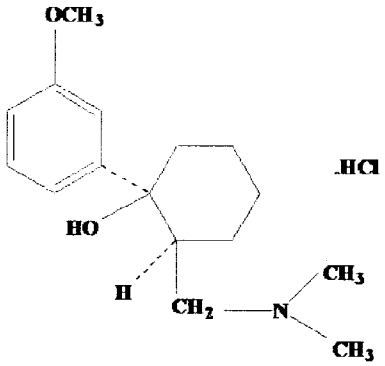

Fig. 1: Chemical Structure of Tramadol HCl.

In past, TmH was complexed with a sulfonic acid cation-exchange by spray-drying method [6]. Matrix sustained release tablets of TmH were developed using natural gums [7]. Therefore, new formulations of Microspheres of TmH were developed using solvent evaporation technique and characterized for drug release pattern and physico-chemical stability. The Kinetic and stability studies of tablets of microspheres of TmH is potential exclusivity of this study.

Results and Discussion

In vitro Drug Release Profile of Microspheres

Increase in concentration of EC, resulted in more sustained release profile. Within 2 hours

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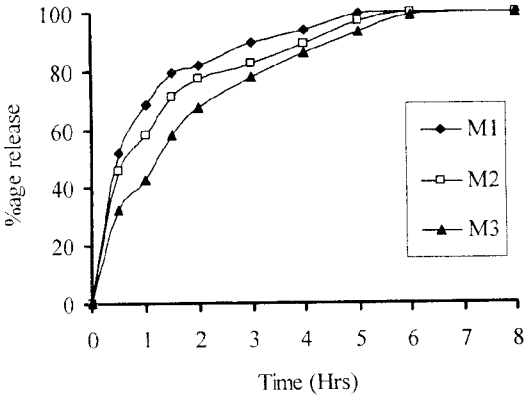

Fig. 2: Release profiles of TmH from Microspheres.

approximately 82 %, 77 % and 68 % of drug was released from M_1, M_2 and M_3, respectively (Fig. 2). Increase in the ratio of EC in the microspheres may reduce the diffusion of water molecules into the polymer, thus reducing the extent of swelling of microspheres, resulting in slower release of drug. At very high ratio of polymer, the microspheres become impermeable to the dissolution medium and give very slow release of drug as observed in the release profile of formulation M_3 (1:3).

In-vitro Drug Release Profile of Tablets

As the ratio of EC was increased, a gradual decrease in the release of the drug was found from the compressed tablets like that of microspheres. About 98.8 %, 86.5 % and 77.3 % drug was released from the tablets T_1, T_2, T_3, respectively, after 12 hours. Microspheres were fine particles and drug can easily be diffused out from them but tablets are large compact masses of particles. Sufficient time is required for the medium to penetrate into the tablets. Moreover, the hardness of all tablet formulations was kept constant (above 9.0 Kg) in order to prevent disintegration of tablets during the drug release study. Similar release pattern from microspheres and tablets of acetylsalicylic acid was found when EC was used as polymer [8].

Comparison of Release Profile of Formulated Tablets with Reference Tablets

In vitro release profile of formulated tablets was compared with commercially available product (Tramal® SR) containing 100 mg of TmH. Drug release from the reference tablets, after 12 hours was 90 %. About 98.8%, 86.5 % and 77.3 % drug was released from the tablets T_1, T_2, T_3, respectively, after the same interval of time. T_2 had the similar release profile with reference (Fig. 3). The similarity factor (f_2 value) was applied to reveal the similarity between the release profile of the reference and the formulated tablets. T_2 showed maximum similarity with reference as shown in Table 1.

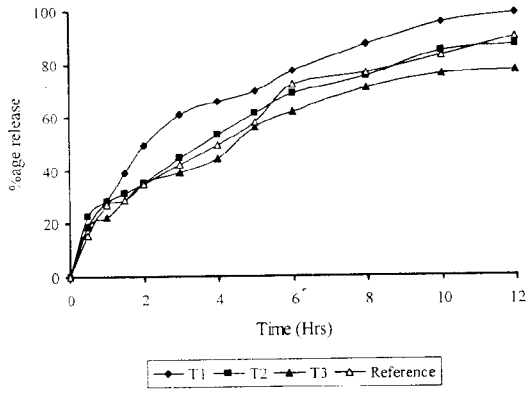

Fig. 3: Comparison of Drug Release from Formulated Tablets and Reference Tablets.

Table-1: f_2-values determined from drug release data of test tablets of different formulations vs reference.

Comparison	f_2-value
Reference Vs T_1	46.69
Reference Vs T_2	72.87
Reference Vs T_3	56.33

Similarity of the formulated tablets was also characterized by applying $T_{50\%}$ (time at which 50% of the drug releases). $T_{50\%}$ of T_2 was close to that of reference (Table 2), that's why it was selected for further studies. $T_{50\%}$ value of T_2 is comparable with that of mixed chitosan-gelatin sponge [9].

Application of Different Kinetic Models

The drug release constant (k) and regression coefficient (R^2) obtained from Zero order; First order, Hixson-Crowell, Korsmeyer-Pappas and Higuchi models are shown in Fig. 4. Drug release kinetics indicated that drug release was best supported by Higuchi's equation. The release of drug from T_2 was because of more than one release mechanism.

Drug release kinetics indicated that drug release was best explained by Higuchi's equation, as

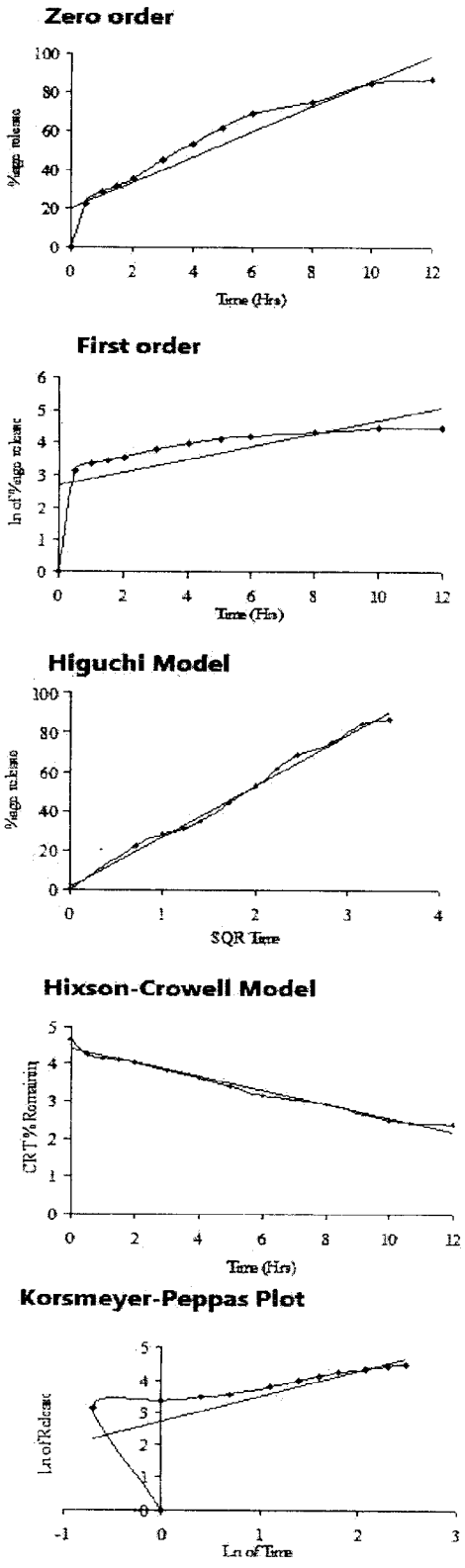

Fig. 4: Kinetic Models Graphs along with Trend Lines.

Table-2: Values of 'K', "R^2" and $T_{50\%}$, Hardness and Friability data of Reference, T_1, T_2 and T_3.

Formulations	K	R^2	$T_{50\%}$	Hardness (Kg) (M±S.D)	Friability (%)
T_1	7.3877	0.8576	3.39	9.2 ± 0.3	0.38±0.4
T_2	6.5329	0.8999	4.52	9.5 ± 0.9	0.33±0.4
T_3	5.8334	0.9121	5.59	9.3 ± 0.7	0.29±0.2
Tramal® SR	6.954	0.9163	4.69	8.9 ± 0.7	0.3±0.3

these plots showed the highest linearity (R^2 = 0.9915), but a close relationship was also noted with zero-order kinetics (R^2 = 0.8999) (Table 3). Drug release from microspheres of theophylline encapsulated by ethyl cellulose, was found to be following Higuchi model [10]. Korsmeyer's plots indicated an n value of 0.78, which was indicative of an anomalous diffusion mechanism or diffusion coupled with erosion; hence, the drug release was controlled by more than one process. Anomalous release of TmH was also observed from alginate-chitosan co-polymer [11]. Similar results were found with a matrix tablet of nicorandil with n = 0.71. [12]. Hixson-Crowell plot indicated a change in diameter and surface area of the tablets with progress in dissolution of formulated tablets as a function of time.

Stability Studies

T_2 stored at 8°C, 25°C and 40°C/75 % relative humidity were evaluated for release profiles after 1, 2, 3 and 6 months (Fig. 5). The results denoted that T_2 were stable at various environmental temperatures because there was no significant difference in release profiles ($p > 0.05$).

The tablets of microspheres of TmH, T_2 presented very good release pattern at all storage temperatures. EC is a stable polymer and the excellent stability of T_2 was because of inert and static nature of EC. At cool (8°C) and room (25°C) temperatures, T_2 were relative more persistent than at extreme temperature with humidity (40°C/75 % RH), this suggested to keep T_2 at cool (8°C) or room (25°C) temperatures for its prolonged storage (Table-4). Although T_2 can bear extreme temperature but its stability is minimum as compared with cool (8°C) /room (25°C), so for maximum storage shelf life of T_2, avoid long term storage in humid vicinity.

Fourier Transform Infrared Spectroscopy

In the FTIR spectrum of TmH, the characteristics of:

- OH shoulders at 3300 cm^{-1},

- aromatic CH stretching at 3050 cm^{-1},
- aliphatic CH stretching at 2900 cm^{-1} and
- aromatic ring stretching at 1600 cm^{-1} can be seen.

It is evident that only slight shift in some of the groups characteristic of drug, took place with overlapping and broadening of similar peaks (Fig. 6). No new bands were detected in the spectra of TmH-EC indicating no interaction between TmH-EC tablets of microspheres, T_2. The drug is chemically stable even after encapsulation. Prolongation of drug release was characterized by polymer-drug conjugation [13].

X-ray Powder Diffractometry

Every crystalline drug has an exclusive XRD pattern, which can be used for its identification.

Table-3: Values of release rate constant 'K' and correlation coefficient 'R^2' of T_2.

Zero Order		First Order		Higuchi Equation		Hixson-Crowell		Korsmeyer-Pappas		
R^2	$K_0(h^{-1})$	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	K_{HC} ($h^{-1/3}$)	R^2	n	K_{KP} (h^{-n})
0.899	1.26	0.43	0.20	0.99	25.76	0.98	0.18	0.43	0.78	0.1309

Table-4: f_2-values of T_2 release profile after stability studies at different storage temperatures.

Comparison	f_2-value			
	After One Month	After Two Months	After Three Months	After Six Months
T_2 Vs 8°C	76.79	79.08	81.99	86.55
T_2 Vs 25°C	82.17	79.18	70.16	71.17
T_2 Vs 40°C+75RH	73.86	82.92	66.14	62.12

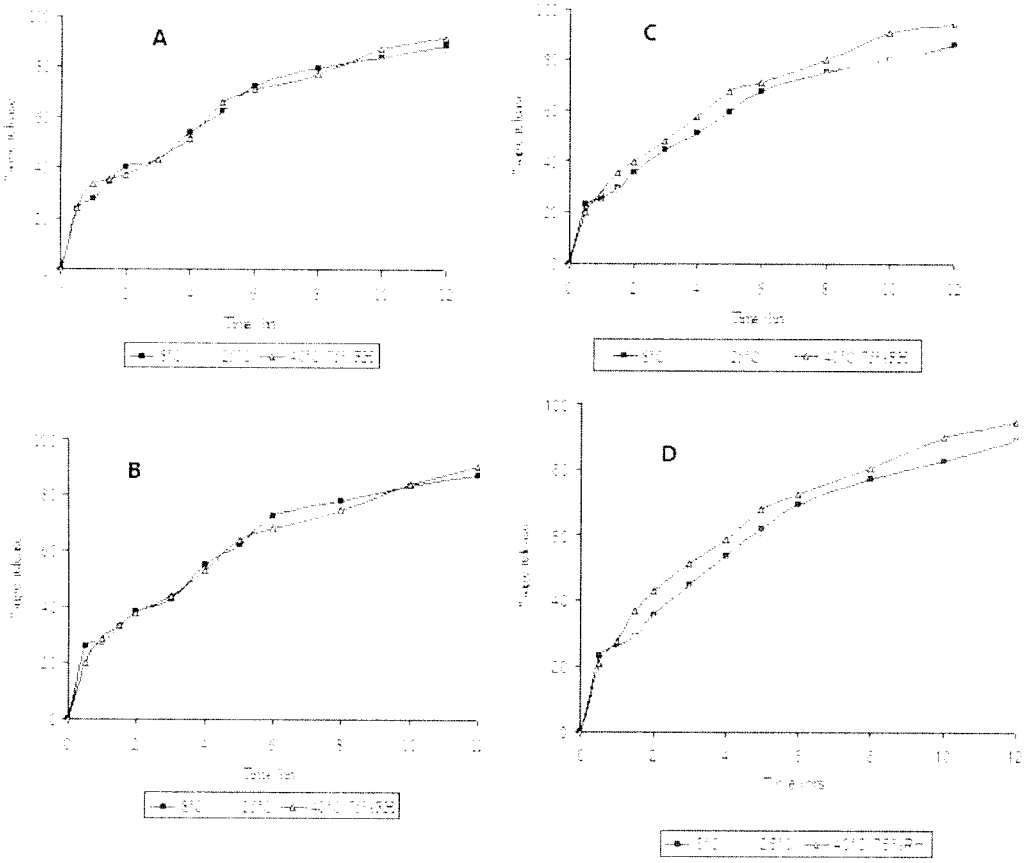

Fig. 5: Release profiles after stability studies.
A: After 1 month; **B:** After 2 months; **C:** After 3 months; **D:** After 6 months.

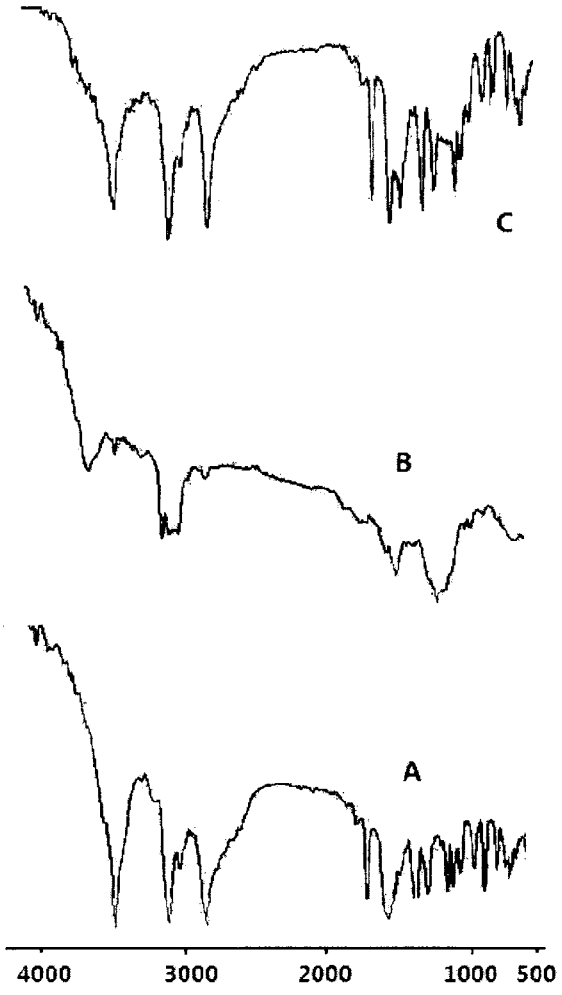

Fig. 6: Fourier Transform Infrared Spectroscopy. **A**=Tramadol HCl; **B**=Ethyl Cellulose; **C**=T_2

XRD was employed to identify crystallographic properties of drug. TmH showed characteristic intense peaks between 2θ of 15^0 and 27^0 due its crystalline nature. The intensity of the peaks is reduced when the drug is encapsulated in polymer, which indicated reduced crystallinity of the drugs (Fig. 7). It may be recognized to the inhibition of nucleus formation and crystal growth due to encapsulation method. The decrease in crystallinity of T_2, also confirmed the physical stability of the drug within the polymeric microspheres [14].

Thermal Analysis

Both the melting temperature and the melting range of TmH and EC are significant. DSC

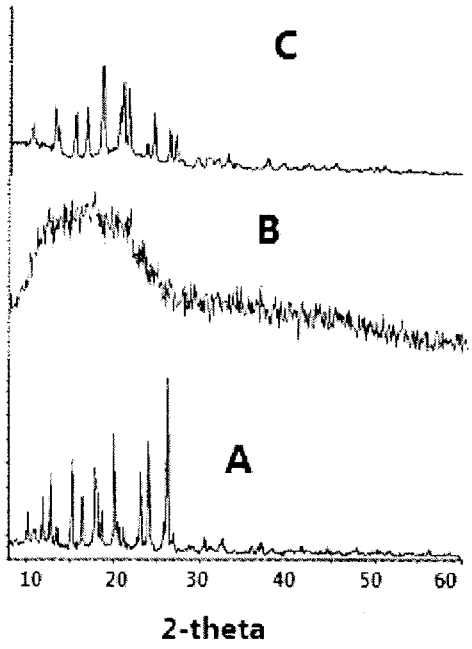

Fig. 7: X-ray Powder Diffractometry. **A**=Tramadol HCl; **B**=Ethyl Cellulose; **C**=T_2

was conducted to explore the melting behavior of drug and polymer and therefore informations about possible drug-polymer interaction were collected (Fig. 8). DSC showed an endothermic peak at 180 °C which is indication of melting temperature of TmH. Similarly endothermic peak of EC was observed at glass transition temperature (132 °C). DSC curve of T_2 also showed peaks at 130°C and 180°C, indicating chemical stability of TmH in the formulation. TGA [15, 16] was conducted to provide complimentary and supplementary characterization information to thermal analysis. TGA measures the amount and rate of change in the mass of a sample as a function of temperature in a controlled atmosphere. The measurements were used to determine the thermal and/or oxidative stabilities of TmH as well as its microspheres. Additionally, TGA was employed to analyze mass loss or gain due to decomposition, oxidation or loss of volatile contents. The loss of mass was identical in TGA curve of TmH, EC and T_2, ensuring chemical stability of drug and polymer.

Experimental

Materials

TmH (Ali Gohar Pharmaceuticals, Pakistan), Methanol (Merck, Germany), Ethyl cellulose 22 cp

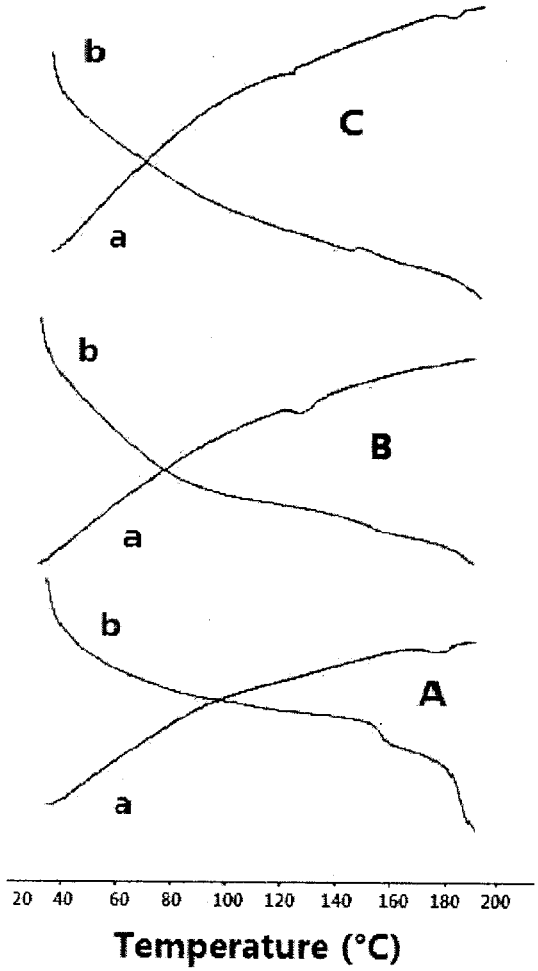

Fig. 8: Differential Scanning Calorimetry and Thermogravimetric Analysis.
A= Tramadol HCl; **B**=Ethyl Cellulose; **C**=T_2 a=DSC; b=TGA

(BDH Chemicals Ltd, Poole, UK), Acetone (Merck, Germany), Mineral oil (Acros Organics, USA), Span 85 (Sigma, Germany), n-Hexane (Merck, Germany). All materials used were of analytical grade.

Preparation of Microspheres

Microencapsulation based on solvent evaporation method was employed to formulate the microspheres of TmH using different ratios of EC. TmH and EC were dissolved in methanol and acetone separately. The solution of TmH was added to EC solution, (Drug-Polymer solution). Mineral oil was taken in suitable size container and Span 85 was added to it and placed at magnetic stirrer (Velp Scientifica, Germany). Drug-Polymer solution was added to mineral oil drop by drop with continuous stirring. After adding Drug-Polymer solution, continue the stirring for about 4 hours to evaporate acetone to obtain microspheres. The microspheres were filtered and washed with n-hexane and distilled water to remove mineral oil and free drug particles from the surface of microspheres respectively. The formed microspheres were dried at 40°C in an oven (Mammert, Germany). The dried microspheres were passed through a fine sieve No. 60 (Mughal Trading Agencies, Pakistan) and stored in an airtight amber glass container at room temperature until their characterization.

Characterization of Microspheres

Size and Shape

To determine the size, microspheres were observed under optical microscope (Nikon, Japan). The mean particle sizes for different formulations of microspheres are given in Table 5. A drop of microspheres dispersion was spread over a SEM stub and dried in a desiccator. To make electrically conductive, microspheres were coated with a thin layer of gold using a vacuum evaporator. Then microspheres were observed under SEM. Microspheres were spherical in shape with smooth surface when studied under Scanning Electron Microscope (Hitachi, S 3000H, Japan) (Fig. 9).

Table-5: Size, Shape and Entrapment Efficiency of Microspheres.

Microspheres	Size (Mean Diameter) (M±S.D) µm	Shape	% Entrapment Efficiency
M_1	131±07	Spherical	67
M_2	136±06	Spherical	72
M_3	144±06	Spherical	77

Entrapment Efficiency

The drug content of microspheres was determined by dissolving known weight of microspheres (M_1, M_2 and M_3) in small amounts of methanol to dissolve the EC layer. To this solution, 15 ml of distilled water was added and the solution was heated to evaporate the methanol. This was filtered to remove insoluble EC and diluted to make volume up to 100 ml with distilled water. 1ml of this

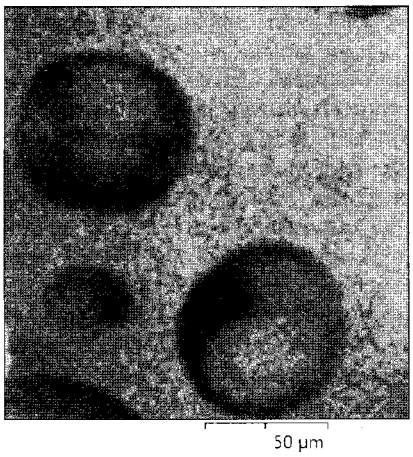

Fig. 9: SEM of Microspheres at 100 Magnification.

was taken and diluted to make volume up to 100ml and analyzed by a UV spectrophotometric method at 270 nm. Entrapment efficiency of all the microspheres was calculated by dividing absorbance values obtained from microspheres to the absorbance value of 100 mg reference TmH at same dilution [17]. Entrapment efficiency increased as the concentration of EC was increased (Table 5).

$$\text{Entrapment Efficiency} = \frac{\text{Absorbance of microspheres equivalent to 100mg TmH}}{\text{Absorbance of 100mg TmH}} \times 100$$

In vitro Drug Release of Microspheres

In vitro drug release of various microspheres was determined using automatic apparatus II USP (Pharma Test, Germany). 5 ml of sample was collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 hours with an automated fraction collector after filtering through 10µm Sintered filters. All samples were diluted up to 25ml and analyzed at 270 nm using a UV-spectrophotometer (Shimadzu 1601, Japan). Percentage drug release at different sampling intervals was calculated.

Preparation of TmH Tablets

TmH tablets, T_1, T_2 and T_3 were prepared by direct compression using microspheres M_1, M_2 and M_3, respectively. Single punch tablet machine (Emmay, Pakistan) was employed for tablets compression. Each tablet contained microspheres equivalent to 100 mg of TmH. The microspheres were blended with 0.5% of magnesium stearate and 0.5% of talc and compressed with a single punch tablet machine.

Physical and Chemical Evaluation of Tablets

Weight Variation of Tablets

In order to determine the uniformity of tablet weight, twenty tablets of each formulation were randomly collected and weighed using 'class A' weight balance (Precisa, Switzerland) and their percentage variation was determined. Weight variation of tablets was within the acceptance limits (<0.5%).

Hardness of Tablets

Hardness of tablets was determined using automatic hardness tester (Curio, Pakistan). Ten tablets of each formulation were used and the average hardness value was calculated (Table 2). Average hardness of all the formulations was above 9 Kg.

Friability of Tablets

The tablets of each formulation were also subjected to friability testing employing friabiliator (Emmy, Pakistan). The weight of twenty tablets prior to their placement in the chamber and at the end of the test was recorded (Table 2). The percentage weight loss was not more than 0.3%.

In vitro Drug Release Studies of Tablets

The apparatus, methodology and specifications adopted for dissolution of microspheres was used except sampling time till 12 h. A commercially available TmH tablets, Tramal® SR was used as a reference.

Reproducibility and Stability Studies at Different Temperatures

In addition, T_2 tablets were packed in an airtight amber glass bottles. The bottles were kept at 8°C (Pel, Pakistan), 25°C (Cold Incubator, Sanyo MIR-153, Japan) and 40°C/75% Relative Humidity

(Hot Incubator Sanyo MIR-162, Japan). The samples of these tablets were withdrawn after 1, 2, 3 and 6 months and evaluated for stability by determining in-vitro release profile.

Application of Kinetic Models

The dissolution data of T_2 tablets was fitted to commonly used models i.e. Zero order, First order, Higuchi [18], Hixson-Crowell [19] and Korsmeyer-Peppas [20, 21] to determine the pattern and mechanism of drug release.

Zero order kinetics $F_t=K_0t$

For zero-order kinetic model, data from in-vitro release was plotted as cumulative amount of TmH released vs time.

First order kinetics
$\ln(1-F)=-K_1t$

In First order model, log cumulative percentage of drug remaining was plotted against time in hours.

Higuchi model
$F=K_Ht^{1/2}$

Here, graph between cumulative percentage of drug released vs square root of time was drawn

Hixson-Crowell
$(1-F)^{1/3}=1-K_{HC}t$

To assess the TmH release with changes in the surface area and the diameter of the tablets, the data were also plotted using the Hixson-Crowell cube root law: as the cube root of the percentage of drug remaining in tablet vs time.

Korsmeyer–Peppas
$F=K_{KP}t^n$

To appraise the mechanism of TmH release from SR tablets via Korsmeyer–Peppas, log cumulative percentage of drug released vs log time was drawn.

In above applied models, K_0, K_1, K_H, K_{HC} and K_{KP} are rate constant for Zero-order, First-order, Higuchi, Hixson-Crowell and Korsmeyer–Peppas, respectively. F and t stand for fraction of drug released and time in hours, respectively. And n is an exponent that judges the mechanism of release. If the exponent n=0.45, then the drug release mechanism is Fickian diffusion and if $0.45 < n < 0.89$, then it is anomalous or non-Fickian diffusion. And n value equal or greater than 0.89 is indicative of zero-order drug release or case-II transport.

Additionally, the similarity factor f_2 [22] was used to match the difference of dissolution profile.

$$f_2 = 50 \log \{ [1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100 \}$$

Fourier Transform Infrared Spectroscopy (FTIR)

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and drug-loaded microspheres using FTIR Midac 2000, USA. Samples were prepared in KBr disks. The scanning range was 500-4000 cm^{-1} and the resolution was 2 cm^{-1}

X-ray Powder Diffractometry (XRD)

Crystallinity of TmH, before and after encapsulation was evaluated by X-ray diffractometer (Bruker D8 Discover, Germany) using Ni-filtered CuK alpha radiation source. The tube voltage of 35KV, current of 35 mA and scanning rate of 5^0 min^{-1}, over a range of 4^0-40^0 diffraction angle (2θ) range.

Thermal Analysis

[Differential Scanning Calorimetry (DSC) and Thermogravimetric analysis (TGA)]. The concurrent DSC and TGA analysis of pure drug and drug-loaded tablets of microspheres were carried out using a SDT Q600. Samples (4-5 mg) were placed in aluminum pans and the lids were crimped. The analysis was performed from 25 ^{0}C to 180 ^{0}C temperature range, at a rate 20 ^{0}C min^{-1} under nitrogen flow of 25 ml per min. The instrument was calibrated with an indium standard.

Conclusion

Among the different formulations prepared, T_2 (1:2) showed comparable release profile with reference product. Higuchi model was found to be the best model fitted for drug release pattern and the

release of drug release was mediated by more than one process. The stability data of T_2 showed no significant difference in drug content and release profile of the drug. This mentioned that T_2 were stable importunate at different ecological temperatures. FTIR, XRD and thermal analytical evaluation also ensured the stability of formulation.

Acknowledgements

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