

Sustained Release Multiple Unit Dosage Form for the Oral Day Delivery of Dexketoprofen Trometamol

Sweed NM¹, Basalious EB² and Nour SA^{2*}

¹Department of Pharmaceutics, Faculty of Pharmacy, Modern University for Science and Arts (MSA), Egypt

²Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, 11562, Egypt

Research Article

Volume 1 Issue 3

Received Date: June 01, 2017

Published Date: June 22, 2017

***Corresponding author:** Samia A Nour, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, 11562, Egypt, E-mail: samia.nour@pharma.cu.edu.eg

Abstract

The aim of this study was to prepare sustained release multiple unit dosage form (MUDF) for the oral day delivery of the frequently administered and the highly water soluble drug, dexketoprofen trometamol (DT). The drug was encapsulated into Eudragit RS100 microspheres using emulsification solvent evaporation technique. A D-optimal design was used to determine the effect of amount of polymer (X_1), the percentage of hydrophobic plasticizer (X_2), the percentage of hydrophilic plasticizer (X_3), and stirring rate (X_4), on the % entrapment efficiency (Y_1), amount of drug released at 1h (Y_2), 4h (Y_3), and 8 h (Y_4). The optimized formulation was prepared using 1.39 g Eudragit, 0.063 g Dibutyl phthalate (DBP), 0.093 g Polyethylene Glycol 400 (PEG 400) and processed using stirring rate of 683 rpm. The optimized microspheres were evaluated by Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD). Results of the optimized formula showed that the microspheres were spherical with a rough surface and a particle size of $32 \pm 1.41 \mu\text{m}$, with entrapment efficiency $76.0 \pm 1.76\%$. The amount of drug released was $33.7 \pm 0.82\%$, $60.2 \pm 0.05\%$, and $75.1 \pm 1.84\%$, at 1, 4, and 8 h, respectively. Complete drug release was achieved after 16 h. The sustained release pattern of DT for up to 16 h with an acceptable initial release suggests that the developed MUDF may be useful for oral day delivery of the highly soluble and frequently administered drug such as DT.

Keywords: Multiple Unit Dosage Form; Differential Scanning Calorimetry; Scanning Electron Microscopy; Dexketoprofen Trometamol; X-ray Diffraction

Introduction

Racemic ketoprofen is used as an analgesic and an anti-inflammatory agent, and is one of the most potent

inhibitors of prostaglandin synthesis. The analgesic effect is due to the S (+)-enantiomer (dexketoprofen), while the R (-)-enantiomer has no analgesic activity. It was found that separation of R (-)-enantiomer from the racemic

mixture resulted in doubling of the analgesic effect of dexketoprofen [1]. DT, a water soluble salt of (S)-(+)-2-(3-Benzoylphenyl) propionic acid, is well absorbed orally throughout the gastrointestinal tract [2]. The usual dose of dexketoprofen is 25 mg (equivalent to 36.9 mg of DT) every 8h or 12.5 mg every 4 to 6 h. A maximum daily dose of 75 mg (equivalent to 110.7 mg of DT) may be applicable [3]. DT peak plasma levels are reached within 0.25 hours to 0.75 hours. The current oral dexketoprofen tablets show several drawbacks, such as rapid elimination due to the short elimination half-life of the drug (1.2-2.5 h) [3,4] which necessitate 3-6 times daily administration. In addition, the immediate release of single oral administration of DT results in gastrointestinal disturbances, such as gastrointestinal discomfort, nausea, diarrhea and gastrointestinal bleeding [5]. The oral dosage form is the most widely accepted route of administration [6]. Therefore, a sustained-release oral formulation of DT can reduce the frequency of administration, adverse effects and improve patient compliance. However, Preparation of sustained release formulation of DT poses a great challenge due to its very high water solubility (~662.37mg/mL) and the frequent oral administration. To the best of our knowledge, no reported trials were so far adopted to manufacture sustained release formulation for the oral day delivery of DT (~16 h release). To reach such criteria, the excipients used should ensure sustainment of drug release throughout the whole GIT irrespective of the pH. Eudragit RS100 is an acrylic polymer commonly used for the preparation of controlled release oral pharmaceutical dosage forms. Eudragit RS100 is insoluble at physiological pH values; therefore it is suitable for the preparation of pH-independent sustained-release drug formulations. Eudragit RS 100 swells in water and becomes permeable to water [7]. The permeability of Eudragit RS100 is due to the presence of a quaternary ammonium group in their structure [8].

Sustained release multiple unit dosage forms (MUDF) such as microspheres are becoming more popular than single unit dosage forms. These systems tend to spread uniformly throughout the gastrointestinal tract (GIT) and thereby, release the drugs more uniformly. The uniform distribution of these MUDF along the GIT could result in more reproducible drug absorption and reduced risk of local irritations than the use of single unit dosage forms. The microspheres can be filled into hard gelatin capsules or compressed into tablets [9].

In this study, sustained release MUDF for the oral day delivery of DT was prepared using emulsification solvent evaporation technique. The effects of formulation and process variables on the entrapment efficiency and

dissolution pattern of the microspheres were studied. A D-optimal design was used to determine the effect of amount of polymer (X_1), the percentage of hydrophobic plasticizer (X_2), the percentage of hydrophilic plasticizer (X_3), and the stirring rate (X_4), on the % entrapment efficiency (Y_1), amount of drug released at 1h (Y_2), 4h (Y_3), and 8h (Y_4). Powder characteristics of the developed microspheres were evaluated.

Materials and Methods

Materials

Dexketoprofen trometamol (DT) was obtained from Marcyl Pharmaceutical Industries (Egypt). Eudragit RS 100 was purchased from Evonik Degussa Corporation (Germany). Magnesium stearate was obtained from Sedico Pharmaceutical Industries (Egypt). Dibutyl phthalate (DBP) and polyethylene glycol 400 (PRG 400) were purchased from Sigma (USA). All other chemicals and solvents used were of analytical grade.

Determination of DT Saturated Solubility

An excess amount of DT was suspended in 10 mL of each of 0.1N HCl, water and phosphate buffer 6.8 in stoppered amber colored glass bottles. The bottles were shaken for 24 h in thermostatically controlled shaker at 37°C [10,11]. The content of bottles was filtered through a 0.45 μ m membrane filter and assayed by a validated in-house HPLC method. A 2 μ L aliquot of the filtrate was injected into HPLC column (Zorbax SB 250mm, 4.6 X 5 μ m). Chromatography was performed using a Waters Alliance system with a UV detector at 233 nm. The column temperature was set at 25°C and the flow rate was 1 mL/min. The mobile phase composed of 48% acetonitrile, 48% water and 4% acetate buffer (pH 3.5). All experiments were conducted in triplicates.

In Vitro Release Studies for DT Powder

The drug dissolution was determined using USP standard dissolution tester, Apparatus II. Dissolution was carried out in 900 ml phosphate buffer, pH 6.8 at 37 \pm 0.5°C for 1 h. 73.8 mg of DT powder was placed in each vessel containing the dissolution medium and the paddle was then rotated at 50 rpm. Aliquots of 4ml were withdrawn at predetermined time intervals; 5, 10, 15, 20, 30, and 60 min. and then filtered through a 0.45 μ m membrane filter. The content of DT was determined spectrophotometrically at 260 nm using a blank of fresh dissolution medium. An equal volume of fresh dissolution medium was added at the specified time intervals to maintain the volume of dissolution medium constant. The experiments were done in triplicates.

Experimental Design

In order to investigate the influence of formulation and process variables on the microspheres characteristics, DT loaded microspheres were prepared adopting a four factor, three level D-Optimal designs (Design Expert® software (Version 7, Stat-Ease Inc., Minneapolis, MN)). The independent and dependent variables of the D-optimal design for the formulation of DTM are shown in table 1. The polymer (Eudragit RS100) amount (X_1), the percentage of hydrophobic plasticizer (DBP) (X_2), the percentage of hydrophilic plasticizer (PEG 400) (X_3), and the stirring rate (X_4) were selected as the four independent variables, while the entrapment efficiency

EE % (Y_1), the amount of drug release at 1 h (Y_2), 4 h (Y_3) and 8 h (Y_4) h were the dependent variables. The software selected a set of candidate points as a base design. These included factorial points (high and low level from the constraints on each factor, centers of edges, constraint plane centroids, axial check point, and an overall center point). The base design consisted of 25 runs as shown in table 2. Design expert software was used to perform the statistical data analysis of the regression model and plot the response surface graphs. Analysis of variance (ANOVA) was carried out to estimate the significance of model and term. Probability p-values ($p < 0.05$) denoted significance.

| Factors (Independent variables) | Levels used | |
|--|-------------------|-------|
| | -1 | 1 |
| X_1 : Eudragit RS100 (g) | 1 | 1.655 |
| X_2 : Dibutyl Phthalate (%w/w of the polymer) | 0 | 10 |
| X_3 : Polyethylene glycol 400(%w/w of the polymer) | 0 | 10 |
| X_4 : Speed of rotation (rpm) | 450 | 750 |
| Response (Dependent variables) | Constraints | |
| Y_1 : Entrapment efficiency | $Y_1 > 70\%$ | |
| Y_2 : DT released after 1 h | $Y_2 < 40\%$ | |
| Y_3 : DT released after 4 h | $40 < Y_3 < 60\%$ | |
| Y_4 : DT released after 8 h | $Y_4 > 75\%$ | |

*Dexketoprofen trometamol amount = 0.333 g

Table 1: The independent and dependent variables of the D-optimal design for the formulation of Dexketoprofen trometamol microspheres (DTM).

| Code | X1: Polymer amount (gm) | X2: Hydrophobic plasticizer (%) | X3: Hydrophilic plasticizer (%) | X4: Stirring rate (rpm) | Y1: % Entrapment efficiency | Y2: % DT released after 1 hr | Y3: % DT released after 4 hr | Y4: % DT released after 8 hr |
|--------|-------------------------|---------------------------------|---------------------------------|-------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|
| DTM-1 | 1.665 | 0 | 5 | 750 | 85.50±0.71 | 35.90±0.42 | 61.05±1.06 | 81.15±1.20 |
| DTM-2 | 1 | 5 | 0 | 750 | 86.50±0.71 | 35.40±0.71 | 61.60±1.98 | 73.55±0.64 |
| DTM-3 | 1.3325 | 5 | 5 | 450 | 82.50±0.71 | 29.54±0.65 | 58.25±0.63 | 74.69±0.87 |
| DTM-4 | 1.665 | 5 | 10 | 750 | 81.00±1.41 | 37.95±0.64 | 62.25±0.64 | 71.20±0.85 |
| DTM-5 | 1.665 | 10 | 0 | 750 | 85.75±1.06 | 22.80±0.28 | 42.65±0.64 | 54.85±0.92 |
| DTM-6 | 1 | 0 | 10 | 750 | 82.65±0.49 | 41.45±0.64 | 83.43±0.74 | 95.98±0.96 |
| DTM-7 | 1.665 | 0 | 10 | 450 | 77.65±0.49 | 28.65±0.49 | 62.95±0.64 | 83.85±0.78 |
| DTM-8 | 1 | 5 | 5 | 600 | 74.00±1.41 | 27.15±1.06 | 59.62±0.87 | 81.95±0.49 |
| DTM-9 | 1.49875 | 7.5 | 7.5 | 600 | 78.15±1.20 | 25.15±0.64 | 45.15±1.06 | 60.18±0.96 |
| DTM-10 | 1.665 | 5 | 0 | 450 | 88.80±0.28 | 27.34±0.62 | 56.45±1.06 | 65.35±0.92 |
| DTM-11 | 1.3325 | 0 | 5 | 600 | 88.85±0.21 | 43.15±0.92 | 74.60±1.27 | 83.50±2.12 |
| DTM-12 | 1.3325 | 0 | 0 | 750 | 93.40±0.85 | 40.30±0.99 | 71.75±0.35 | 81.95±0.64 |
| DTM-13 | 1.665 | 0 | 0 | 600 | 92.80±1.13 | 28.05±1.06 | 54.15±1.20 | 64.50±0.71 |
| DTM-14 | 1.3325 | 5 | 5 | 750 | 75.50±0.71 | 28.35±0.64 | 51.99±1.11 | 71.14±0.91 |
| DTM-15 | 1 | 5 | 10 | 450 | 72.00±1.41 | 34.65±0.92 | 61.45±1.48 | 80.65±0.49 |
| DTM-16 | 1 | 10 | 10 | 750 | 75.10±1.27 | 34.00±0.28 | 60.00±1.27 | 80.25±1.05 |
| DTM-17 | 1 | 0 | 0 | 450 | 93.50±0.71 | 40.25±0.92 | 72.20±1.13 | 82.65±0.92 |
| DTM-18 | 1.3325 | 5 | 0 | 600 | 87.50±0.71 | 26.24±0.76 | 52.03±0.75 | 67.45±2.19 |
| DTM-19 | 1.665 | 10 | 10 | 450 | 77.00±0.01 | 35.15±0.92 | 57.99±1.26 | 72.75±1.06 |

| | | | | | | | | |
|--------|-------|----|----|-----|------------|------------|------------|------------|
| DTM-20 | 1 | 10 | 10 | 750 | 73.60±0.57 | 34.70±0.85 | 60.45±0.78 | 81.40±0.85 |
| DTM-21 | 1.665 | 10 | 0 | 750 | 84.50±0.71 | 21.30±0.85 | 38.45±0.78 | 50.65±0.92 |
| DTM-22 | 1 | 10 | 0 | 450 | 86.50±0.71 | 34.25±0.64 | 59.00±1.13 | 71.50±0.71 |
| DTM-23 | 1 | 0 | 10 | 750 | 86.05±0.07 | 41.75±0.64 | 78.45±0.64 | 89.23±0.75 |
| DTM-24 | 1 | 0 | 0 | 450 | 87.50±0.71 | 40.55±0.64 | 72.60±0.99 | 84.99±1.26 |
| DTM-25 | 1 | 10 | 0 | 450 | 83.50±0.71 | 32.15±0.21 | 55.35±0.78 | 66.30±0.42 |

Table 2: The formulations of D-Optimal design and their characterization results.

Preparation of Microspheres

DT microspheres (DTM) were prepared by emulsification solvent evaporation technique [12]. Different amounts of Eudragit RS 100 polymer were dissolved in 20 ml of acetone by using a magnetic stirrer. For formulations containing plasticizers, different amounts of Dibutyl phthalate and /or polyethylene glycol 400 were added to the polymeric solution and stirred. Powdered DT (0.333 g) was dissolved in 5 ml methanol and added to the prepared polymeric solution. Magnesium stearate (0.2 g) was dispersed in the polymer solution. The resulting dispersion was then poured into a 250 mL vessel containing a mixture of 90 ml liquid paraffin and 10 ml n-hexane while stirring with a mechanical stirrer with four blades (VELP Scientifica, Italy). Stirring was continued for four h till complete evaporation of acetone and methanol. The formed microspheres were collected by filtration in vacuum, washed 3 times with 50 ml n-hexane each and dried at room temperature for 24 h. All microsphere formulae were prepared in triplicates.

Characterization of Microspheres

Determination of production yield: The formulated microspheres were recovered and weighed accurately. The yield of microspheres was determined by comparing the whole weight of the formed microspheres with the combined weight of the starting materials using the equation below:

% Yield of microspheres = Total weight of the formed microspheres / Total weight of the starting materials

Determination of the mean particle size: The particle size of 100 microspheres was measured by optical microscope with a digital camera (JVC, Victor, Yokohama, Japan), and the mean particle size of 100 microspheres was determined.

Determination of Flowability: The bulk density of the microspheres was calculated by dividing the weight of 50 g powder by the volume (in cm³) of the powder in a 100-ml graduated cylinder [13]. The tapped density was determined dividing the weight (in g) by the volume (in cm³) obtained after tapping of the powder within the

graduated cylinder until constant volume. The bulk, and tapped densities were measured to evaluate the flowability of microspheres. Hausner's ratio [14] and Carr's index [15] were estimated from equations below:
Hausner's ratio = ρ_t / ρ_o . Where, ρ_t = Tapped density, ρ_o = Bulk density

Compressibility Index (C.I.) % = $(\rho_t - \rho_o) / \rho_t \times 100$

Determination of entrapment efficiency (EE %) (Y1):

Methanol was selected as an appropriate solvent for the lysis of the prepared microspheres [17]. Total amount of the drug (drug content) of the prepared formulations was determined by dissolving 50 mg of the prepared DTM in methanol and then measuring the UV absorbance using spectrophotometer at the predetermined λ_{max} of DT in methanol after performing the necessary dilution. The entrapment efficiency (EE %) was calculated as follows:

EE (%) = QP/QT X 100, Where QP is the quantity of drug entrapped in the microspheres, and QT is the initial quantity of drug added.

In vitro release of dexketoprofen trometamol from MUDF:

The drug dissolution was determined using USP standard dissolution tester, Apparatus II. An accurately weighed sample of DTM equivalent to 73.8 mg of DT was filled into hard gelatin capsules (HGC) size 00 forming MUDF and then were added to 900 mL dissolution medium phosphate buffer, pH 6.8 kept at $37 \pm 0.5^\circ\text{C}$. At different time intervals (0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, and 16 h), 4 mL aliquots were withdrawn and replaced by an equal volume of dissolution medium to maintain constant volume. After suitable dilution, the samples were analyzed spectrophotometrically at λ_{max} 260 nm. All measurements were conducted in triplicates. The amounts of DT released at 1h (Y₂), 4h (Y₃), and 8h (Y₄) were compared between the different formulations prepared.

Kinetic Analysis of the in vitro Drug-release Data: The mean in vitro drug release data were fitted to different kinetic models: zero order [17], Higuchi [18], and Korsmeyer-Peppas [19] to evaluate the kinetics of drug release from the prepared microspheres. The highest value of the coefficient of determination (R²) indicated a

superiority of the dissolution profile fitting to a particular dissolution model.

Formulation Optimization of DTM

The aim of the optimization was the preparation of DT microspheres (DTM opt) that has high EE % ($Y_1 > 70\%$), release less than 40% of DT content after 1 h corresponding to the loading dose ($Y_2 < 40\%$), release ~50% of the dose after 4 h ($40 < Y_3 < 60\%$), and finally release more than 75% of the dose after 8 h ($Y_4 > 75\%$). Desirability was calculated and considered to optimize the studied responses depending on the provided results. The significant responses were taken into considerations while the non-significant factors were not. The DT microsphere formula with the highest desirability value (close to 1) was subjected to further investigation. The optimized formula was prepared and characterized using the previously mentioned tests.

Scanning Electron Microscopy (SEM): The surface properties of the optimized microsphere formula were examined by the scanning electron microscope using (Jeol JSM-6400, Tokyo, Japan). The microcapsules were fixed on a sample holder, and coated with gold palladium using sputter coater for 1min under argon gas before electron microscopic scanning.

Differential Scanning Calorimetry (DSC): The thermal behavior of DT, Eudragit RS100, optimized microspheres formula, and its corresponding blank formula were traced by differential scanning calorimetry (Shimadzu differential scanning calorimeter, DSC TA-50 ESI, Koyoto, Japan), where one mg of each sample was hermetically sealed in a flat-bottomed aluminum pans and were heated over the temperature range of 25 to 350°C under a nitrogen purge (20 mL/min) at a constant heating rate of 10°C/min.

Powder X-ray Diffraction (XRD): XRD patterns of DT, Eudragit RS100, optimized microspheres formula, and its corresponding blank formula were measured on a Philips PW/170 diffractometer with a Cu-filtered radiation ($\lambda = 1.542 \text{ \AA}$). The patterns were recorded on a quartz plate at a tube voltage of 40 kV and a current of 35 mA°, applying a scan rate of 0.02° 2 θ /s in the angular range of 3-80°2 θ .

Stability Study of the Optimized MUDF: The optimized MUDF was placed in HDPE bottle with HDPE cap and packed into a stability chamber. An accelerated stability

study was conducted as per ICH guidelines at 40°C/75% relative humidity. Samples were withdrawn after three months of storage and were evaluated for drug content and dissolution.

Results and Discussion

Saturated Solubility of DT

Saturated solubility studies have shown that DT has pH-dependent solubility where the highest solubility was observed in the alkaline environment ($892.38 \pm 34.45 \text{ mg/mL}$ at pH 6.8). The solubility of DT in distilled water and 0.1 N HCl were 662.37 ± 21.25 and $0.226 \pm 0.008 \text{ mg/mL}$, respectively. The pH dependent solubility is attributed to the acid nature of the drug being propionic acid derivative ($pK_a = 4.55$), where the drug molecules are unionized at low pH values and ionized at higher pH values [20,21]. It is worth mentioning that the solubility of DT at pH 1.2 is still high enough to retain a sink condition in the dissolution media of 900 mL of 0.1 N HCl. Therefore, solubility is not a limiting factor for the release of DT even at pH 1.2 which indicates the possibility of dissolution and absorption of DT in the stomach.

In vitro Dissolution of Pure Drug

The *in vitro* dissolution of DT in phosphate buffer (pH 6.8) was very rapid where 95% of the drug was dissolved in 10 min and 100% of DT was dissolved in 15 min. This is due to the previously mentioned high solubility of the DT in phosphate buffer (pH 6.8). The very rapid dissolution of DT indicated that there was a challenge for the preparation of sustained release formulation that extends the release of the drug for a period of 16 h.

Production Yield of DTM

The preparation of matrix type microspheres of DT (DTM) was achieved using emulsification solvent evaporation technique. The yield for the prepared DT microspheres was generally high ranging from $70.95 \pm 0.77\%$ to $90.97 \pm 0.52\%$ as shown in table (3). The high percentage yield of most microspheres formulae indicates that the procedures and parameters employed for the preparation of DTM were effective and efficient. These results have shown that Eudragit RS 100 is a good polymer for the preparation of microsphere formulation containing a hydrophilic drug by the emulsification solvent evaporation technique which was also reported by Sengel Turk, et al. [22].

| Formula Number | Production Yield | Particle Size | Carr's index | Hausner's ratio |
|----------------|------------------|-------------------|------------------|-----------------|
| | (%) | (μm) | | |
| DTM-1 | 80.65 \pm 0.49 | 34.26 \pm 2.88 | 15.46 \pm 2.21 | 1.18 \pm 0.02 |
| DTM-2 | 72.95 \pm 0.78 | 24.80 \pm 3.41 | 15.03 \pm 1.24 | 1.18 \pm 0.05 |
| DTM-3 | 71.35 \pm 0.49 | 43.89 \pm 9.49 | 13.71 \pm 1.19 | 1.16 \pm 0.03 |
| DTM-4 | 73.25 \pm 0.35 | 25.52 \pm 5.44 | 12.17 \pm 0.38 | 1.14 \pm 0.01 |
| DTM-5 | 77.20 \pm 0.28 | 38.99 \pm 9.92 | 15.91 \pm 2.31 | 1.19 \pm 0.04 |
| DTM-6 | 83.80 \pm 0.57 | 27.05 \pm 4.04 | 13.53 \pm 0.66 | 1.16 \pm 0.02 |
| DTM-7 | 87.65 \pm 0.49 | 46.65 \pm 4.51 | 16.72 \pm 2.71 | 1.2 \pm 0.05 |
| DTM-8 | 83.30 \pm 0.71 | 36.36 \pm 5.07 | 10.60 \pm 3.85 | 1.12 \pm 0.01 |
| DTM-9 | 84.32 \pm 0.46 | 53.28 \pm 14.00 | 15.24 \pm 0.21 | 1.18 \pm 0.03 |
| DTM-10 | 82.67 \pm 0.18 | 48.63 \pm 7.31 | 14.16 \pm 0.18 | 1.16 \pm 0.02 |
| DTM-11 | 85.25 \pm 3.89 | 36.22 \pm 8.36 | 17.22 \pm 0.12 | 1.21 \pm 0.04 |
| DTM-12 | 78.30 \pm 0.85 | 35.33 \pm 7.31 | 17.39 \pm 0.21 | 1.21 \pm 0.03 |
| DTM-13 | 76.60 \pm 0.85 | 36.69 \pm 3.1 | 11.92 \pm 3.24 | 1.14 \pm 0.05 |
| DTM-14 | 77.65 \pm 0.92 | 45.13 \pm 7.81 | 17.21 \pm 1.39 | 1.21 \pm 0.02 |
| DTM-15 | 86.60 \pm 0.85 | 38.18 \pm 7.52 | 14.77 \pm 0.64 | 1.17 \pm 0.01 |
| DTM-16 | 90.97 \pm 0.52 | 37.47 \pm 6.93 | 13.26 \pm 5.66 | 1.16 \pm 0.04 |
| DTM-17 | 81.85 \pm 0.49 | 25.31 \pm 6.87 | 14.94 \pm 2.45 | 1.18 \pm 0.02 |
| DTM-18 | 76.20 \pm 0.85 | 29.58 \pm 6.12 | 14.02 \pm 1.05 | 1.16 \pm 0.01 |
| DTM-19 | 87.50 \pm 0.42 | 49.55 \pm 10.42 | 16.47 \pm 1.95 | 1.21 \pm 0.03 |
| DTM-20 | 80.70 \pm 0.99 | 36.41 \pm 7.08 | 13.95 \pm 3.85 | 1.16 \pm 0.05 |
| DTM-21 | 85.05 \pm 0.64 | 35.24 \pm 13.48 | 14.47 \pm 0.06 | 1.17 \pm 0.02 |
| DTM-22 | 78.70 \pm 0.57 | 23.80 \pm 4.30 | 19.45 \pm 0.27 | 1.24 \pm 0.01 |
| DTM-23 | 79.90 \pm 0.42 | 26.60 \pm 2.85 | 15.95 \pm 2.95 | 1.19 \pm 0.04 |
| DTM-24 | 70.95 \pm 0.78 | 31.34 \pm 2.64 | 16.82 \pm 0.22 | 1.20 \pm 0.01 |
| DTM-25 | 71.95 \pm 0.35 | 36.69 \pm 9.09 | 13.9 \pm 3.02 | 1.16 \pm 0.03 |

Table 3: Yield, micromeritics and Flowability characteristics of dexketoprofen trometamol microspheres (DTM).

Particle Size Analysis

The particle size for the prepared microspheres ranged from 23.804 \pm 4.299 μm to 53.275 \pm 13.995 μm as shown in table (3).

Flow Properties

Micromeritics of the 25 DTM formulae are shown in table 3. The bulk densities of DT microspheres ranged from 0.383 \pm 0.009 g/cm^3 to 0.526 \pm 0.005 g/cm^3 . The tapped densities ranged from 0.45 \pm 0.014 g/cm^3 to 0.61 \pm 0.007 g/cm^3 . Carr's index and Hausner's ratio for all microspheres formulae were <20 and \leq 1.25, respectively,

indicating that the microspheres show good flow properties [8,23].

Statistical Analysis of the Experimental Design

The D-optimal design was applied in this study in order to obtain the optimum DTM formula. All the responses observed for the 25 formulae prepared were fitted to various models using design-expert software. It was observed that the best-fit model was the linear model for all responses because its PRESS was the smallest. The results of regression analysis for the responses are summarized in table 4.

| Response | Model | R ² | Adjusted R ² | Equation |
|----------------|--------|----------------|-------------------------|--|
| Y ₁ | Linear | 0.7235 | 0.6682 | Y ₁ = 82.2550 + 0.9843 X ₁ - 3.6801 X ₂ - 5.2478 X ₃ + 0.5224 X ₄ |
| Y ₂ | Linear | 0.5345 | 0.4414 | Y ₂ = 32.1551 - 3.2827 X ₁ - 3.8465 X ₂ + 1.6399 X ₃ + 0.2226 X ₄ |
| Y ₃ | Linear | 0.7751 | 0.7301 | Y ₃ = 59.1546 - 5.7673 X ₁ - 8.8381 X ₂ + 3.1774 X ₃ - 0.3987 X ₄ |
| Y ₄ | Linear | 0.8664 | 0.8396 | Y ₄ = 73.7170 - 6.3954 X ₁ - 7.7941 X ₂ + 5.5154 X ₃ - 0.5592 X ₄ |

Table 4: Reduced regression equations in term of coded factor of the measured responses.

The values of the coefficients X₁, X₂ and X₃, X₄ are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative sign indicates an antagonistic effect upon the response [24]. The larger coefficient means that the independent variable has more potent influence on the response. ANOVA was applied to estimate the significance of the linear model at the 5% level. 3D response surface plots were used to study the interaction effects of two factors on the response at a time, when the third and the fourth factors are kept at a constant level [25].

Effect of formulation and process variables on entrapment efficiency % (Y₁): It was observed that the entrapment efficiency for all DT microspheres formulae ranged from 72 ± 1.41% to 93.50 ± 0.71% as shown in table 2. The observed high entrapment efficiency of the

drug in all prepared microspheres may be due to the poor solubility of the drug in liquid paraffin, which minimized leakage of the drug into the external hydrophobic phase during the emulsification process, and thus resulted in high entrapment efficiency. It can be inferred that the terms X₂ and X₃ have significant antagonistic effect on the percentage entrapment efficiency (p<0.05), as confirmed by the negative values of X₂ and X₃ coefficients.

Figure 1 illustrates the 3D response surface plot showing the effect of factors X₂ and X₃ on the drug entrapment efficiency (Y₁). As shown in figure 1, the entrapment efficiency was significantly decreased as the amount of both plasticizers increased. The decrease in the entrapment efficiency may be due to the enhancement of drug solubility in liquid paraffin by plasticizers.

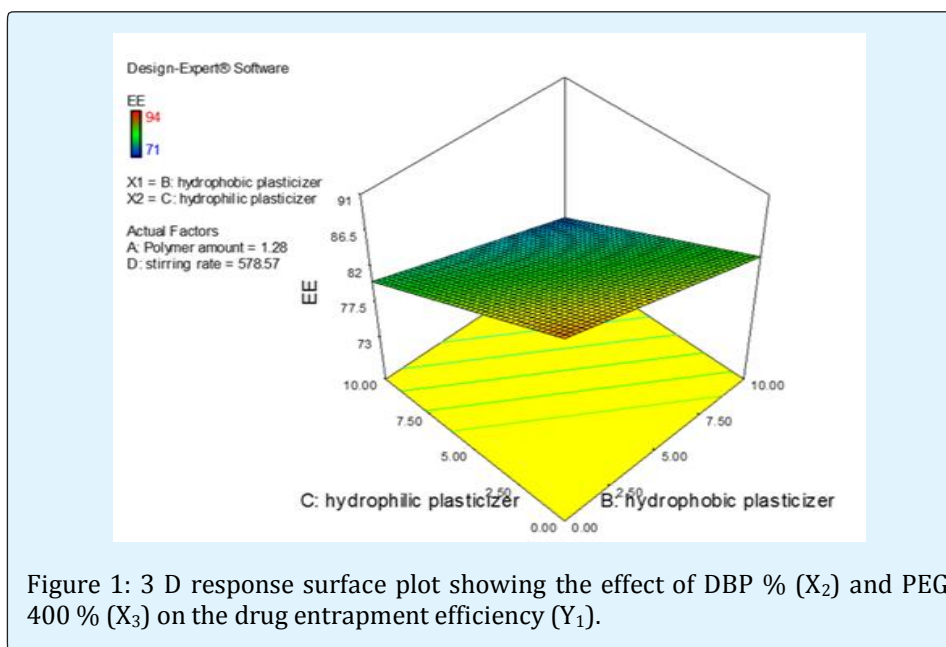


Figure 1: 3 D response surface plot showing the effect of DBP % (X₂) and PEG 400 % (X₃) on the drug entrapment efficiency (Y₁).

Effect of formulation and process variables on in vitro release of DT from DTM: Plasticizers are generally used to improve the mechanical properties of a polymer matrix [26]. In addition, plasticizers are used also to obtain polymeric systems with controlled drug permeation [27]. The release of DT was studied as a function of plasticizer

type. It was noticed that the release rate was affected with the chemical nature of plasticizer.

As shown in figure 2a, complete release was noticed for PEG 400-containing microspheres, where all formulae released 100% after 16 hours. A high burst release was

observed at 1 h, as the amount of DT released ranged from $28.65 \pm 0.49\%$ to $43.15 \pm 0.92\%$. The amount of DT released ranged from $61.05 \pm 1.06\%$ to $83.43 \pm 0.74\%$ and from $81.15 \pm 1.20\%$ to $95.98 \pm 0.96\%$, at 4 and 8 h, respectively. The rapid release profiles for formulae containing PEG 400 only as a plasticizer could be attributed to the hydrophilic and leaching property of PEG, which increased the magnitude of the voids and consequently the channels from the microsphere surface, resulting in the formation of a heavily structured microporous matrix [28]. Other investigators already described a similar effect of hydrophilic nature of the plasticizer on the drug release mechanism [29].

On comparing different microspheres formulae containing PEG 400, it was noticed that DTM-1 showed the slowest release profile, while DTM-6 showed the fastest release profile. The slower release profile for DTM-1 is due to the high polymer amount (1.655 g) and the low amount of PEG 400 (5%), in comparison to low polymer amount (1 g) and high amount of PEG400 (10%) in DTM-6, while keeping the stirring speed constant in both formulae (750 rpm).

The *in vitro* release profile of DT from DTM containing DBP is shown in Figure 2b. Not all DBP-containing microspheres showed complete drug release after 16 hours (DTM-5, DTM-18, DTM-21 and DTM-25). A low burst release was observed after 1 h as the amount of DT released ranged from $21.30 \pm 0.84\%$ to $35.40 \pm 0.71\%$. The amount of DT released ranged from $38.45 \pm 0.77\%$ to $61.60 \pm 1.98\%$ and from $50.65 \pm 0.91\%$ to $73.55 \pm 0.64\%$ at 4 and 8 h, respectively.

By comparing different microspheres formulae containing DBP, it was noticed that DTM-21 showed the slowest release profile, while DTM-2 showed the fastest release profile. The slower release profile for DTM-21 is due to the high polymer amount (1.655 g) and the high amount of DBP (10%), in comparison to low polymer amount (1 g) and low amount of DBP (5%) in DTM-2, while keeping the stirring speed constant in both formulae (750 rpm). The slower release rate observed by formulae containing DBP as the only plasticizer, as compared to those containing PEG 400 as the only plasticizer, could be related to the hydrophobicity of DBP compared to the hydrophilic character of PEG 400. This

was in good agreement with Siepmann, et al. [30] who reported that plasticizers with phthalate groups should be chosen when a more prolonged release is desired.

Figure 2c illustrates the release profiles of DT from DTM containing combinations of PEG 400 and DBP plasticizers, which shows that a moderate burst release was observed after 1 h as the amount of DT ranged from $25.15 \pm 0.64\%$ to $37.95 \pm 0.64\%$. The amount of DT released ranged from $45.15 \pm 1.06\%$ to $62.25 \pm 0.64\%$ and from $60.18 \pm 0.96\%$ to $81.95 \pm 0.49\%$ at 4 and 8 h, respectively. Finally all formulae released almost 100% after 16 h except DTM-9. The moderate release profiles for formulae containing combinations of PEG 400 and DBP plasticizers could be attributed to the antagonistic effect of the hydrophilic and leaching property of PEG, which increased the magnitude of the voids and consequently the channels from the microsphere surface, and the hydrophobicity of DBP which slowed the release of the hydrophilic drug [28].

By comparing different microspheres formulae containing both PEG 400 and DBP plasticizers, it was noticed that DTM-9 showed the slowest release profile, while DTM-4 showed the fastest release profile. The slower release profile for DTM-9 may be due to the low stirring speed during preparation of microspheres (600 rpm) which resulted into larger particle size and smaller surface area exposed to dissolution medium. Also DTM-9 has a lower amount of PEG than DTM-4 (7.5 % versus 10 %, respectively).

Figure 2d illustrates the release profiles of DT from DTM containing no plasticizer. The microspheres prepared without the use of plasticizers exhibit moderate burst release after one hour which ranged from $28.05 \pm 1.06\%$ to $40.55 \pm 0.64\%$. The amount of DT released ranged from $54.15 \pm 1.20\%$ to $72.60 \pm 0.99\%$ and from $64.50 \pm 0.71\%$ to $84.99 \pm 1.26\%$ at 4 and 8 h, respectively. Finally all formulae released almost 100% after 16 h except DTM-13. By comparing different microspheres formulae containing no plasticizers, it was noticed that DTM-13 showed the slowest release profile, while DTM-24 showed the fastest release profile. The slower release profile for DTM-13 is due to high polymer amount (1.655 g) as compared to (1 g) in DTM-24.

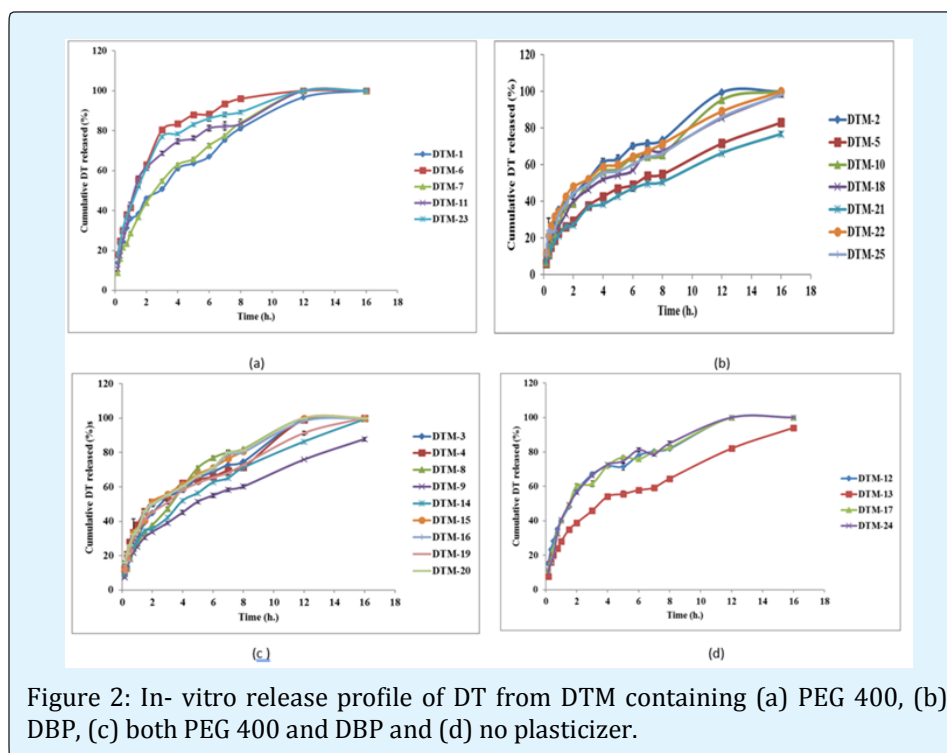


Figure 2: In- vitro release profile of DT from DTM containing (a) PEG 400, (b) DBP, (c) both PEG 400 and DBP and (d) no plasticizer.

From table 4, it can be inferred that the terms X_1 and X_2 have significant antagonistic effect on the amount of DT released after 1 h, 4 h, and 8 h ($p < 0.05$). X_3 had a significant synergistic effect on the amount of DT released after only 4 h, and 8 h ($p < 0.05$).

Figure 3a-e shows the effect of X_1 , X_2 and X_3 on the amount of DT released after 1 h, 4 h, and 8 h. It can be inferred that the amount of drug released was significantly increased with decreasing the amount of the polymer. This could be due to the formation of a short diffusion path, and hence faster drugs release [31]. It is also clear from figure 3, which the amount of drug released at 1, 4, 8 h decreased significantly with increasing the % of the hydrophobic plasticizer ($p < 0.05$). This was in accordance with Lecomte, et al. [32] who compared a hydrophobic (dibutyl sebacate) and hydrophilic (triethyl citrate) plasticizers and reported that the hydrophilic plasticizer, triethyl citrate rapidly leached from a polymer coat affecting film permeability and drug diffusion. In contrast the hydrophobic plasticizer, dibutyl sebacate remained in the polymer layer, reducing the drug release rate. Figure 3c and 3e shows the amount of drug released at 4 h and 8 h that was increased significantly with decreasing the amount of the hydrophilic plasticizer ($p < 0.05$). This could be attributed to the hydrophilic and the leaching property of PEG400, which increased the magnitude of the voids and consequently the channels from the microsphere surface,

resulting in the formation of a heavily structured-microporous matrix [28].

In vitro Drug-Release Kinetics and Mechanism

The kinetic analysis of the in-vitro drug-release data from the 25 DTM formulae were evaluated using the various mathematical models. According to the determination coefficient (R^2), the in vitro release data were in favor of Higuchi release kinetics (for formulae DTM 1, DTM 2, DTM 3, DTM 5, DTM 7, DTM 9, DTM 10, DTM 13, DTM 14, DTM 18, DTM 19, DTM 21, and DTM 22). Eudragit RS 100 does not dissolve at physiological pH values, instead, it becomes permeable to water and form a gel diffusion layer in the microsphere matrix structure when in contact with dissolution medium [7].

The in vitro release data were in favor of Korsmeyer-peppas release kinetics (for formulae DTM 4, DTM 6, DTM 8, DTM 11, DTM 12, DTM 15, DTM 16, DTM 17, DTM 20, DTM 23, DTM 24, and DTM 25). The values of $n > 0.43$ and < 0.85 indicate non-fickian (anomalous) transport for most formulae which means that the drug release mechanism was mainly due to the combination of diffusion of drug through the polymeric matrix and polymer erosion of the Eudragit RS100 microspheres. This is in accordance with the findings of Alai and Lin, 2013 [33]. The values of $n \leq 0.43$ for formulae DTM-16 and DTM-20 indicate a fickian release mechanism.

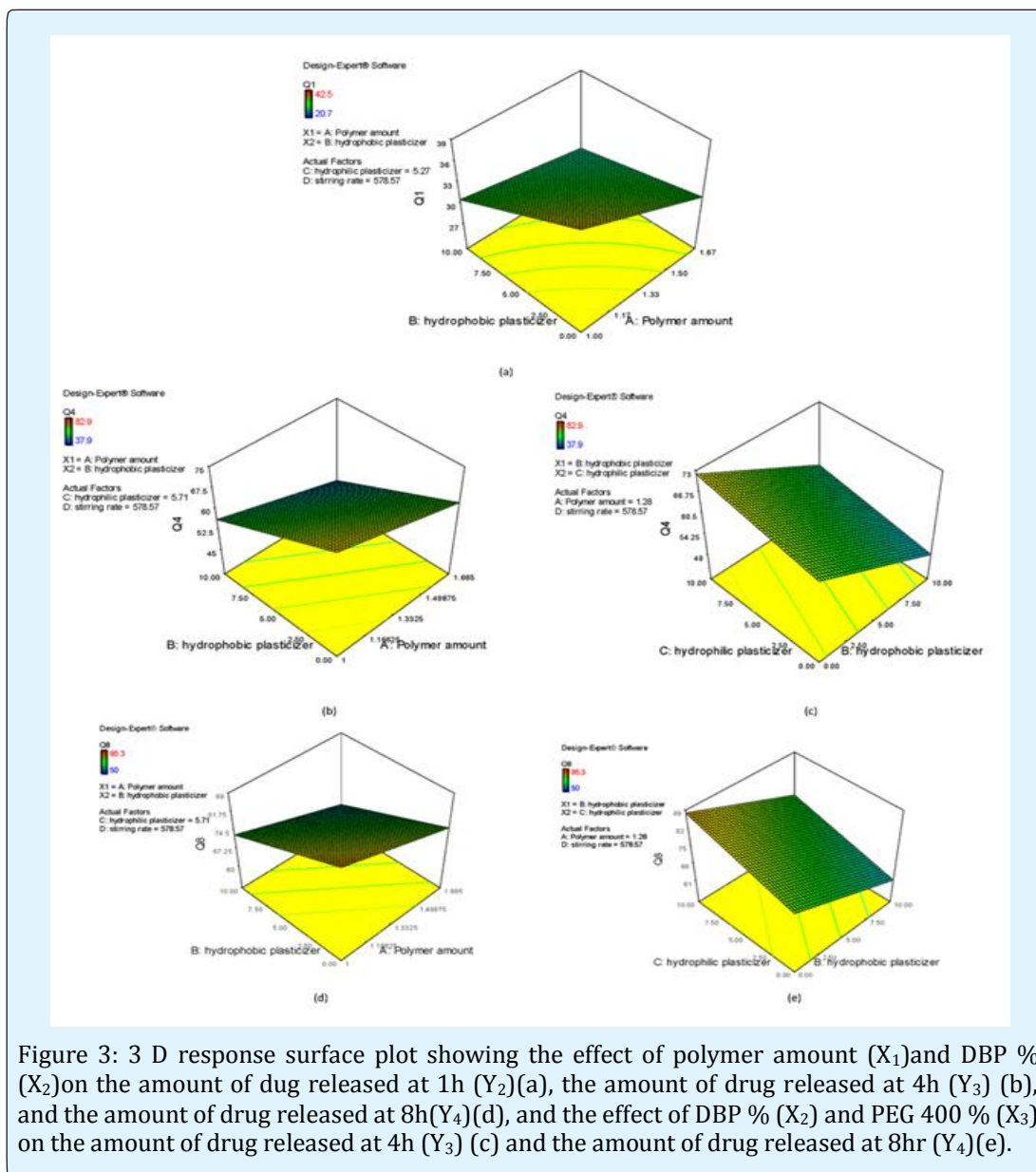


Figure 3: 3 D response surface plot showing the effect of polymer amount (X_1) and DBP % (X_2) on the amount of drug released at 1h (Y_2) (a), the amount of drug released at 4h (Y_3) (b), and the amount of drug released at 8h (Y_4) (d), and the effect of DBP % (X_2) and PEG 400 % (X_3) on the amount of drug released at 4h (Y_3) (c) and the amount of drug released at 8hr (Y_4) (e).

Optimization of DTM and Preparation of MUDF

The aim of the optimization of pharmaceutical formulations is to determine the levels of variables required to produce a high quality product. In the present study, desirability was calculated by Design-Expert® software and considered to optimize the studied responses depending on the provided results. The entrapment efficiency and the total amount of drug released are the most important factors regarding the properties of microspheres. The optimum formulation (optimum for the oral day delivery of DT) was selected to have high EE ($Y_1 > 70\%$), release less than 40% of DT content after 1 h corresponding to the loading dose

($Y_2 < 40\%$), release ~50% of the dose after 4h ($40 < Y_3 < 60\%$), and finally release more than 75% of the dose after 8 h ($Y_4 > 75$). The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert software and based on the criterion of desirability [34]. Therefore, the optimized formula of DTM with the predicted levels of formulation and process variables were prepared to confirm the validity of the optimization procedure. The composition of the optimized formula was 1.39 g, 0.063 g, 0.093 g and 683 rpm for X_1 , X_2 , X_3 and X_4 , respectively. The observed responses were 76.0%, 33.7%, 60.2 and 75.1% for Y_1 , Y_2 , Y_3 and Y_4 , respectively.

The optimized DTM was prepared and filled into HGC forming MUDF. The in vitro release profile of DT from MUDF was performed in phosphate buffer pH 6.8 compared to DT powder. The optimized DTM showed a high initial burst release where $33.7 \pm 0.82\%$ of DT was

released after 1 h which corresponds to the initial loading dose (Figure 4). The amount of DT released at 4, 8 and 16 h were $60.2 \pm 0.05\%$, $75.1 \pm 1.84\%$, and 100% , respectively. However, DT powder showed complete drug release in 15 min.

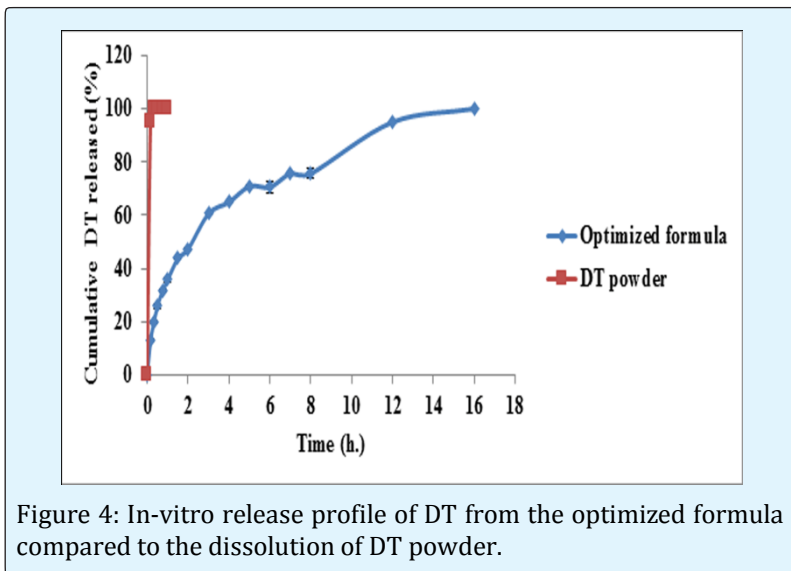


Figure 4: In-vitro release profile of DT from the optimized formula compared to the dissolution of DT powder.

Kinetic analysis of the in vitro release data of the optimized formulation was performed using zero order, Higuchi and Korsmeyer-Peppas models. It was found that the in-vitro release data followed the Korsmeyer-Peppas model as it showed the highest value of R^2 (0.9905). The value of n was between 0.43 and 0.85, suggesting non-Fickian diffusion kinetics. Hence, it is concluded that the drug release mechanism was mainly due to the combination of diffusion of drug through the polymeric matrix and polymer erosion of the Eudragit RS100 microspheres [33].

Scanning Electron Microscopy (SEM)

The morphology of the optimized formulation and its corresponding blank microspheres formula were observed by scanning electron microscopy and the photomicrographs are shown in figures (5a,b), respectively. SEM studies showed that the microspheres were spherical with a rough surface. This is in accordance with the findings of [31] Kilicarslan & Baykara and [35] Joshi et al. No drug crystals were observed at the surface of the microspheres.

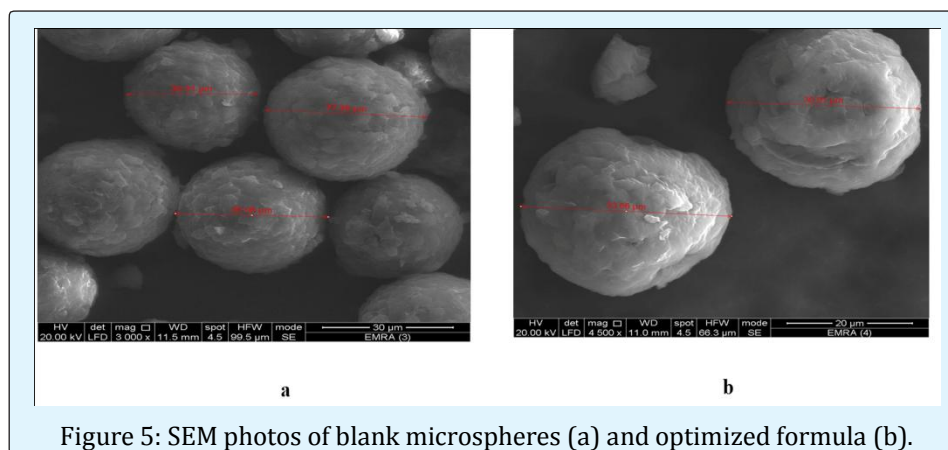


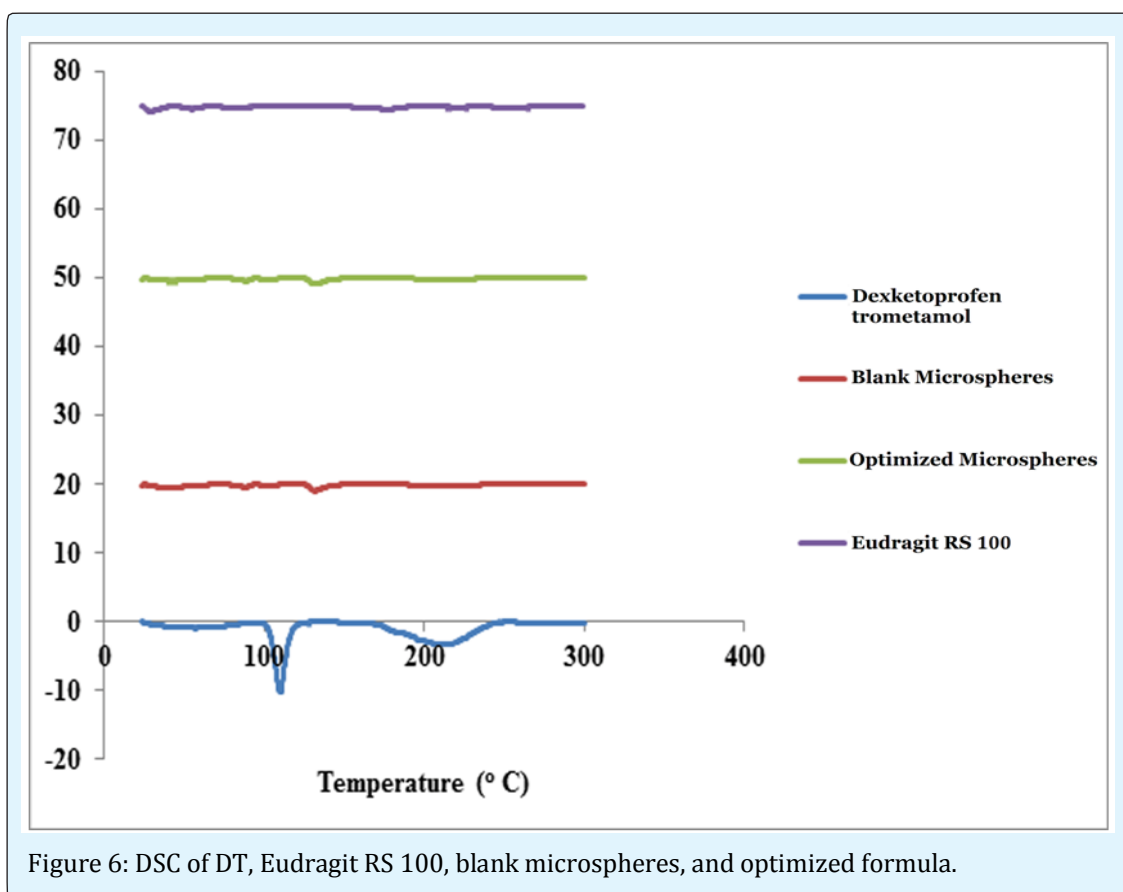
Figure 5: SEM photos of blank microspheres (a) and optimized formula (b).

Differential Scanning Calorimetry (DSC)

DSC was performed for the optimized formulation as well as for its corresponding blank, DT powder, and Eudragit RS100 in order to evaluate the phase transformation of DT during preparation of the microspheres.

As illustrated in figure 6, the DSC curve of pure drug was characterized by a sharp endothermic peak at 109°C,

corresponding to its melting point and indicating its crystalline nature [3]. The thermal curve of Eudragit® RS100 was typical of amorphous substances showing no distinct peak. DSC thermograms of the optimized formulation showed complete disappearance of the characteristic peak of the drug at 109°C, indicating that the drug was completely dispersed at the molecular level in the microspheres. It also indicates the conversion of DT from the crystalline form to the amorphous form.



Powder X-ray Diffraction (XRD)

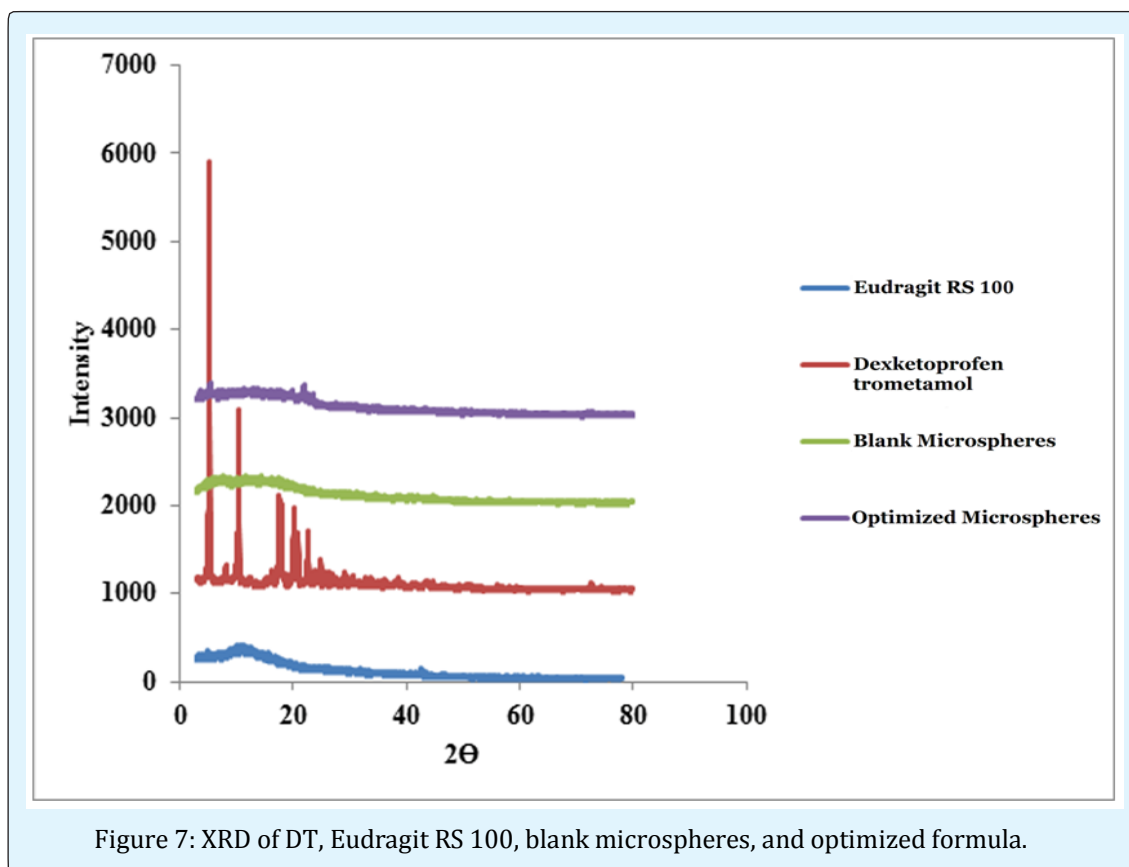
As indicated in figure 7, the diffraction spectrum of pure DT showed that the drug is crystalline in nature as demonstrated by numerous distinctive peaks. Eudragit RS100 diffraction pattern showed no characteristic peaks indicating that they are amorphous in nature [21]. The X-ray diffractogram of the optimized formulation was characterized by disappearance of the distinct diffraction peaks, signifying a drug amorphization or its dissolution in the amorphous polymer. X-Ray results demonstrated consistency with the findings obtained from DSC analysis.

Concisely, the DSC and XRD studies verified decrease of drug crystallinity in the microspheres.

Stability studies

The optimized formulation was subjected to accelerated stability study by storing at 40°C /75% RH for 3 months as per ICH guidelines. The results revealed that no considerable differences in drug content of the microspheres inside MUDF where the drug content was $98.20 \pm 0.22\%$. The in vitro release profiles of both stored

and fresh samples were comparable as indicated by the high similarity factor ($f_2 = 74$).



Conclusion

A sustained release MUDF of DT with satisfactory release characteristics was successfully prepared using the emulsification solvent evaporation technique. The novel multiple unit formulation composed of 1.39 g Eudragit, 0.063 g DBP, 0.093 g PEG 400 and processed using stirring rate of 683 rpm. The sustained release pattern of DT for up to 16 h with an acceptable initial release suggests that the developed MUDF may be useful for oral day delivery of the highly water soluble and frequently administered drug such as DT.

References

1. Moore RA, Barden J (2008) Systematic review of dexketoprofen in acute and chronic pain. *BMC Clin Pharmacol* 8(1): 1-11.
2. Mauleon D, Artigas R, Garcia ML, Carganico G (1996) Preclinical and clinical development of dexketoprofen. *Drugs* 52(5): 24-46.
3. Moffat AC, Osselton MD, Widdop B, Galichet LY (2004) Clarke's analysis of drugs and poisons. London Pharmaceutical Press.
4. Barbanoj MJ, Antonijoan RM, Gich I (2001) Clinical pharmacokinetics of dexketoprofen. *Clin pharmacokinet* 40(4): 245-262.
5. Laporte J-R, Ibanez L, Vidal X, Vendrell L, Leone R (2004) Upper gastrointestinal bleeding associated with the use of NSAIDs. *Drug saf* 27(6): 411-420.
6. Gupta VK, Beckert TE, Price JC (2001) A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. *Int J pharm* 213(1-2): 83-91.

7. Pignatello R, Consoli P, Puglisi G (2000) In vitro release kinetics of Tolmetin from tableted Eudragit microparticles. *J microencapsul* 17(3): 373-383.
8. El Say KM, El Helw AR, Ahmed OA, Hosny KM, Ahmed TA, et al. (2015) Statistical optimization of controlled release microspheres containing cetirizine hydrochloride as a model for water soluble drugs. *Pharm dev technol* 20(6): 738-746.
9. Soppimath K, Kulkarni A, Aminabhavi T (2001) Encapsulation of antihypertensive drugs in cellulose-based matrix microspheres: characterization and release kinetics of microspheres and tableted microspheres. *J microencapsul* 18(3): 397-409.
10. Basalious EB, Abdullah A, Ibrahim M (2014) Utility of Mannitol and Citric Acid for Enhancing the Solubilizing and Taste Masking Properties of β -Cyclodextrin: Development of Fast-Dissolving Tablets Containing Extremely Bitter Drug. *J Pharm Innov* 9(4): 309-320.
11. El-Setouhy DA, Basalious EB, Abdelmalak NS (2015) Bioenhanced sublingual tablet of drug with limited permeability using novel surfactant binder and microencapsulated polysorbate: In vitro/in vivo evaluation. *European Journal of Pharmaceutics and Biopharmaceutics* 94: 386-392.
12. Haznedar S, Dortunc B (2004) Preparation and in vitro evaluation of Eudragit microspheres containing acetazolamide. *International journal of pharmaceutics*.269(1): 131-140.
13. Martin A (1993) Physical pharmacy: physical chemical principles in the pharmaceutical sciences: BI Waverly. Pvt Ltd.
14. Hausner H (1967) Friction conditions in a mass of metal powder. Polytechnic Inst. of Brooklyn. Univ. of California, Los Angeles.
15. Carr RL (1965) Classifying flow properties of solids. *Chem Eng* 72: 163-168.
16. Comoglu T, Gonul N, Dogan A, Basci N (2008) Development and in vitro evaluation of pantoprazole-loaded microspheres. *Drug delivery* 15(5): 295-302.
17. Wagner JG (1969) Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. *J pharm sci* 58(10): 1253-1257.
18. Higuchi T (1963) Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J pharm sci* 52(12): 1145-1149.
19. Peppas N (1985) Analysis of Fickian and non-Fickian drug release from polymers. *Pharmaceutica acta Helvetiae* 60(4): 110-111.
20. Pai R, Kohli K, Jain G, Srivastava B (2011) In vitro and in vivo evaluations of ketoprofen extended release pellets prepared using powder layering technique in a rotary centrifugal granulator. *Arch Pharm Res* 34(7): 1135-1142.
21. El-Kamel A, Sokar M, Al Gamal S, Naggar V (2001) Preparation and evaluation of ketoprofen floating oral delivery system. *Int J pharm* 220(1-2): 13-21.
22. Sengel Turk CT, Hascicek C, Gonul N (2008) Microsphere-based once-daily modified release matrix tablets for oral administration in angina pectoris. *J Microencapsul* 25(4): 257-266.
23. Khamanga SM, Walker RB (2012) The use of response surface methodology in the evaluation of captopril microparticles manufactured using an oil in oil solvent evaporation technique. *J Microencapsul* 29(1): 39-53.
24. Huang YB, Tsai YH, Lee SH, Chang JS, Wu PC (2005) Optimization of pH-independent release of nifedipine hydrochloride extended-release matrix tablets using response surface methodology. *Int J pharm* 289(1): 87-95.
25. Woitiski CB, Veiga F, Ribeiro A, Neufeld R (2009) Design for optimization of nanoparticles integrating biomaterials for orally dosed insulin. *Eur J pharm biopharm* 73(1): 25-33.
26. Sadeghi F, Hijazi H, Garekani HA (2011) Production of ibuprofen pellets containing high amount of rate retarding Eudragit RL using PEG400 and investigation of their physicochemical properties. *Iran J of basic med sci* 14(4): 383-390.
27. Meier MM, Kanis LA, Soldi V (2004) Characterization and drug-permeation profiles of microporous and dense cellulose acetate membranes: influence of plasticizer and pore forming agent. *Int J Pharm* 278(1): 99-110.
28. Avachat AM, Bornare PN, Dash RR (2011) Sustained release microspheres of ropinirole hydrochloride:

- effect of process parameters. *Acta pharm* 61(4): 363-376.
29. Frohoff-Hülsmann MA, Schmitz A, Lippold BC (1999) Aqueous ethyl cellulose dispersions containing plasticizers of different water solubility and hydroxypropyl methylcellulose as coating material for diffusion pellets: I. Drug release rates from coated pellets. *Int J pharm* 177(1): 69-82.
 30. Siepmann J, Lecomte F, Bodmeier R (1999) Diffusion-controlled drug delivery systems: calculation of the required composition to achieve desired release profiles. *J Control Release* 60(2-3): 379-389.
 31. Kilicarslan M, Baykara T (2003) The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. *Int J pharm* 252(1-2): 99-109.
 32. Lecomte F, Siepmann J, Walther M, MacRae R, Bodmeier R (2004) Polymer blends used for the aqueous coating of solid dosage forms: importance of the type of plasticizer. *J Control Release* 99(1): 1-13.
 33. Alai M, Lin WJ (2013) A novel once daily microparticulate dosage form comprising lansoprazole to prevent nocturnal acid breakthrough in the case of gastro-esophageal reflux disease: preparation, pharmacokinetic and pharmacodynamic evaluation. *J Microencapsul* 30(6): 519-529.
 34. Basalious EB, Shawky N, Badr-Eldin SM (2010) SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: development and optimization. *Int J pharm* 391(1): 203-211.
 35. Joshi AS, Patil CC, Shiralashetti SS, Kalyane NV (2013) Design, characterization and evaluation of Eudragit microspheres containing glipizide. *Drug Invention Today* 5(3): 229-234.