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Formulation Study for Enteric Microspheres of Tenoxicam Using Cellulose Acetate Phthalate Part-II: Modulation of Ulcerogenic Effect¶

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One of the most significant adverse effects of oral dosage forms is their gastric irritancy. This problem can be solved by enteric coating. In this project, cellulose acetate phthalate, a polymer usually utilized for gastrointestinal film coating of tablets, was used to prepare enteric microspheres. Tenoxicam, an antiinflammatory drug causing irritation on stomach mucosa on oral administration, was selected as a model drug. Cellulose acetate phthalate microspheres of tenoxicam were prepared with solvent evaporation technique. After preformulation studies with differential thermal analysis and thermal gravimetry in the first part of this project, *in vitro* dissolution studies and *in vivo* investigations testing the gastric irritancy and antiinflammatory effect of microspheres were carried out in this part. Data indicate that enteric microspheres reduced the gastric irritancy of the pure drug. Microsphere formulation didn't influence the therapeutic efficacy (antiinflammatory activity) of tenoxicam. The data obtained from *in vitro* dissolution studies revealed the controlled release behaviour of tenoxicam from the microspheres in intestinal medium. Consequently, cellulose acetate phthalate is an appropriate polymer for preparation of enteric microspheres of tenoxicam.

Key Words: Tenoxicam, Cellulose acetate phthalate, Microspheres, Enteric microspheres, Ulcerogenic effect, Antiinflammatory effect.

INTRODUCTION

It is well-known fact that most conventional nonsteroidal antiinflammatory drugs (NSAIDs) cause gastric irritancy upon oral administration of conventional dosage forms[1-3]. Their pharmacological activity relies on the inhibition of prostaglandin synthesis[4,5]. Although the inhibition of

¶This work is dedicated to Prof. (Dr.) Ahmed Cevdet Ekinci.

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cyclooxygenase, leading to depletion of endogenous prostaglandins (PGs), is a major pathogenic element in the development of gastric lesions. There are other factors including neutrophil activation, hypermotility, microcirculatory disturbances and oxygen free radicals[2,3]. Several attempts have been made to reduce the incidence of gastrointestinal damage of NSAIDs such as the formulations of prodrugs, the preparation of amphoteric gel formulations and solid dispersions[6-11]. The, enteric dosage forms may be suggested to overcome their gastric irritancy problem[12,13] as they are soluble in intestinal fluid rather than gastric fluid, avoiding gastric mucosal damage[5,6]. These dosage forms may be prepared in the form of enteric-coated tablets or microparticulate systems.

Microparticulate dosage forms have been of great interest in oral drug delivery, as they show several advantages over single unit dosage forms[14-16]. Microspheres are a class of microparticulate systems in which drug is distributed in a polymeric matrix. Enteric microspheres are more advantageous than enteric-coated tablets as these microparticulate systems possess better formulation properties and absorption[16-24]. Enteric microspheres are designed to resist the acidic environment of the stomach and to disintegrate in the higher pH environment of the intestinal fluid. Regarding the preparation of enteric microspheres, methods based on phase separation are quite attractive, due to their simplicity and modest requirements in equipment[19-23]. Some of the polymers for enteric coating have been used for the preparation of enteric microspheres[15,16]. Cellulose derivatives are some of the most widely used polymers for the preparation of enteric dosage forms[13,25-37]. Cellulose acetate phthalate (CAP) has been used as an enteric coating polymer for preparation of enteric tablets and also utilized in microsphere formulation studies[25-28].

In this study, tenoxicam, 4-(hydroxy-pyridin-2-ylamino-methylidene)-3-methyl-2,2-dioxo-2u{6},7-dithia-3-azabicyclo[4.3.0]nona-8,10-dien-5-one, (TNX) with analgesic and antiinflammatory effects, was used as the model drug. It has been used orally in the treatment of rheumatic disorders and causes gastric irritancy as other NSAIDs[38,39]. In present studies, preparation of enteric microspheres of tenoxicam with CAP was suggested to overcome its gastric side effect. In the first part of present study, the appropriateness of the enteric polymer was tested using differential thermal analysis (DTA) and thermal gravimetry (TG) analysis[40].

On the basis of the results obtained from preformulation study with DTA/TG analysis, a hypothesis was set as cellulose acetate phthalate is an appropriate polymer to prepare enteric microspheres of tenoxicam. In order to test our hypothesis, *in vitro* dissolution studies and *in vivo* investigations including gastric effect and antiinflammatory activity of microspheres bearing tenoxicam were carried out in the second part.

EXPERIMENTAL

Tenoxicam was gift from Roche, Istanbul, Turkey. Cellulose acetate phthalate was donated by Eastman Kodak, UK. Magnesium stearate was obtained from Merck, Germany. Span 80 was from Fluka, Switzerland. All other reagents and chemical substances were of analytical grade.

Preparation of CAP microspheres: The microspheres were prepared utilizing solvent evaporation method[24]. Cellulose acetate phthalate (CAP) was dissolved in acetone at the concentration of 10 % (w/v). Light mineral oil with 1 % (w/v) of Span 80 was placed in a beaker and mixed at 400 rpm using two-bladded propeller stirrer (Heidolph RZR 2021, Germany). Drug was dispersed in polymer solution at a ratio of 1:1. Subsequently, this mixture was poured into the oil phase containing 0.5 % magnesium stearate and mixed until all solvent was evaporated at room temperature. Microspheres were filtered, washed with ether and collected with a buchner filter. Collected microspheres were allowed to dry at room temperature for overnight. Dry microspheres were collected in a scintillation vial and kept in a desiccator for further analysis.

Particle size: Particle size distribution of microspheres was determined using a set of sieves apertures with mesh size between 1 mm and 125 μm and having a vibratory shaker (Retsch, Germany).

Characterization of enteric-microspheres microscopically: Enteric-TNX-microspheres were characterized by means of scanning electron microscope. Each sample was mounted on stubs using conductive double-sided carbon tape and sputter-coated with gold/palladium in sputter coater (Polaron SC7620, UK) for 90 s at 9 mA. The samples were examined and digital images captured using a Jeol JSM-5500 (Tokyo, Japan) scanning electron microscope (SEM) at an accelerating voltage of 5 kV.

***In vitro* release studies:** Drug efflux from microspheres was determined employing USP dissolution apparatus (paddle) (Aymes, Istanbul, Turkey) at 37 ± 0.5ºC[41]. Alcohol-phosphate buffer (1:24) was used as dissolution medium. The release experiments were conducted at various pH values (1.2, 6.0 and 7.2). The stirring rate was maintained at 100 rpm. At certain time intervals, aliquots of 5 mL were withdrawn and replaced by equal volume of fresh dissolution medium in order to maintain sink conditions. The amount of TNX released from microspheres was determined spectrophotometrically (TU-1880 Double Beam UV-VIS spectrophotometer, Spain) at 366 nm. Each measurement was repeated 5 times.

Drug loading capacity: Adequate amount of microspheres was weighed and dissolved in 100 mL of 0.2 M NaOH solution. An aliquot of 0.2 mL was taken and the final volume was completed to 10 mL using an alkaline solution. Subsequently, the amount of TNX encapsulated was determined by spectroscopy at 366 nm. The experiment was repeated three times for each batch and per cent drug loaded was calculated.

Modeling of the drug release: Data collected from *in vitro* release studies were fitted to both the matrix model and to the first-order kinetic model. The matrix model can be described by using the equation below[14]:

$$\frac{M_1}{M_\infty} = k.t_{1/2} \quad (1)$$

where M_1/M_∞ is the fraction of the total drug released by time t and k is a constant. On the other hand, the first order model has been described using the following equation[14]:

$$\frac{M_1}{M_\infty} = 1 - e^{-kt} \quad (2)$$

The best fitted model was determined using non-linear regressions with the least square method.

***In vivo* studies**

Animals: Male albino Wistar rats (180-230 g) were used to study ulcerogenic effect. The animals were housed and fed in a laboratory at constant temperature of 22°C under the standard conditions (L:D cycle, 12:12 h; standard pellet diet, tap water). Each experimental group consisted of 6 animals/dose and all the animals were used only once. Treatment of the used laboratory animals in this study was in full accordance with the respective European regulations and was approved by the Local Ethics Committee of Mersin University.

Ulcerogenic activity in rats: Rats (n = 6 for each group) were fasted for 24 h prior to the experiments. However, they were allowed free access to water. The animals were divided into 3 groups. Empty microspheres were administered as control group. For the test groups, TNX (25 mg/kg) or its microsphere formulations were administered orally by means of gavage following the fasting period. After 24 h drug administration, rats were injected 1 mL of 1.5 % Tryphan blue solution in distilled water from the tail vein[42]. The animals were sacrificed 10 min after the injection. The stomachs were removed, rinsed with saline and immersed in 1 % formaline. Later, they were opened along the greater curvature and mucosa was examined for lesions with a binocular microscope (Magnification: 11, Model: SZH 10 Research Stereo-Olympus, Japan). Each lesion was graded from 1 to 3 based on the size; 1 = less than 1 mm, 2 = 1-2 mm, 3 = more than 2 mm. Subsequently, lesion indices were calculated and statistically analyzed by Anova.

Determination of antiinflammatory activity of enteric-microspheres: Groups of eight rats weighing 180-220 g were injected with 0.1 mL of a 1.5 % carragennan suspension in 0.9 % NaCl solution into the sub-planar region of the right hind paw[43-45]. TNX containing enteric microspheres (dose

per kg adjusted according to the drug content equivalent to 5 mg/kg) and pure drug (dose: 5 mg/kg) were administered orally by the aid of a gavage. Saline was injected into the left hind paw as control group. The volume of hind paws was measured by a differential meter (Model 7101, Ugo Basile, Italy). 0, 1, 2, 3, 4, 5 and 6 h following the carragennan injection, percentage of edema inhibition was calculated and the data were analyzed by unpaired Student t-test.

RESULTS AND DISCUSSION

Preparation of microspheres: Based on the DTA/TG data, CAP was used for the preparation of enteric microspheres of TNX. The microspheres were easily obtained with solvent evaporation technique. The loading capacity of the microspheres was found as 42 ± 1.5 %. Photomicrographic observations revealed that well-defined microspheres of spherical shape have been prepared (Fig. 1). It is apparent that the surface contains no adhering drug. In all batches, more than 78 % (by weight) of the microspheres were within the range of 250-1000 µm. Mean diameter of microspheres was *ca*. 623 ± 1.38 µm.

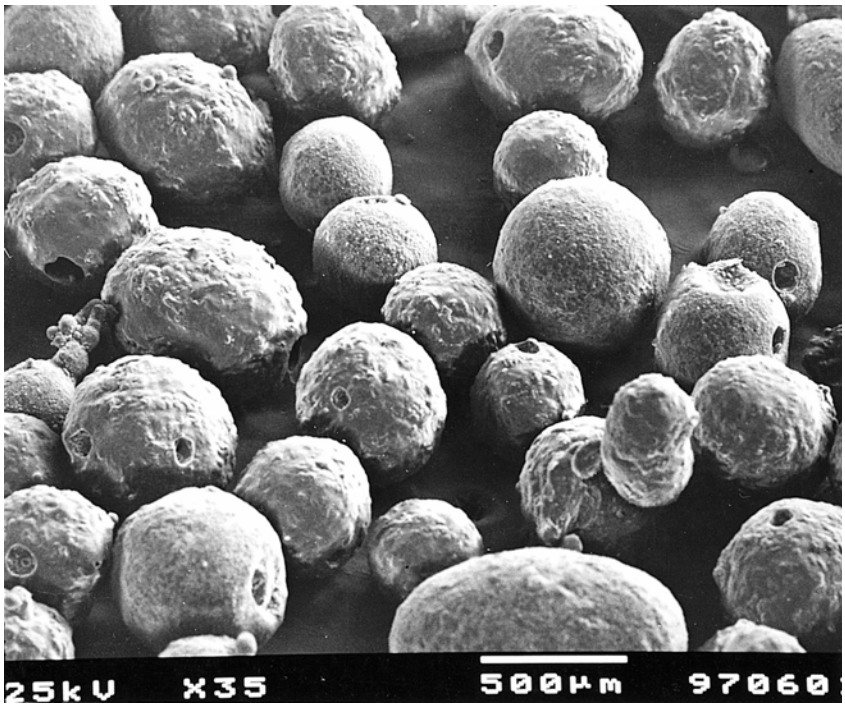

Fig. 1. SEM picture of TNX microspheres

***In vitro* dissolution studies:** Absorption of a drug formulated in an oral dosage form is preceded by *in vitro* release and dissolution in gastrointestinal media. Dissolution is an important property for a drug with limited solubility such as TNX. The *in vitro* dissolution studies were carried out at various pH values (pH = 1.2, 6.0 and 7.2). Significant effect of pH on the release rate of TNX was observed (Fig. 2). The rate of dissolution for microspheres was negligible at pH 1.2 for 1 h (< 8.6 %). This may be due to the enteric coating formed with CAP around the drug particles by means of microspherization. The amount of drug released was significantly high at higher pH values since CAP becomes soluble above pH 6.0. The dissolution profiles of microspheres are shown in Fig. 2. At pH 7.2, the maximum amount of the drug released from microspheres within 1 h was only 34.6 %.

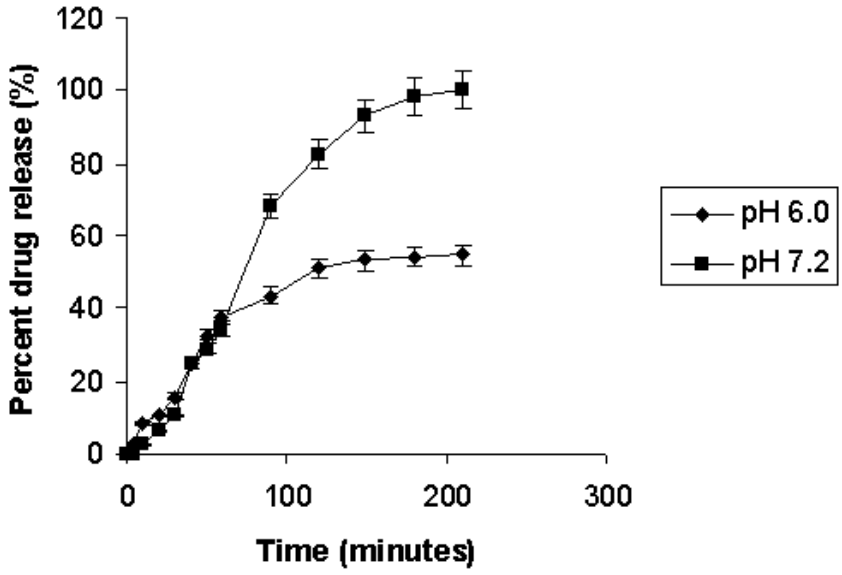

Fig. 2. Cumulative release of TNX from enteric microspheres at various pH values (pH 6.0 and 7.2) in phosphate buffered media.

Modeling of the drug release: The release of tenoxicam from the microspheres were tested for both matrix model and the first-order kinetics. The release curve showed an excellent fitting to the matrix model, with correlation coefficient (r^2) above 0.99.

***In vivo* studies on enteric-microspheres of TNX**

Ulcerogenic effect: To study the effect of enteric encapsulation on the gastrointestinal mucosa, tryphan blue was used as a marker. The staining

of ulcerative lesions upon permeation of the dye was reported as the evidence of mucosal damage. In this study, the rats were treated with the plain drug, encapsulated tenoxicam, or empty microspheres. It was determined that the gastric irritation caused by pure drug was significantly reduced by means of enteric-microspherization (Table-1).

TABLE-1
ULCEROGENIC EFFECT OF PURE DRUG AND FORMULATIONS

Sample	Number of rats	Score	Mean length of ulcers (mm)	Inhibition of ulcers (%)
TNX powder (Control group)	6	3	3.00 ± 0.79	3.60 ± 0.82
Empty microspheres (Negative control group)	6	1	0.56 ± 0.12	–
TNX-EMC[a] (Test group)	6	1	0.32 ± 0.14	89.20 ± 0.27

[a]TNX-EMC: Enteric microspheres of TNX.

Determination of antiinflammatory activity of enteric-microspheres: The values of percentage of oedema inhibition for pure drug and enteric-microspheres were compared with each other (Table-2). No statistically significant difference was found among the values. This indicates that encapsulation process does not reduce the activity of the drug.

TABLE-2
ANTIINFLAMMATORY ACTIVITY OF PURE DRUG AND MICROSPHERES OF TENOXICAM, USING HIND-PAW OEDEMA TEST[a]

Sample	Time (h)					
	0	1	2	3	20	24
Pure drug	-	29.3	40.1	45.8	60.3	63.1
Enteric microspheres bearing TNX	-	30.6	42.4	47.6	62.3	66.9

[a]The values shown in the table are corresponding to the percent oedema inhibiton ($p < 0.05$).

Conclusions

In present studies, the hypothesis made on that the preformulation studies using DTA/TG analysis of the drug and enteric polymer should be a useful tool to choose the suitable polymer for microsphere formulation was tested by means of drug design and pharmacology. The DTA/TG data obtained in the first part of this project indicated that no chemical interaction should take place between pure drug (TNX) and polymer (CAP) while forming the microspheres[40]. Consequently, CAP was used as the polymer to prepare enteric microspheres of TNX, assuming that CAP will

not interfere with the pharmacological activity of the drug, but improve absorption, providing release in intestinal medium. Thus, in the second part of the project, *in vitro* the dissolution profile of the enteric microspheres were investigated, following *in vivo* tests in which pharmacological activity and gastric side effects were tested. *In vitro* dissolution profile at various pH values showed pH-dependent modified release behaviour. Negligible drug release in gastric medium was obtained. On the other hand, higher dissolution rate with controlled release was achieved at pH values approximating the intestinal medium. This is typical to enteric matrix type microspheres. *In vivo* studies indicated that microspherization process did not alter the pharmacological effect (*i.e.* antiinflammatory effect) of the drug and CAP can be suggested as an enteric polymer to overcome the gastric side effects of TNX. All the data obtained from both *in vitro* and *in vivo* studies performed on CAP microspheres containning TNX confirms our hypothesis. The polymer of choice is suitable to prepare enteric microspheres of tenoxicam as it doesn't interact with the drug to influence its pharmacological activity.

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