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Preparation and Characterization of a Novel Injectable Vesicles

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ABSTRACT

Tenoxicam (TX), a non-steroidal anti-inflammatory drug, is used for treatment of many inflammatory conditions. In this work novel injectable vesicle formulations (IVFs) encapsulating TX were prepared using the emulsion method. The aim of this work was to identify the effect of varying the type of oil in the emulsion system on the properties of the prepared formulations. The IVFs were prepared using different oils, namely Capmul, cotton seed, linseed and sesame oils. The prepared formulations were characterized for emulsion formation, shape of the obtained vesicles and *in-vitro* drug release profiles. Results revealed the formation of homogenous emulsions in all prepared formulations. Microscopical investigation revealed spherical vesicles with definite margins. Formulation containing Capmul as the external oily phase exhibited the lowest initial release characteristics. Based on the above results, formulation of TX as injectable vesicle formulations (IVF) using Capmul as the oil phase presents a promising controlled release delivery system for the drug.

Keywords: Vesicles, non-ionic surfactants, Capmul, in-vitro drug release

Introduction

Tenoxicam (TX) is a non-steroidal anti-inflammatory drug (NSAID) (Bird *et al.,* 1983). It is a member of the oxicam family (Woolf and Radulovic, 1989). TX is characterized by its potent anti-inflammatory (Morof *et al.*, 1988) , antipyretic and analgesic effect (Todd and Clissold, 1991), thus, it is widely used in the treatment of rheumatic diseases (Gonzales and Todd, 1987), acute gout (Waterworth and Waterworth, 1987), enkylosing spondylitis, primary dysmenorrhea (Thadikonda *et al.,* 1995), extra-articular diseases (Huang *et al.,* 2002), as well as back pains and post-operative (Sporn and Suh, 2000) besides postpartum uterine contraction pain (Huang *et al.,* 2002). The pharmacological and metabolic behavior of TX is induced by blocking prostaglandins (PGs) biosynthesis, hindering phagocytosis and leukocyte migration and preventing human metalloproteinases which provoke cartilage breakdown (Gonzales and Todd, 1987). TX side effects' resemble other NSAIDs; influencing GIT causing epigastric pain, nausea, dyspepsia, indigestion, vomiting, GI ulceration (Gonzales and Todd, 1987) and may lead to renal failure or bleeding (Brittain, 1994). It also has serious side effects on the biliary tract and liver that may lead to hepatitis in high doses, in addition to increasing liver enzyme activity .
Parenteral controlled release of drugs presents an approach for an efficient delivery of many drugs. They offer many advantages including increased bioavailability, extended drug release period, constant drug plasma concentration and localized delivery of the drug (Luan, 2006).

Vesicular systems are innovative means of delivering drug in controlled manner to enhance bioavailability and prolong their therapeutic effect (Ruckmani and Sankar 2010 ; Vyas *et al.,* 2005; Hiruta, 2006; Negi, 2009; Sankar *et al.,* 2009; Kumar and Rajeshwarrao, 2011). Microspheres and nanoparticles as liposomes and niosomes are common systems used as carriers to encapsulate an active drug. The properties of these systems can be varied to maximize their therapeutic efficacy leading to increased absorption, efficacy and stability and reduced toxicity of the active ingredient (Chien *et al.,* 1989). Niosomes are examples of vesicular systems which are amphiphilic in nature and well characterized for favorable permeation of drugs through biological membranes. They are capable of delivering the drug in a controlled manner to augment bioavailability and extend the therapeutic effect. Niosomes are superior to other micro and nano encapsulation technologies through the higher stability of non-ionic surfactants compared to phospholipid molecules used in liposome formulations. Niosomes possess longer shelf-life than other nanocarrier systems. They are stable at room temperature and exhibit less light susceptibility (Sahin, 2007). Owing to the various disadvantages of classical methods of drug upload into these vesicular systems; complicated processes, difficult scale-up and encapsulation efficiencies, an

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increasing number of new *in-situ* forming systems have been developed as alternatives. Injectable vesicle formulations (IVFs) offer many advantages over the *in-situ* forming implants. The lower viscosity of the used emulsion in their formation reduces pain during injection compared to the pure polymer solution used in implants. In addition, they offer a controlled initial rapid release because of the presence of an external oil phase. Furthermore, IVFs form multiparticulates and could thus minimize the variation of implant morphology and thus offer a more consistent and reproducible drug release (Luan, 2006).

In this work, injectable non-ionic surfactant based formulations encapsulating tenoxicam were developed using the emulsion method. The effect of varying the type of oil in the external phase on the release pattern of the prepared formulations was studied. The obtained vesicles were characterized for emulsion formation, shape of the obtained vesicles and *in-vitro* drug release profiles.

Materials and Methods

Materials

Tenoxicam was a kind gift from EIPICO Co., 10th of Ramadan, Egypt. Sorbitan monooleate (Span 80), polyoxyethylene sorbitan monooleate (polysorbate 80; Tween 80), cholesterol and dialysis bag (2.2 cm wide, molecular weight cut off 12-14,000 Daltons) were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Mono/diglycerides of caprylic acid (Capmul MCM C8) was kindly donated from Abitec Corporation, Janesville, USA. Linseed oil, sesame oil, cotton seed oil, ethyl acetate, disodium hydrogen orthphosphate anhydrous and sodium dihydrogen orthphosphate-1-hydrate were bought from ADWIC, Cairo, Egypt.

Methods

Preparation of tenoxicam injectable vesicles formulations (IVFs)

Accurately measured amounts of the non-ionic surfactant were dissolved in ethyl acetate (internal phase) in the desired concentration (Table 1). The mixture was then sonicated until a clear solution was obtained (Branson 3510, USA). Exactly, 1 mg of tenoxicam was added to the ethyl acetate solution (internal phase) and then sonicated until well dispersed. The ISVs were prepared by emulsifying the non-ionic surfactant-drug mixture into the oil phase stabilized with 4% w/w Tween 80 (external phase) with sonication for 10 min at room temperature (Kranz and Bodmeier, 2008; Kranz *et al.,* 2008).

Table 1: Composition of the tenoxicam injectable vesicle formulations

Formula	Phases ratios (v/v) Internal : External	Internal phase*		External phase**
		Non-ionic surfactant	Solvent type	Oil type
F1	1:4	Span 60	Ethyl acetate	Capmul
F2	1:4	Span 60	Ethyl acetate	Cotton seed
F3	1:4	Span 60	Ethyl acetate	Linseed
F4	1:4	Span 60	Ethyl acetate	Sesame

** All formulations contain 2% w/v non-ionic surfactants and 1mg tenoxicam added to the ethyl acetate (internal phase)*
*** All formulations contain 4% w/v Tween 80 in the external phase (oil)*

In-vitro drug release studies

In-vitro drug release pattern of the prepared formulations was carried out by means of the dialysis bag method, where a pretreated dialysis bag (6 cm long, 2.2 cm wide, molecular weight cut off 12-14,000 Daltons) was used as the donor compartment (Aggarwal and Kaur, 2005). Exactly, 0.5 mL tenoxicam injectable vesicle formulation was injected into the dialysis bag and placed into 10 ml phosphate buffer (pH 7.4) as the receptor compartment, at 37 °C ± 0.5 ˚C in an incubation shaker apparatus (n=3) operated at a rate of 100 strokes per minute (IKA, KS4000IC, Germany). At predetermined time intervals, 3 ml of the medium were withdrawn and replaced by fresh medium. The withdrawn samples were analyzed for drug content spectrophotometrically at 369 nm.

Percentage of mean cumulative drug release was plotted against time. The obtained data from the release studies were analyzed using different kinetic models and the order of drug release from different formulations was determined by applying linear regression analysis. Kinetics of drug release were assessed via fitting the data to the different equations representing zero order (Garrett and Carper 1955), Higuchi diffusion model (Higuchi, 1963) and Korsmeyer Peppas model (Korsmeyer *et al.,* 1983; Peppas, 1985). T_{50} and T_{90} values were calculated from the obtained data.

Characterization of tenoxicam injectable vesicles formulation(IVFs)

Investigation of emulsion formation

Emulsification was carried out by mixing the non-ionic surfactant-drug mixture (internal phase) into the oil phase stabilized with 4% w/w Tween 80 (external phase) with sonication for 10 min at room temperature. The formed emulsions were examined visually.

Morphological examinations

The formed vesicles in the dialysis bag from the IVFs in the *in-vitro* drug release study were used for further characterizations. The dialysis bags were opened and the obtained systems were centrifuged (RPM 15000, 4°C for 15 minutes) followed by removal of the oily phase. The obtained vesicles were suspended in 1 mL phosphate buffer solution (pH 7.4) and a drop of the obtained vesicle suspension was placed on a glass slide and air dried then visualized under the inverted microscope (Olympus Inverted microscope, CKX41, Japan).

Results and Discussion

In-vitro drug release studies

The *in-vitro* drug release study was carried out according to dialysis bag method (Aggarwal and Kaur, 2005). The cumulative amount of the drug released was plotted against time and release parameters were calculated to compare the investigated formulations (Fig. 1).
Formulations F2, F3 and F4 exhibited a rapid drug release pattern with an initial drug release of 67.73%, 67.43% and 74.21%, respectively in the first hour. F1 compromising Capmul as the external oily phase showed the best release pattern with an initial release of 41.63% of the drug in the first hour, followed by moderate drug release pattern across 24 hours. This could be attributed to the fact that Capmul as a medium chain monoglyceride, possesses a high HLB value (HLB = 6) which will likely increase the interfacial fluidity resulting in enhancing the diffusion of the solvent (ethyl acetate) and formation of the vesicles upon dilution with aqueous medium (Taha *et al.,* 2004). This leads to the rapid encapsulation of the drug inside the vesicles and controlling the drug release. Consequently, the initial drug release was decreased in case of Capmul compared to that for the other oils (cotton seed, linseed and sesame oils) used in formulations F2, F3 and F4, respectively, which did not possess the same self emulsifying properties as Capmul.

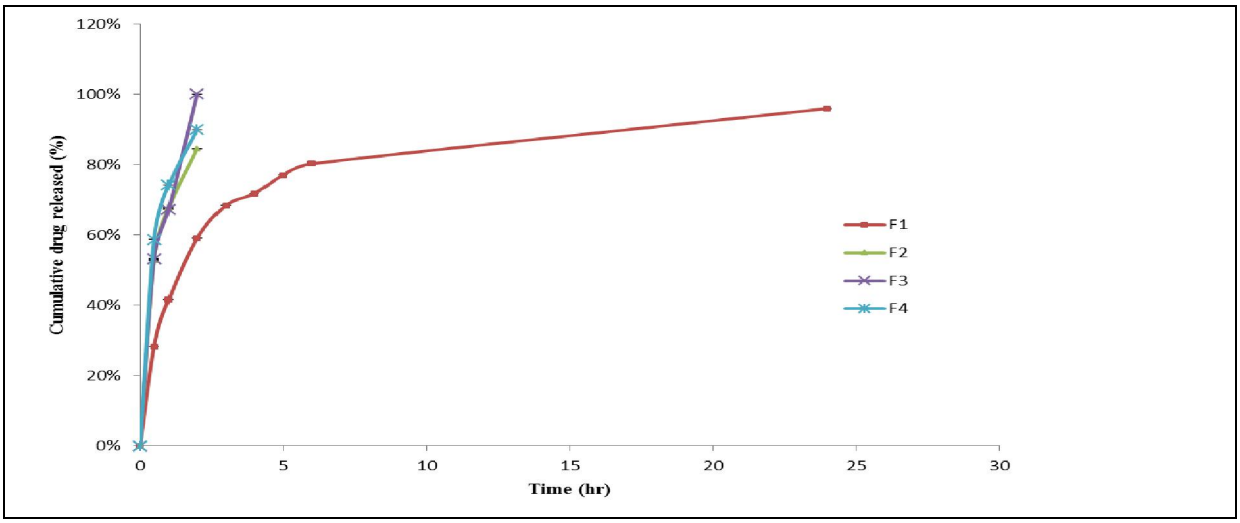

Fig. 1: Release profiles of tenoxicam from the prepared formulations

By fitting the *in-vitro* drug release data into different kinetic models, the release pattern of F1 followed the Korsmeyer-Peppas model (R^2 = 0.971) with n-value equals 0.49 indicating fickian diffusion drug transport mechanism. This is correlated with the reported release mechanism of various controlled release formulations (Dash *et al.,* 2010). The T_{50} and T_{90} values for F1 were 245.69 ± 0.02 and 442.56 ± 0.04 hours, respectively.

The release kinetics of the other formulations (F2, F3 & F4) could not be studied due to the release of more than 60% of the drug in the first 1 hour.

Characterization of tenoxicam injectable vesicles (IVFs)

Investigation of emulsion formation

All prepared IVFs revealed the formation of homogenous emulsion with no lumps. This may be attributed to the moderate viscosities of the used oils and the emulsifying effect of the non-ionic surfactant.

Morphological examinations

Micrographs of the prepared formulations were produced using inverted microscope (Fig. 2). All formulations revealed the formation of vesicles. In general, nearly spherical, vesicles were identified.

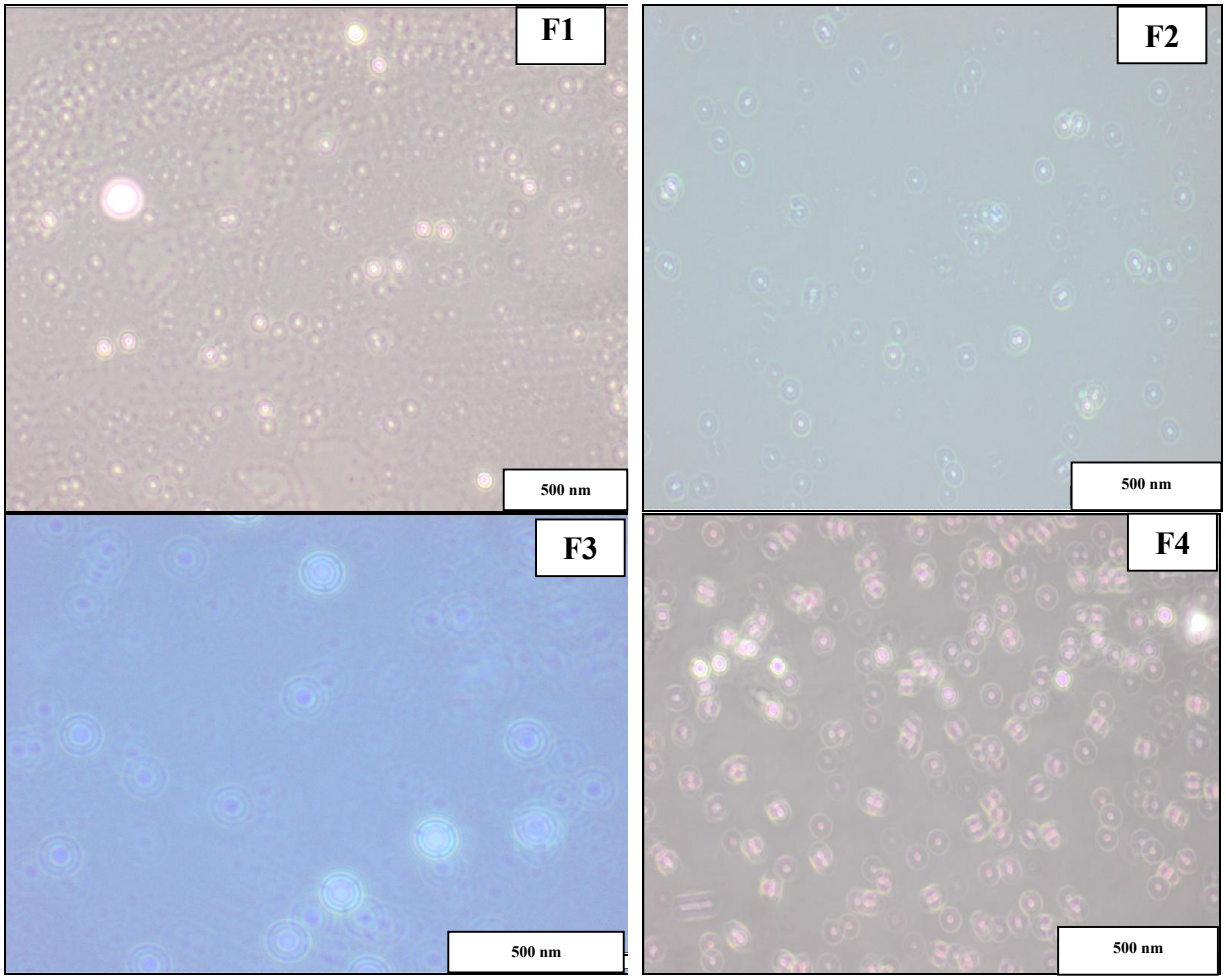

Fig. 2: Inverted microscope micrographs for F1, F2, F3 and F4

Conclusion

In conclusion, incorporation of self-emulsifying oil as Capmul as the external phase improved the properties of the produced IVFs which presents a promising dosage form of the drug that can be further modified.

Conflict of Interest

The authors report no declarations of interest

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