TOPICAL W/O/W DOUBLE EMULSIONS OF PIROXICAM: IN VITRO DRUG RELEASE STUDY

LAVINIA VLAIA¹, VICENŢIU VLAIA², LENUŢA-MARIA MICLEA¹, IOANA OLARIU¹, GEORGETA CONEAC¹

University of Medicine and Pharmacy „Victor Babes”, Faculty of Pharmacy, P-ta Eftimie Murgu, No. 2, 300041, Timișoara, Romania
¹Department of Pharmaceutical Technology
²Department of Organic Chemistry
*corresponding author: laviniaursica@yahoo.com

Abstract
The aim of this work was to study the in vitro release of piroxicam suspended in the internal phase of some W/O/W (water/oil/water) double emulsions and to investigate the effect of hydrophilic macromolecules (hydroxyethylcellulose and Carbopol 940) in the external aqueous phases of W/O/W double emulsions on the diffusion profiles of piroxicam through synthetic membranes. W/O/W double emulsions, containing 1% piroxicam and 80% W/O primary emulsion, were prepared using liquid paraffin, Span 80 and Tagat S2, by a two-step emulsification procedure. The results of the in vitro study showed a slow release of piroxicam from W/O/W double emulsions; the release was affected by the presence of hydrophilic macromolecules.

It was concluded that the release of piroxicam from the studied W/O/W double emulsions occurs either by molecular diffusion through the oily intermediary film as well as by the rupture of this film and is controlled by the viscosity and the stability of the systems.

Keywords: piroxicam; W/O/W double emulsion; release rate

Introduction
Piroxicam is one of the most potent non-steroidal anti-inflammatory agent used for the treatment of acute and chronic rheumatoid arthritis or osteoarthritis, traumatic contusions and different regional inflammatory disorders such as muscle pain or low-back pain. Although piroxicam is well
absorbed following oral administration, its use has been associated with a number of gastro-intestinal disorders, dizziness and headache. Due to these side effects correlated with the oral use of piroxicam, development of various topical dosage forms such as gels, creams, ointments and cataplasms was proposed. The physicochemical characteristics of piroxicam make it suitable for topical delivery [1, 2, 3, 4, 5, 6].

Multiple emulsions are vesicular and complex systems considered as “emulsions of emulsions”. Double emulsions are the simplest multiple emulsions and have two different interfaces because their dispersed phase itself is an emulsion [7, 8].

The enhancement of dermal absorption and prolonged release of the entrapped active substances ranked among the potential pharmaceutical applications of double emulsions. Despite their potential usefulness, the applications of double emulsions have been limited because of their pronounced thermodynamic instability generated by the same ternary structure [7, 9, 10, 11].

The stability and release characteristics of W/O/W (water/oil/water) double emulsions are influenced by different factors including surfactant type and ratio, nature of hydrophilic macromolecules and some physical properties of the system (e.g. globule size, viscosity, phase volume ratio, etc.) [7, 8, 12, 13, 14].

The major purpose of this work was to develop a topical stable W/O/W double emulsion containing piroxicam. The present paper studied the in vitro release of piroxicam suspended in the internal phase of various W/O/W double emulsions and examined the effect of the hydrophilic macromolecules (hydroxyethylcellulose and Carbopol 940) in the internal and/or external aqueous phases of W/O/W double emulsions on the diffusion profiles of piroxicam through synthetic membranes.

Materials and methods

Materials

Piroxicam, the active pharmaceutical substance, was generously provided by Terapia S.A. (Cluj-Napoca, Romania); liquid paraffin (Merkur Vaseline) as oily phase; Span 80 (sorbitan monooleate, HLB=4.3, Fluka) as primary, lipophilic surfactant; Tagat S2 (polyethylene glycol 20-glyceryl monostearate, HLB=15, T.H.Goldschmidt A.G. Essen) as secondary, hydrophilic surfactant; hydroxyethylcellulose (HEC, Merck) as additive for both internal and external aqueous phases; Carbopol 940 (BF Goodrich) as additive for external aqueous phase; hydrated magnesium sulfate (MgSO\(_4\) \cdot 7H\(_2\)O, F.R. X; Reactivul Bucureşti) as stabilizing substance entrapped in the
internal aqueous phase; triethanolamine 85% (Steria Chemicals, București) as neutralizing agent for Carbopol 940; preservative solution (F.R. IX) as vehicle for aqueous phases. All chemicals used were of pharmaceutical grade.

**Methods**

*Preparation of W/O/W double emulsions*

The compositions of piroxicam W/O/W double emulsions used in this study, named 1P, 2P, 3P, are shown in table I.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Composition (g) of the piroxicam W/O/W double emulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>W/O/W double emulsion code</strong></td>
<td><strong>Constituents</strong></td>
</tr>
<tr>
<td>1P</td>
<td>Piroxicam</td>
</tr>
<tr>
<td>2P</td>
<td>Oily phase</td>
</tr>
<tr>
<td>3P</td>
<td>Span 80</td>
</tr>
<tr>
<td>1P</td>
<td>Internal aqueous phase</td>
</tr>
<tr>
<td>2P</td>
<td>Hydrated magnesium sulfate</td>
</tr>
<tr>
<td>3P</td>
<td>Hydroxyethylcellulose</td>
</tr>
<tr>
<td>1P</td>
<td>Preservative solution to</td>
</tr>
<tr>
<td>2P</td>
<td>W/O primary emulsion</td>
</tr>
<tr>
<td>3P</td>
<td>Oily phase</td>
</tr>
<tr>
<td>1P</td>
<td>External aqueous phase</td>
</tr>
<tr>
<td>2P</td>
<td>Tagat S2</td>
</tr>
<tr>
<td>3P</td>
<td>Carbopol 940</td>
</tr>
<tr>
<td>1P</td>
<td>Hydroxyethylcellulose</td>
</tr>
<tr>
<td>2P</td>
<td>Triethanolamine</td>
</tr>
<tr>
<td>3P</td>
<td>Preservative solution to</td>
</tr>
</tbody>
</table>

For the preparation of the internal aqueous phases of W/O/W double emulsions, piroxicam, previously pulverized (sieve IX, F.R. X), was suspended into a preservative solution inside which hydrated magnesium sulfate (1P and 2P formula) or both hydrated magnesium sulfate and HEC (3P formula), were dissolved.

For the preparation of the external aqueous phases of W/O/W double emulsions, the hydrophilic surfactant and HEC (2P formula) or Carbopol 940 (1P and 3P formula) were dissolved into the same preservative solution.

W/O/W double emulsions were prepared by a two-step emulsification procedure. In the first step, a primary W/O emulsion was prepared as follows: the oily mixture of Span 80 and liquid paraffin was
heated at 80°C and then the aqueous internal phase brought at the same
temperature was added progressively to this mixture by stirring (at 2000
rpm) with a laboratory mixer (Eurostar digital, Ika-Werke, Germany) for 30
minutes, until complete cooling (25°C). The second emulsification step
provided the formation of the W/O/W double emulsion as follows: the
freshly prepared W/O primary emulsion (80 g) was reemulsified in an
external aqueous phase (20 g) containing the hydrophilic surfactant, by
stirring at low speed (600 rpm) for about 40 minutes to prepare the W/O/W
double emulsion.

**In vitro release studies**

The release study of piroxicam from the double emulsions was
performed through 0.2 μm cellulose nitrate membranes (Sartorius AG,
Germany), using a system of six Franz diffusion cells, with a diffusional
area of 1.767 cm² (Microette-Hanson, 57-6AS9 Model). The membrane was
mounted between the donor and the receptor compartment.

0.3 g of the double emulsion were placed on the membrane surface
in the donor compartment which was sealed from the atmosphere with a
plastic film, in order to ensure sink conditions during the experiment. The
receptor compartment of the cell was filled with 7 ml of degassed phosphate
buffer (pH 7.4).

During the experiments, the solution in the receptor side was kept
at 37±1°C and it was stirred magnetically at 600 rpm. The samples from the
receptor compartment were withdrawn at 30, 60, 120, 180, 240, 300 and 360
minutes intervals and immediately replaced by an equal volume of fresh
buffer solution (to keep the diffusion medium constant). The collected
samples were analyzed for the piroxicam assay spectrophotometrically at a
wavelength of 252 nm. The piroxicam concentration (c, mg/ml) of the
collected samples was calculated using the equation \( c = 38.93 \cdot A \), which
describes the linear domain of the calibration curve (r = 0.9999).

All release studies were carried out in triplicate.

**Data analysis of in vitro release studies**

Piroxicam release rates were calculated using the Higuchi equation
which is valid when the release of drug from the vehicle is under 30%:

\[
Q = 2C_0 \sqrt{Dt/\pi} \quad \text{or} \quad Q = k\sqrt{t}
\]

where \( Q \) is the amount of drug released per unit area (mg/cm²); \( C_0 \) is the
initial concentration of drug in the vehicle (mg/cm³); \( D \) is the apparent
diffusion coefficient (cm²/s); \( t \) is time (s); \( \pi \) is a constant; \( k \) is the release
rate constant and was determined from the slope of the amount of drug
released per unit area versus square root of time. The apparent diffusion coefficient of the drug (D) was estimated from the release constant value.

The *in vitro* membrane permeation rate or flux (J) is expressed by the Fick’s second law as

\[ J = \frac{C_0 K_p D}{l} = C_0 P \]

where \( K_p \) is the partition coefficient of drug between membrane and vehicle; \( l \) is the thickness of the membrane (cm); \( P \) is the permeability coefficient of the drug (cm/s).

The flux (J) was calculated from the slope of the linear plot of the cumulative amount of drug (μg) permeated per unit diffusion surface (cm²) as a function of time, in the steady state region. The permeability coefficient (P) was estimated from the flux and donor drug concentration.

**Results and discussion**

The main characteristics and the physical stability of the double emulsions were investigated in a previous study by macroscopic, microscopic and globule size analysis and viscosity measurements as a function of time. Macroscopically, all formulations were homogeneous, very compact and viscous, and white-yellowish. The microscopic and globule size analysis have shown the emulsions type, the double character and the mean double globule sizes in the range of 2.74 and 3.95 μm. All formulations were physically stable more than 3 months [14, 15].

The *in vitro* release profiles of piroxicam from W/O/W double emulsions 1P, 2P and 3P are presented in figure 1.

**Figure 1**

Release profiles of piroxicam from W/O/W double emulsions: a) immediately after preparation; b) 3 months after preparation
All release profiles exhibited an initial rapid release phase and then a slower release of the drug molecules. The first phase of the release profiles (the first two hours of release profile) might be due to piroxicam existence in the external aqueous phase during the manufacturing; during the second emulsification, piroxicam molecules probably leaked out of the internal aqueous phase. The drug particles from the external aqueous phase were released gradually as they were dissolved in this phase. The release of piroxicam from the external aqueous phase of W/O/W emulsions was completed within approximately 3 hours. After this period, piroxicam suspended in the internal aqueous phase was released slowly at a rate governed by its low solubility in water as well as by the oily barrier between the inner and outer aqueous phases because piroxicam, although is a lipophilic drug, could not pass freely through the oily layer. So, the second phase might be attributed to a prolonged release of piroxicam from the internal aqueous phase.

Also, from figure 1 it can be seen that after 3 months, the amount of piroxicam released suffered a very small increase for 1P and 3P formulations, while it almost doubled for the 2P formulation. This finding indicated that the physical stability of 2P formulation was smaller than the ones of 1P and 3P formulations, increasing the transport of piroxicam from the internal to the external aqueous phase by oily membrane breakdown.

The piroxicam release rates (k) and diffusion coefficient (D) values across the synthetic membranes are listed in table II.

### Table II
Piroxicam release rates and diffusion coefficient values across the synthetic membranes

<table>
<thead>
<tr>
<th>W/O/W double emulsion code</th>
<th>k (μg/cm² min²)</th>
<th>D · 10⁷ (cm² min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1P</td>
<td>7.34 ± 0.11**</td>
<td>4.27 ± 0.12**</td>
</tr>
<tr>
<td></td>
<td>7.77 ± 0.12***</td>
<td>4.79 ± 0.14***</td>
</tr>
<tr>
<td>2P</td>
<td>4.12 ± 0.22**</td>
<td>1.52 ± 0.14**</td>
</tr>
<tr>
<td></td>
<td>8.30 ± 0.43***</td>
<td>5.42 ± 0.56***</td>
</tr>
<tr>
<td>3P</td>
<td>7.21 ± 0.10**</td>
<td>4.12 ± 0.12**</td>
</tr>
<tr>
<td></td>
<td>7.81 ± 0.20***</td>
<td>4.94 ± 0.24***</td>
</tr>
</tbody>
</table>

*Values are the mean±SD of 3 measurements at 37°C
** immediately after preparation
*** 3 months after preparation

For freshly prepared W/O/W double emulsions, the release rates values of piroxicam from 1P and 3P formulas were almost 1.8 times higher than from 2P formula. However, after 3 months, the release rate values of
piroxicam from 1P and 3P formulas were nearly unchanged, while for 2P formulation the value of this parameter doubled, almost equalizing the ones of 1P and 3P formulas. This difference in the release rate values of piroxicam from 2P formulation can be explained through the loss of system stability mentioned above. Unlike this evolution, the constancy of the release rate values of piroxicam from 1P and 3P formulas indicated the stability of these systems during aging.

The plot of the cumulative amount of piroxicam that permeates through the synthetic membrane per unit area versus time is given in figure 2. The steady state flux (J) and the permeability coefficient (P) values are summarized in table III.

![Figure 2](image)

Permeation profiles of piroxicam through synthetic membrane from W/O/W double emulsions: a) immediately after preparation; b) 3 months after preparation.

<table>
<thead>
<tr>
<th>W/O/W double emulsion code</th>
<th>Steady state flux, J·10⁻² (μg·cm⁻²·min⁻¹)</th>
<th>Permeability coefficient, P·10⁻⁵ (cm·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1P</td>
<td>99.47 ± 1.33&quot;** 104.98 ± 1.49***</td>
<td>9.947 ± 0.133&quot;** 10.498 ± 0.149***</td>
</tr>
<tr>
<td>2P</td>
<td>54.47 ± 2.85*** 108.88 ± 0.53***</td>
<td>5.447 ± 0.285*** 10.888 ± 0.053***</td>
</tr>
<tr>
<td>3P</td>
<td>96.93 ± 1.33&quot;** 104.05 ± 2.19***</td>
<td>9.693 ± 0.133&quot;** 10.405 ± 0.219***</td>
</tr>
</tbody>
</table>

*Values are the mean±SD of 3 measurements at 37°C
** immediately after preparation
*** 3 months after preparation

The data obtained were similar to the ones observed in the release study. The steady state flux values for freshly prepared 1P and 3P W/O/W
double emulsions were 1.8 times higher compared with the 2P formulation. After three months, the flux value for 1P and 3P formulations suffered a very small increase, while that for the 2P formulation increased to such an extent that it was higher than for the other two systems.

Conclusions

The release of piroxicam from W/O/W double emulsions through synthetic membranes is characterized by an initial rapid release followed by a much slower rate of release. Because of their role as stabilizing and thickening agents, hydrophilic macromolecules in the internal and/or external aqueous phases of W/O/W double emulsions, affected the piroxicam release and permeation parameters through synthetic membranes.

The investigations presented above lead us to conclude that stable, concentrated W/O/W double emulsions, containing 80% W/O simple emulsion and hydrophilic macromolecules as thickening agents in the external aqueous phase, can be used as potential topical prolonged release dosage forms for piroxicam due to their appropriate viscosity, and physical stability. Between the tested hydrophilic polymers (Carbopol 940 and HEC), Carbopol 940 slowed in greater extent the release of piroxicam from the studied W/O/W double emulsions, increasing the viscosity of the external aqueous phase, and consequently the systems stability.

Further in vitro examinations such as different type of oily phase or different surfactants concentrations are still required and further formulation studies are necessary to obtain the most stable topical W/O/W double emulsion as vehicle for piroxicam.

References

Manuscript received: 04.05.2009