

IN VITRO AND IN VIVO INVESTIGATION OF DRUG RELEASE FROM SEMISOLID PRODUCTS IN CASE OF SYNTHETIC MEMBRANE AND RAT SKIN



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Introduction

In vitro and in vivo testing of diclofenac sodium containing creams, hydrogels and organogels for dermal use were investigated compared to 2 marketed medicinal products used as reference preparations.

In vitro drug release experiments - which are quality indicators of the products -, can be used for screening of compositions prior to in vivo animal testing.

The main aims of our study were

- * examination of drug release and penetration,
- * investigation of hydrophilic and lipophilic membranes,
- * comparison of in vitro results between the Franz vertical diffusion cell and paddle over disk method,
- * to carry out in vivo studies,
- * finding in vitro-in vivo correlation (IVIVC).

Methods

Sameness

FDA **FIP-AAPS**

Method
Franz vertical diffusion cell

Membrane
Synthetic cellulose acetate



Difference

FDA **FIP-AAPS**

Receiving medium
Water Buffer Alcohol

Temperature
? 32°C

Sample amount
300 mg Specific dose

Sample number
5<x ?

Sampling time
6 hours ?

In vitro

Franz glass diffusion cell system (Hanson Research Co., USA) containing six cells and equipped with autosampler (Hanson Microette Autosampling System) was used. Membrane filters were mounted to glass cells. The diffusion area was 1.767 cm².

In case of paddle over disk method containing 8 cells, the sampling procedure was manual. Dosage forms in ointment cells were dropped into glass vessels with 70 ml dissolution media.

Experiments were run at 32 ± 0.5°C. 800 µl samples in case of Franz cell and 2 ml samples in case of USP 2 were taken after 0.5, 1, 2, 3 and 6 hours replaced with fresh receiving medium. Phosphate buffer (pH 5.4 ± 0.1) was chosen for receptor medium. Synthetic cellulose acetate membrane (Porafil, Macherey-Nagel, Germany and Pall Life Sciences, USA) with a pore size of 0.45 µm was soaked in buffer solution or in isopropyl myristate (IPM). Absorbance was measured by UV Spectrophotometer (Unicam Helios Alfa UV-Vis Spectrophotometer, England) at 275 nm.

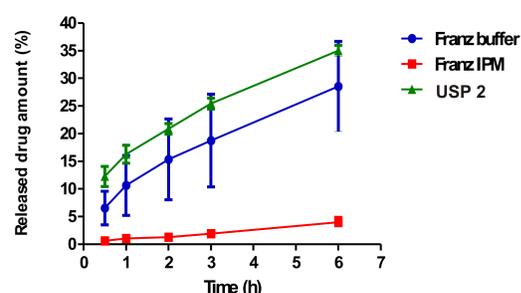


Fig. 1. In vitro drug release of 1 % diclofenac sodium in 70 % o/w cream

Conclusion

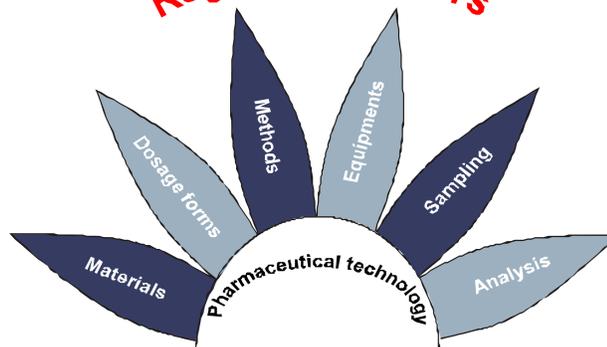
In vitro drug release and penetration studies are not acceptable without in vivo feedback. The order of different dosage forms concerning release data, is as follows: o/w creams > reference gel emulsion > hydrogels > reference hydrogel > w/o creams > organogels. Diffusion through IPM soaked membrane decreased in the following order: hydrogels > organogels > w/o creams > o/w creams > reference gel emulsion > reference hydrogel.

The hydrophilic and lipophilic character of the membrane such as selection of dissolution apparatus have an influence on the drug release rate.

We recommend the use of Franz cell prior to in vivo animal testing and USP 2 for in vitro quality control (QC) investigations.

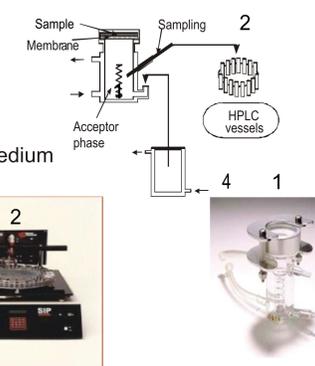
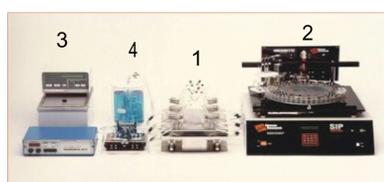
Our developed products with less additives than in reference gels reduced paw oedema in rats. Evaluating in vitro, in vivo and IVIVC data, we offer our hydrogels and o/w creams for clinical use.

Regulatory affairs



Franz cell

- 1 Vertical diffusion cell
- 2 Autosampling system and sample collector
- 3 Thermostat
- 4 Replacement of receptor medium



In vivo

In vivo studies were carried out on with Forane solution anaesthetized male Wistar rats (150-181 g). All experiments were performed at 24 ± 1 °C in an air-conditioned room. Local inflammatory response was elicited by 0.1 ml subplantar injection of carrageenan (Viscarin, Marine Colloids Inc., Springfield, USA) solution given into the right hand paw 1 hour after treatment. Concentration of carrageenan solution was 0.5 %. The left paw, used as control, was treated with physiological saline solution without carrageenan. Paw volume was measured with plethysmometer 5 h after the carrageenan injection. The volume difference between the carrageenan and saline injected paws was used for the evaluation of inflammatory response.

The carrageenan paw oedema decreasing effect of 12 different formulations and 2 reference gels was measured in comparison with a control group. 300 mg from each cream was applied onto the depilated dorsal skin of the rat.

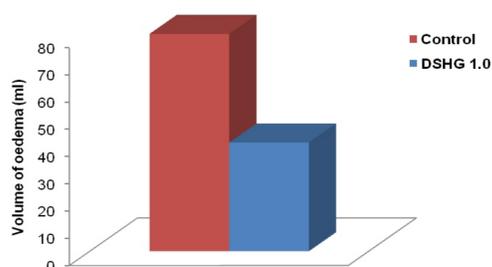


Fig. 2. In vivo study of 1 % diclofenac sodium in 1 % Carbomer gel

Acknowledgement

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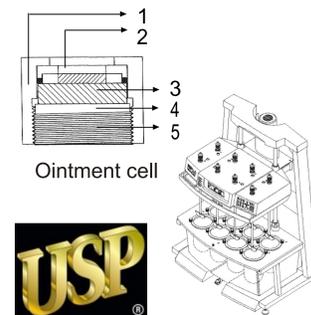
Materials

Active agent: 1 % diclofenac sodium

Basements	Compositions	Sign
Hydrogels	Polymer content: 0.8-0.9-1.0%	DSHG 0.8 DSHG 0.9 DSHG 1.0
Organogels	Gel oil with unorganic colloid Colloid substance: 25-30-35%	DSOG 25 DSOG 30 DSOG 35
o/w creams	Water content: 65-70-75%	DSOW 65 DSOW 70 DSOW 75
w/o creams	Water content: 40-45-50 %	DSWO 40 DSWO 45 DSWO 50

USP Apparatus 2

- 1 Sample holder
- 2 Membrane
- 3 Sample
- 4 Low plate
- 5 Fixing screw



Results

In vitro drug release rate in all products was significant higher in case of USP Apparatus 2 than in case of Franz cell. Cumulative drug release amount in all products was lower measured with membrane soaked in IPM than soaked in buffer solution (Fig. 1.).

All selected preparations from the in vitro experiments reduced paw oedema in rats, although we found significant differences among them both in vitro and in vivo. 58 % of our products reached the oedema decreasing effect of one of the reference gels. The highest oedema swelling inhibition rate was measured in case of 35 % organogel, the lowest in 45 % w/o cream. All other formulations seemed to be effective in treatment of oedema (Fig. 2.).

In case of buffer soaked membrane the hydrogels and organogels had the best IVIVC. In case of IPM soaked membrane the highest IVIVC was detected in organogels and in o/w creams (Table I.).

Compositions	R ² with linear fitting	R ² with power trend line fitting
Hydrogels	0.9055	0.9395
Organogels	0.9091	0.7936
o/w creams	0.3947	0.3394
w/o creams	0.0455	0.0121

Table I. IVIVC coefficients in case of buffer soaked membrane