

DIFFUSIONAL AND RHEOLOGICAL EVALUATIONS AS VALUABLE QUALITY CONTROL AND *IN VIVO* PERFORMANCE PROGNOSTIC TOOLS FOR TOPICAL DRUG PRODUCTS

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Abstract

The current paper describes a combined rheological and diffusion approach to the *in vitro* evaluation of four commercially available topical semisolid dosage forms containing 0.5% piroxicam. The experimental conditions represent an adaption of the USP Topical/Transdermal Ad Hoc Advisory Panel stimuli for revision process, for the considered low solubility, high permeability drug, enclosed in hydrophilic gel matrix. The cumulative mass release profiles in hydro-alcoholic receptor media, using two hydrophilic membranes as a procedure applicable for scale-up post-approval changes are correlated with the study of rheological behavior. The static character of vertical diffusion cell and the flow indexes of the topical drug product are analyzed as key factors for the *in vivo* performance.

Rezumat

Lucrarea reprezintă o abordare combinată a evaluării *in vitro*, de difuzie și reologie, pentru patru formulări topice semisolide disponibile comercial, conținând 0.5% piroxicam. Condițiile experimentale constituie o adaptare a documentului de inițiere a revizuirii ghidului emis de Topical/Transdermal Ad Hoc Advisory Panel, pentru o substanță medicamentoasă caracterizată prin solubilitate redusă și permeabilitate înaltă, inclusă într-o matrice de tip gel hidrofil. Profilele de cedare în medii hidro-alcoolice, utilizând două tipuri de membrane hidrofile, ca procedură de monitorizare a transferului de tehnologie sau de control a schimbărilor de proces, sunt corelate cu studiul comportamentului reologic. Caracterul static al celulelor verticale de difuzie și indicele de curgere caracteristic produselor topice sunt analizate ca factori majori ai performanțelor *in vivo*.

Keywords: piroxicam, vertical diffusion cell, topical drug product.

Introduction

Either in static or flow-through conditions, the Franz diffusion cells had been used for the evaluation of drug release rate from liquid and semisolid dosage forms [5]. In 2009, The Topical/Transdermal Ad Hoc

Advisory Panel for the USP Performance Tests of Topical and Transdermal Dosage Forms issued a key stimuli article [10], describing major aspects for standardization of compendial vertical diffusion cell (VDC) test conditions. In the same time, a first draft monograph is proposed, for USP Hydrocortisone Cream Reference Standard.

In the case of solid oral dosage forms, the *in vitro* drug release methodologies allow the use of surfactants for low solubility active ingredients and strongly discourage the hydro-alcoholic media, although several non-official methods are reported by the literature (e.g. for glibenclamide, a Biopharmaceutical Classification System (BCS) Class II drug [4]). The use of a certain quantity of alcohol is accepted for diffusion cells, considering a solubility-base justification (compliance with sink conditions criteria) and a demonstrated discriminatory character of the entire group of experimental parameters (including membrane nature and compatibility with the active ingredient i.e. lack of adsorption phenomenon, stirring rate, temperature etc.) on the role and biopharmaceutical impact of formulation and manufacturing variables.

The current paper describes the optimisation of the conditions mentioned in the stimuli article to topical drug products containing a typical BCS class II active ingredient (piroxicam) in a hydrophilic matrix and the comparison of the release profiles as a procedure applicable for scale-up post-approval changes (SUPAC).

Materials and methods

The active ingredient release profile and the rheological behavior of four commercially available topical semisolid dosage forms containing 0.5% piroxicam were investigated, the composition for the innovator product (Foldene, considered reference and noted R) and generic formulations (noted T1, T2, T3) is detailed in table I.

Table I
Composition of topical semisolid products

Product	Feldene* (R)	T1	T2	T3
Piroxicam (%)	0.5	0.5	0.5	0.5
Composition	Carbomer 980	Carbomer 940	Hypromellose	Carbomer 980
	Hydroxyethyl cellulose		Macrogol 7 glycerol cocoate	Hydroxypropylmethyl cellulose K15M
	Ethanol, propilenglycol	Ethanol, propilenglycol	Propilenglycol, isopropyl alcohol	Ethanol, propilenglycol
	Diisopropanolamine	Triethanolamine	Sodium hydroxide, Potassium dihydrogen phosphate	Triethanolamine
	Benzyl alcohol	Benzyl alcohol	Sodium metabisulfite	Methyl p-hydroxy benzoate Benzyl alcohol
	Purified water	Purified water	Purified water	Purified water
Batch no.	G10051331	0350022	103725	130608

The *in vitro* drug release profile was evaluated using a Hanson Microette System, Hanson Research, USA, equipped with 6 vertical diffusion cells, having a 12 mL nominal volume (10 mL effective volume, considering the magnetic stirrer and upper stainless steel helix) and a diffusion surface of 15 mm diameter. The receptor media consisted of a 1:1 (v:v) mixture of absolute ethanol and purified water, degassed by filtration through 0.45 μ m regenerated cellulose membranes under vacuum, then by stirring for 2 min (a longer period of stirring is considered to change the hydro-alcoholic media composition). The vertical diffusion cells were filled with the receptor media and stirred at 1000 rpm for 30 min prior to drug product application, in order to eliminate the resident air bubbles and to allow the system to equilibrate. The individual volume was previously determined by weighing the empty and water filled cells.

The temperature was set at $32 \pm 0.2^\circ\text{C}$, using a Lauda Ecoline Staredition E100 / 090. Two types of hydrophilic membranes were used: cellulose mix esters, Teknokroma, batch no. 133895 and Tuffryn® - polysulfone, PALL Life Sciences HT-450, batch no. T72556. The membranes, having 0.45 μ m pore diameter and comparable filtration surface, were soaked for 30 min in the receptor media and gently pressed between filter paper sheets, then the teflon o-rings were attached and the semisolid drug product was applied, using a teflon spatula (approximately 300 mg). Glass cover slides, aluminium pressing rings and sealing clamps were attached after the application of drug-product / o-ring / membrane assembly. The experiments were conducted on 6 units for each product and the stirring rate was set at 400 rpm.

Sampling was performed manually, using 1 mL Hamilton syringe, at 30, 60, 90, 120, 150, 180, 210, 240, 300 and 360 min after semisolid drug product application. The stirring was stopped for 30 sec prior to each sampling procedure. Volumes of 1.0 mL were injected in the receptor compartment over 1 min, the last 0.5 mL being collected in a pre-labeled vial.

The quantitative determination of piroxicam was performed using a Jasco UV-Vis V-530 spectrophotometer (equipped with Spectra Manager software for Windows 95/NT, version 1.54.03), at 357 nm (correlation coefficient > 0.9999 , 0.05 to 10 $\mu\text{g/mL}$). The samples were diluted 1:9 with the receptor media, prior to analysis.

Since the membrane is currently assumed to be mainly the support of semisolid products in diffusion studies, one of the main factors affecting drug release being the diffusion resistivity of the formulation itself, the rheological profile was recorded using a rotational Rheometer type RC1,

RheoTec GmbH, Germany, with CC14 coaxial cylinder (shear rate values interval: 0 – 250 s⁻¹, on ascending and descending routes; volume: 3 mL). Data recording and modeling was performed using the Rheo 3000 software, version 1.2.1328.1. RheoTec Messtechnik GmbH - Brookfield Engineering Labs., Inc.

Piroxicam and ethanol were purchased from Sigma-Aldrich. The purified water was prepared using a SGW Ultraclear UV PlusTM system.

Results and discussion

Evaluation of cumulative mass released from each formulation per unit of diffusion area plotted against square root time indicated the robustness of the procedure implemented as a quality control test (coefficient of variation: CV% < 10%, for each sampling point), as well as the linearity of profiles ($R^2 > 0.98$), using the Higuchi model for solutions [1]:

$$Q = 2C_0\sqrt{D_m t / \pi}$$

where Q is the quantity of drug released per unit of diffusion surface, C_0 is the concentration of drug in the product matrix, D_m is the diffusion coefficient through the matrix and t is the time. The release profiles are presented in Figure 1a,b.

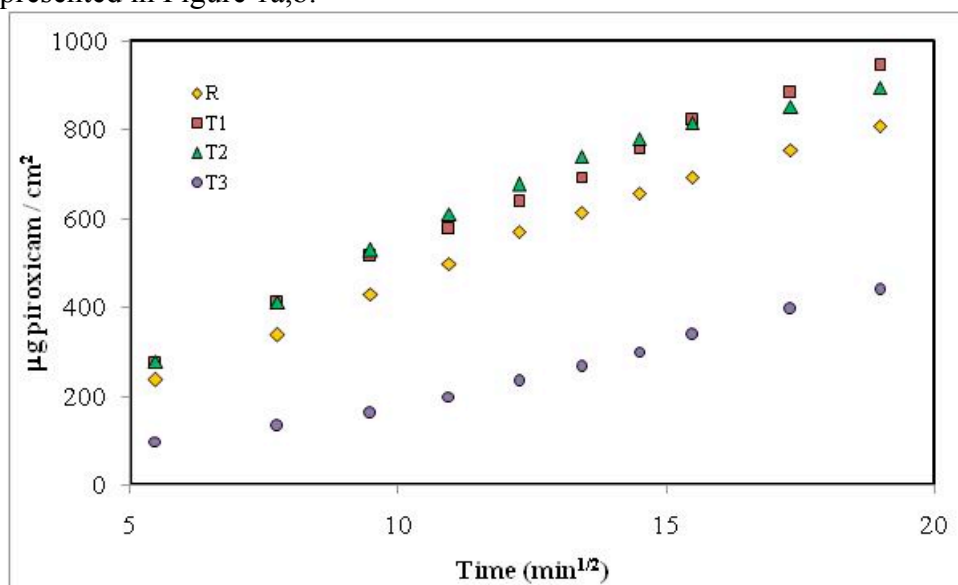


Figure 1a

In vitro piroxicam cumulative release profiles in hydro-alcoholic media, using a cellulose mix ester, hydrophilic membranes with 0.45 µm pore size

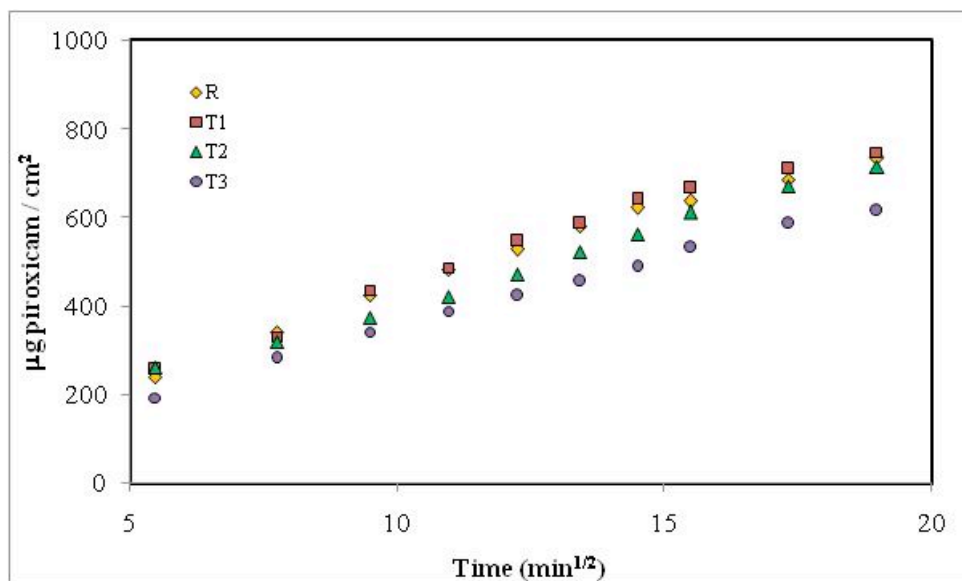


Figure 1b

In-vitro piroxicam cumulative release profiles in hydro-alcoholic media, using a polysulfone, hydrophilic membranes with 0.45 µm pore size

Both types of membranes discriminated between the compositions of the four products. The diffusion coefficient values ranged between 26.33 and 49.55 µg/cm²/min^{1/2} for the cellulose mix ester and between 31.35 and 37.93 µg/cm²/min^{1/2} for polysulfone membranes (Table II).

Table II

Membrane	Product	R	T1	T2	T3
Cellulose mix ester	Regression				
	Slope (µg piroxicam/cm ² /min ^{1/2})	43.107	49.553	46.519	26.334
	Intercept	17.838	28.339	73.905	-74.816
	Correlation coefficient	0.996	0.997	0.982	0.991
Polysulphone	Regression				
	Slope (µg piroxicam/cm ² /min ^{1/2})	36.608	37.926	35.284	31.358
	Intercept	67.161	64.253	48.805	36.657
	Correlation coefficient	0.992	0.992	0.997	0.998

The comparison of the release profiles was performed according to current recommendations, using the paired ratio matrix for the diffusion coefficient for each cell, ordered from the lowest to the highest. The similarity has been concluded based on the 8th and 29th test vs. reference ratio, extracted and converted to percentage, if fallen within 75 and 133% (5;10).

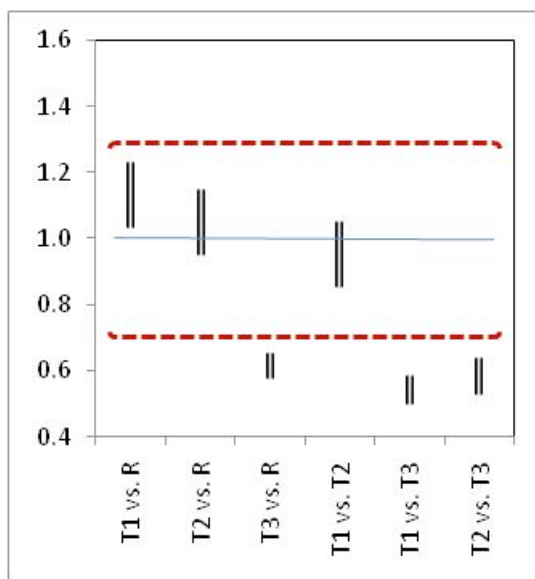


Figure 2

8th and 29th diffusion coefficient ratio, extracted and converted to percentage, obtained for cellulose mix ester membranes (dotted lines indicate the 75 – 133 % acceptance interval)

The most discriminatory conditions are induced by the cellulose mix ester membrane, clearly indicating the lowest diffusion coefficient values obtained for T3 and the similarity of the release profiles for the other three products. It must be pointed out the difference of regulatory approach in comparison of the release rate between the similarity metrics implemented for solid dosage forms and the ratio comparison for topical dosage forms. In the first case, a point to point evaluation of mean dissolution profiles obtained on several units of each product (8;9), the later comparing a regression parameter characterizing the entire release profile, between each unit of both tested products (10). No comparison of the release pattern, e.g. difference of profile in the initial 30 to 60 min time interval, is provided.

Nevertheless, the hydro-alcoholic media could be a better option for the large majority of hydrophobic active ingredients enclosed in topical dosage forms, providing better approach to the sink condition requirements, compared to widely used phosphate buffer, pH 5.4 to 7.2 or 7.4 (with doubtful physiological relevance, considering at least the complex composition and character of *stratum corneum*). Preliminary unpublished results from similar experiments conducted by our group indicate two interesting facts: the hydrophobic membranes are over-discriminatory when applied to creams and ointments and, due to the assumed inert character of the membrane, the clear *in vivo* role of absorption promoters, key

formulation components (2;3), cannot be revealed using currently accepted, SUPAC-based methodology.

It concerns the rheological profiles, all the investigated topical formulation displayed pseudoplastic characteristics, with more intense viscosity decrease at lower share rate values for T3.

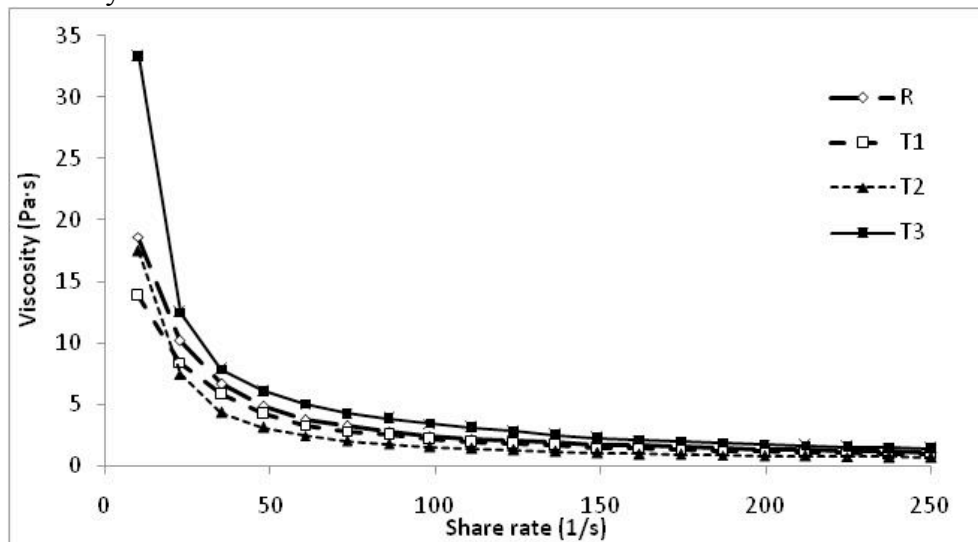


Figure 3
Rheological profiles of piroxicam topical drug products

In fact, VDC are lacking the share stress factor characteristic for the *in vivo* drug administration, which could be assumed as a release under-estimating factor (2;3;7). The recorded profiles were further fitted by Ostwald de Waele model (6):

$$\tau = m(\dot{\gamma})^n,$$

where τ is the share stress (Pa), $\dot{\gamma}$ is the share rate (s^{-1}), m is the flow consistency index ($Pa \cdot s^{-1}$) and n is the flow behavior index (adimensional).

Table III
Ostwald de Waele model parameters

Product	R	T1	T2	T3
m	59.88	217.33	133.46	156.13
n	0.34	0.01	0.04	0.13
Standard deviation	3.97	31.84	10.64	1.95
Stability index	0.9999	0.9826	0.9960	1.0000

The fitting results presented in table III confirm the pseudoplastic characteristics, by flow behavior index values lower than 1. Good

correlations were observed between the diffusion coefficient values and flow consistency index values ($R^2=0.994$ for cellulose mix ester membranes) in the case of the three products for which the release profiles similarity has been previously concluded (R, T1, T2). This fact further supports the importance of combined, diffusional and rheological evaluations, as valuable quality control and *in vivo* performance prognostic tools.

Conclusions

The simple procedure implemented for the evaluation of piroxicam *in vitro* release profile from hydrophilic semisolid matrix clearly marked out the formulations difference of the topical products. Although recommended to be an inert matrix within the diffusion process, the membrane influenced the release profile parameters, therefore it could be included as an important factor in the validation of rapid, discriminatory test for either batch to batch or SUPAC quality control. Nevertheless, the use of hydro-alcoholic receptor media could be considered a better approach compared to either saline isotonic solutions or plasma / skin pH-simulating buffer systems. Moreover, the good correlation obtained between diffusion coefficients and flow consistency indexes, although limited in sample size, could be integrated in the process of development and selection of formulation.

Acknowledgments

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