ORIGINAL ARTICLE

IN VITRO ASSESSMENTS OF POTENTIAL TOPICAL APPLICATION FOR ORGANOMETALLIC COMPLEXES OF COPPER WITH OXICAMS

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Abstract

The paper presents the assessment of the solubility, distribution coefficient and *in vitro* release from liquid and semisolid vehicles for organometallic complexes of copper with oxicam ligands. Aqueous buffers systems with physiological pH and biorelevant organic solvents such as n-octanol and isopropyl-myristate were used in developing simple experimental procedures. The substances were described as model entities for increased hydrophobicity and the results were discussed in terms of the utility and limitations of these methodologies for screening the feasibility of delivery across the skin.

Rezumat

Lucrarea prezintă analiza solubilității, a coeficienților de distribuție și a cedării *in vitro* din vehicule lichide și semisolide pentru complecși organometalici ai cuprului cu liganzi de tip oxicami. În dezvoltarea de proceduri experimentale simple s-au utilizat sisteme tampon apoase cu pH fiziologic și solvenți organici biorelevanți ca n-octanolul și izopropil-miristatul. Substanțele au fost descrise ca entități model pentru hidrofobicitate crescută, iar rezultatele au fost interpretate din perspectiva utilității și limitărilor acestor metodologii pentru evaluarea fezabilității distribuției prin piele.

Keywords: organometallic complexes, copper, topical application, *in vitro* release, semisolids

Introduction

Due to the particular nature of the biological barrier, the products applied onto the skin are subjected to a distinct physicochemical and bio-pharmaceutical characterization, as recommended by current regulation and guidelines [2, 5]. The qualitative and quantitative composition is considered to be critical, because the excipients are known to change, either in transient or permanent way, the structure and permeability of the *stratum corneum*, the first barrier that acts as the main, limiting and rate controlling interface. The depth of distribution and the amount of active pharmaceutical ingredient which is delivered from a liquid or semisolid vehicle, as well as the pattern of exposure of the pharmacological target, is controlled by several other factors [1]. The status of the skin (whether or not its integrity is altered by the pathological process and to which extent, the local temperature), the state of aggregation of the drug (completely solubilized, partitioned between two or more phases or partially suspended), the concentration gradient between the vehicle and first layer at the site of administration are only few factors mentioned in the description of this complex delivery across a highly specialized barrier.

The excipients selection takes into account not only their influence on the kinetics of permeation or depth of penetration, but also the fact that they display their own pharmacodynamic effects [2]. Therefore, the clinical endpoint studies are the reference approach in comparing two or more formulations. For the selection of the best candidates during the research and development phase, one should consider the thermo-dynamic activity of the drug, as well as the diffusional resistance that the active moiety will face from the vehicle, especially in case of a semisolid matrix. Depending upon the hydro-lipophilic balance and volatility, some excipients may evaporate or penetrate the skin to a different

extent. Their concentration within the thin layer applied onto the skin will continuously change, altering the solubility of the drug.

The importance of the *in vitro* release tests is continuously increasing. Despite the use of an artificial membrane as a support for a topical formulation applied in infinite dose and occluded conditions, it is assumed that the diffusion profile can adequately reflect the combined effect of several critical factors. This is the assumption on which an adapted Topical drug Classification System was proposed by Shah VP *et al.* [3, 4], which does not take into consideration the solubility and permeability characteristics of the drug. The current regulatory applications of the *in vitro* release limited only comparative evaluation of formulations with similar qualitative and quantitative composition [5], preventing the comparison across manufacturers with few notable exemptions [6]. However, it is frequently applied based on its assumed sensitivity for formulation factors. The utility in the selection of the most appropriate candidate for further *in vitro* skin permeation or *in vivo* assessment depends upon integration of other complementary assessments.

Many of the drugs intended to be delivered into or through the skin are lipophilic; therefore if a completely dissolved state is deemed necessary for rapid and consistent distribution from the pharmaceutical vehicle, solubility enhancers are the first choice. As mentioned earlier, one should consider their potential multiple roles, including promotion of absorption [10] but also changes in the inner interaction between the matrix components [11].

The current paper presents the results of the *in vitro* characterization of hydrophobic drugs, i.e. metal complexes of the oxicams, with prospected application onto the skin. The type of evaluations and the testing parameters were selected in order to illustrate the relations between the physicochemical properties of the drug, vehicle composition and *in vitro* release profiles, useful for adequate screening of topical delivery.

Materials and Methods

For the assessment of the distribution coefficients in biorelevant organic solvents at physiological pH, stock solution of the two organometallic compounds were prepared in dimethyl-sulfoxide (200 μg/mL). Working standards having a concentration of 80 μg/mL were prepared by dilution with aqueous buffer systems (phosphate buffers with pH 5.4 and 7.4, having a concentration of 50 mM and, respectively 10 mM). The final concentration of dimethyl-sulfoxide was 10% (v/v). Equal volumes of 600 μL of the working standard and organic solvent were transferred into Eppendorf polypropylene vials of 1.5 mL and sealed with paraffin. Previously, both buffer solution and organic solvents were reciprocally saturated for at least 24 hours using a volume percentage of 2%, in order to prevent further mass variation during the test. The vials were stirred for 240 minutes using a BiosanMultiBio RS-24 programmable rotator (Biosan, Latvia), using the following parameters: 100% vibrations, 8 seconds rotation at 90°, with 170 seconds agitation at 5°. For phase separation, the samples were centrifuged for 10 minutes at 14000 rpm and 32 or 37°C, in a Sartorius Sigma 2-16K equipment (Sartorius GmbH, Germany). Volumes of 400 μL were collected from the aqueous layer, immediately diluted with the corresponding buffer and homogenized using an IKA Genius vortex at 6000 rpm.

The distribution coefficients of the two compounds were calculated as the logarithm of the ratio between the concentration of the analyte in the organic *versus* aqueous layer ($LogD_{7.4}^{n\text{-}Oct}$ for n-octanol/phosphate buffer pH = 7.4 10 mM mixture, respectively $LogD_{5.4}^{IPM}$ for the isopropyl-myristate/phosphate buffer pH = 5.4 50 mM mixture).

The diffusion across the lipophilic barriers was assessed *in vitro* using a system of six static vertical diffusion cells (Hanson Microette™, Hanson Research Inc, United States). The individual volume was determined as difference between the individual units having the receptor compartment filled with purified water and the empty cells. As a general approach, typical physiological membranes were simulated using a hydrophilic membrane comprising mixtures of cellulose esters (Teknokroma® membrane filters, code TR-200240, batch 133895), soaked in either isopropyl-myristate or n-octanol for 60 minutes. At the end of the time interval, both sides of the hydrophobized interface was washed with the corresponding aqueous buffers, then attached to specific liquid adaptors as donor compartments and mounted on the cells, using adjustable clamps. The tests were conducted in triplicate, at 32 ± 0.5°C for mimicking the conditions of the skin (simulated by isopropyl-myristate), and at 37 ± 0.5°C (in case of n-octanol). The receiver having magnetic bars and attached stainless still helix was filled with degassed buffer systems under intense stirring. The individual cells were serially connected to a thermostatic circulator (EcoLaudaStarEdition, Lauda GmbH, Germany). Volumes of 3 mL of each working standard, previously diluted to 40 μg/mL, were heated to either 32 ± 0.5°C or 37 ± 0.5°C and slowly transferred on top of the membranes using an automated pipette. The upper donor was sealed with plastic screw caps. Samples of 0.5 mL were collected manually in a closed system and under static conditions at 15, 30, 45, 60, 90, 120, 150 and 180 minutes after initiation of stirring (400 rpm).

As a preliminary screening for the feasibility of topical application, two experimental semisolid formulations were prepared based on the same mixture of cellulose

derivatives, alcohols and solubilizing polymer. The preparation method included dispersion of the macromolecular agents (1.5% carboxymethyl cellulose and 1% hydroxypropyl-methyl cellulose) into the total quantity of water under mild stirring with storage at 4 - 8°C for 24 hours for complete swelling and structuring. After neutralization with triethanolamine (0.5%), the resulting gel was added to the alcoholic mixture containing a dispersed block polyoxypropylene-polyoxyethylene copolymer (either Poloxamer 407 for compositions coded F1 or Poloxamer 188 for formulas coded F2) and the organometallic complex (0.574% piroxicam-copper, respectively 0.569%). All specified amounts of ingredients correspond to their final concentrations in the semisolid vehicle. The *in vitro* evaluation protocol was the same as described previously, with adaption in terms of dosing in Teflon wafers with occluding glass disks and receptor solution (50% absolute ethanol in water, v/v). The artificial membranes were used without pretreatment, except for a preliminary soaking in the receiver. For the calculation of in release parameters, the Higuchi (square root) model was applied [7].

The quantitative evaluation of each organometallic compound in the collected or processed samples was performed using a double beam, single monochromator Jasco spectrophotometer model V-530, equipped with VWS-580 Spectra Manager software. The calibration samples were prepared by sequential dilution of the stock solution in each receptor media. The specific absorption maxima λ_{max} was within the 355 and 360 nm interval.

All reagents were of analytical grade and used as received. The organometallic complexes were synthesized and characterized at the National Institute for Chemical - Pharmaceutical Research and Development - ICCF Bucharest, Romania.

Results and Discussion

The results of the solubility assessment were comparable and below 1 μg/mL, with the notable exception of meloxicam complex in phosphate buffer pH = 7.4 (Table I). This confirmed the hydrophobicity of the two compounds, related to changes in the acidic character following complexation. The hydroxyl group specific to carboxyamide-enolic ligand is blocked (Figure 1), therefore the pH dependence of the solubility in aqueous buffers was reduced.

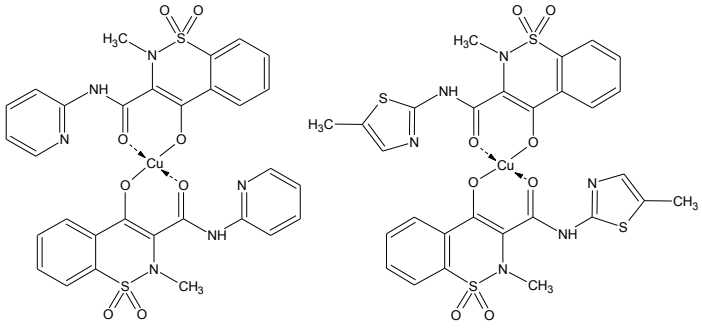

Figure 1.
Possible chemical structures for the piroxicam-copper (left) and meloxicam copper complexes (right)

In a correlated manner, the values of the distribution coefficient were much higher compared to the corresponding organic ligand [7]. The composition of the binary mixture had only a mild influence of the experimental data for the piroxicam complex ($LogD_{7,4}^{n\text{-}Oct}$ = 1.69, $LogD_{5.4}^{IPM}$ = 0.97). This may be due to particular interactions developed by the exposed moiety with n-octanol, including the increased ability of the biorelevant organic solvent to develop hydrogen bonds [12].

Table I
The results of the *in vitro* characterization of the organometallic compounds

Compound	Piroxicam-copper		Meloxicam-copper	
Distribution coefficient (LogD)	1.6902 ± 0.0277	0.9706 ± 0.0218	1.3310 ± 0.0146	1.2386 ± 0.0162
Solubility in the receiver (μg/mL)	0.3667 ± 0.0115	0.2500 ± 0.0100	1.1167 ± 0.0058	0.3500 ± 0.0350
Organic solvent used for pretreatment	n-Octanol	Isopropyl-myristate	n-Octanol	Isopropyl-myristate
Receptor medium (Phosphate buffer)	pH = 7.4, 10 mM	pH = 5.4 50 mM	pH = 7.4, 10 Mm	pH = 5.4 50 mM
Diffusion coefficient ($\mu g/cm^2/min^{1/2}$)	0.2333	0.2066	0.6610	0.2487
Lag time ($min^{1/2}$)	2.8943	5.1989	1.4600	1.7144
Correlation coefficient (R^2)	0.9973	0.9933	0.9958	0.9974

On the other hand, the lowest solubility combined with a decreased value of the distribution coefficient observed in the phosphate buffer pH 5.4 – isopropyl-myristate binary mixtures generated a decreased

diffusion rate with highest lag time during the *in vitro* release testing (5.2 $min^{0.5}$). It is important to point out that the latter parameter illustrates the kinetic particularities in reaching the steady state transfer across the membranes. In this experimental design, the interface is not an inert barrier, separating the two compartments. More than that, it represents a distinct, slowly equilibrating compartment and is apparently acting as a reservoir for the receiver (Figure 2). A series of partition processes occurs which are only partially controlled by the solubility in the buffer systems. A direct correlation was concluded between the solubility and the values of the diffusion coefficient ($R^2 > 0.99$).

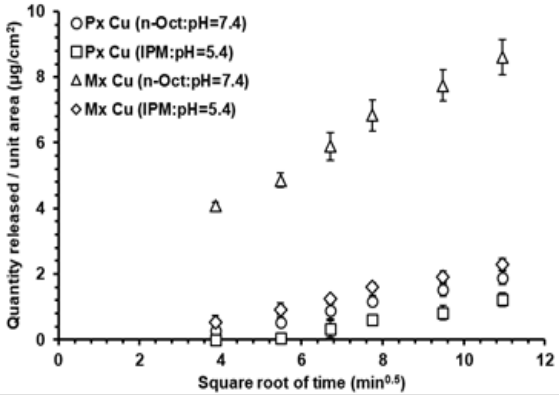

Figure 2.

The mean *in vitro* diffusion profiles of the oxicam-copper complexes from solutions across model hydrophobic membranes (mean ± standard deviation, n = 3)

Px – piroxicam; Mx – meloxicam; n-Oct – n-octanol; IPM – isopropyl-myristate. The pH value corresponds to the buffer system used as receptor medium.

However, both donor and receptor were solutions of the same analyte in the same solvent; therefore the relative affinity of the analyte between the aqueous and organic layers is the limiting factor.

The relevance of these findings relies on the common use of n-octanol or isopropyl-myristate for screening the partition across the lipid barriers. In terms of *in vitro* release, the latter one has been considered for simulation of skin properties [8]. At the same time, it provides an adequate contact angle between the lipid vehicles and hydrophilic receptor media, therefore facilitating the attainment of steady state transfer [9]. It should be considered that prolonged lag time usually indicates that separating membranes, rather than the formulation controls the process; therefore the results are not direct descriptors of interactions within a semisolid formulation. The selection of testing parameters should rely on the aims of the study. The previously mentioned difference in solubility for organometallic complex of meloxicam points out that the frequent selection of an aqueous buffer with pH of 7.4 (e.g. phosphate buffer saline) may not be reflective for the *in vivo* conditions, especially at the surface of the skin.

The evaluation of the *in vitro* release from the gel formulations was included in the experimental plan with changes in the testing parameters. The use of a hydro-alcoholic mixture provided sink conditions throughout the test duration, whereas the inert membrane without pretreatment with organic solvent precluded its reservoir function. In this standard approach, the lag time was decreased (0.93 to 2.85 $\mu g/cm^2/min^{0.5}$; Table II), considering the supplementary diffusional resistance expressed by the semisolid matrix.

The diffusion profiles were majorly altered by the composition only in case of meloxicam-copper complexes, the difference in the grade of the solubilizing block-copolymer generating a ratio of 2.5 for the corresponding release rates.

Table II

The mean *in vitro* diffusion profiles of the organometallic compounds from the topical semisolid formulations (n = 3)

Formulation	**F1**		**F2**	
Compound	Piroxicam-copper	Meloxicam-copper	Piroxicam-copper	Meloxicam-copper
Diffusion coefficient ($\mu g/cm^2/min^{1/2}$)	2.84	8.90	2.72	22.75
Lag time ($min^{1/2}$)	0.93	2.47	1.93	2.85
Correlation coefficient (R^2)	0.9935	0.9938	0.9890	0.9947

The release process was adequately described by the Higuchi model throughout the 180 minutes of the test, indicating that the diffusion occurs from a pseudo-infinite reservoir across a non-limiting support into a sink compartment (Figure 3).

However, minor shifts of the mean profiles during the last 60 minutes indicated that back diffusion, especially of the ethanolic component of the receiver, might have occurred. This observation is common for anhydrous compositions, as well as for the formulations with a reduced content of free water.

The results indicate that the delivery of hydrophobic entities from semisolid vehicles needs not only adapted selection of the composition, but also careful selection of the experimental conditions. The assessment of solubility and distribution coefficients in a relevant setup may point out potential limiting steps of the *in vivo* delivery. Increased lipophilicity may trigger significant storage of the active moiety within the *stratum corneum* or deeper layers. Many absorption promoters display also a solubilizing effect, but their effect is dependent on both amount and dose (quantity *per* surface unit).

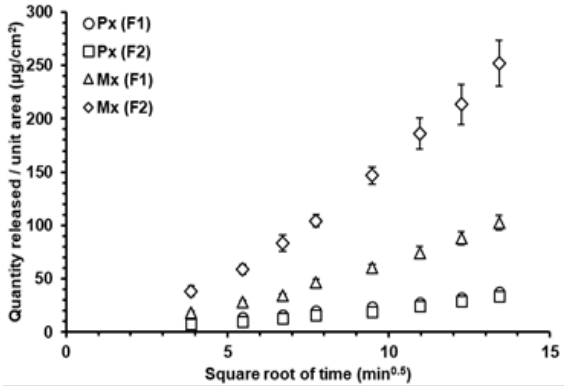

Figure 3.
The mean *in vitro* release profiles of hydrophobic compounds from experimental topical formulations (mean ± standard deviation, n = 3)
Px – piroxicam; Mx – meloxicam

Large quantities of volatile lower alkanols are usually used for lipophilic compounds, but evaporation without concurrent delivery of the active ingredient may induce advanced precipitation onto the skin and decreased bio-availability. The distribution into the skin is not always the goal of topical application [13]. The depth of the final pharmacological target and the exposure pattern (both amount and rates) need to be considered. The storage into the upper layer and slow distribution at low concentrations may not lead to effective treatment. The use of an artificial membrane in case of topical dosage forms with high quantities of active excipients may seem useless, even for the research and development phases. However, it represents a simple and reliable approach for analysing the thermo-dynamic activity of the drug, i.e. whether or not the semisolid formulation is able to deliver it toward the skin.

Conclusions

A series of *in vitro* evaluations was performed for screening the feasibility of topical application of organometallic complexes of copper with oxicam ligands. This included the biorelevant assessment of aqueous solubility and distribution coefficient in various mixtures of organic solvent and buffers, as well as *in vitro* release studies from solutions and gel systems. The results indicated that the hydrophobicity of the ligand is increased by coordination and controlling the distribution across the lipophilic barrier. The membranes pre-treated with organic solvents such as n-octanol or isopropyl-myristate may be used for simulation of the skin reservoir function, whereas the use of inert interfaces combined with sink conditions may reflect the ability of the vehicle to deliver the drugs toward the skin.

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