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IN VITRO EVALUATION OF SEMISOLID TOPICAL FORMULATIONS CONTAINING ORGANOMETALLIC COMPLEXES OF COPPER AND COBALT WITH OXICAMS

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ABSTRACT: The paper presents the results of correlated in vitro release and rheological assessment of hydrophilic gels containing organometallic complexes of copper and cobalt with piroxicam and tenoxicam. The semisolid experimental formulations intended for local delivery after application onto the skin were prepared based on polyoxyethylene-polyoxypropylene copolymers (Poloxamer 407). The results confirmed that the diffusion process of the hydrophobic compounds had long lag-times and release rates depending upon the affinity of the complex for the semisolid matrix. Higher consistency due to particular internal interactions generated decreased release rates. All formulations presented a pseudoplastic behavior, adequately described by Ostwald de Waele model.

Keywords: organometallic complexes, cobalt, copper, oxicam ligand, in vitro release, hydrogels.

INTRODUCTION:

The in vitro testing is currently described as a simple, reliable and relevant methodology for the quality control and assessment of the performance of topical semisolid formulations (SUPAC-SS, 1997; USP 1724, 2016). The qualitative and quantitative compositions are key factors which determine the state of aggregation for the active pharmaceutical ingredient, as well as the nature and amplitude of changes that the excipients may induced in contact with the skin. One of the critical process that influences the bioavailability for topical application is the transformation occurring into the delivering matrix. This points out the role of the microstructural assessments, usually performed by a set of rotational or oscillatory measurements (USP 912, 2016). In brief, the vehicle response to controlled, ideally relevant stress or strain, is recorded and discussed in terms of changes that may occur during and after in vivo administration. For accurate interpretation of the results, it is important to consider not only the pattern and amplitude of deformations induced by the thermal or mechanical stress, but also the subsequent dynamic changes of both arrangement of the matter and composition (Chank RK *et al.*, 2013a,b).

The paper presents the results obtained by correlated in vitro release and rheological assessment of hydrophilic gels containing organometallic complexes of copper and cobalt with piroxicam and tenoxicam, as extreme case where hydrophobic drugs request specific formulations factors and generates distinct interactions with the semisolid vehicle.

MATERIALS AND METHODS:

The preparations of hydrogels with organo-metallic complexes of oxicams

The organometallic complexes of copper and cobalt with oxicams were synthesized by a research group at the National Institute for Chemical Pharmaceutical Research and Development (INCDCF-ICCF, Bucharest, Romania), as part of a project financed by National Authority for Scientific Research. Details on the sythesis and physicochemical characterization were previously presented by Niță S *et al.* (2011). Based on preliminary evaluation of the solubility profile (Tudosa PC *et al.*, 2017), Poloxamer 407 (a polyoxyethylene-polyoxypropylene copolymer) was selected for the design of hydrophilic gels, based on its biocompatible and tensioactive properties. The qualitative and quantitative compositions are detailed in table I, the main difference being the co-solvent mixture containing absolute ethanol andone of the following non-ionic tensioactives: isopropyl myristate (Scherlau Chemie SA, Spain), Cremophor EL (PEG 35 Castor oil ;BASF SE, Germany), Saboderm G20 (dodecanol-octyl; SABO S.p.A., Italy) or Saboderm SHO (PEG-7 cocoate glyceryl and PEG-200 hydrogenated palmitate glyceryl; SABO S.p.A., Italy).

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Tab. 1.
The qualitative and quantitative compositions of topical semisolid hydrogels (g)

Excipient	**F1**	**F2**	**F3**	**F4**
Poloxamer 407	20	20	20	20
Ethanol	10	10	10	10
Isopropyl myristate	5	-	-	-
Cremophor EL	-	5	-	-
Saboderm G20	-	-	5	-
Saboderm SNO	-	-	-	5
Purified water ad	100	100	100	100

The first phase of preparation was the dispersion of each substance into the mixture of alcohol and non-ionic tensioactive compound. The quantity of active substance was calculated by equimolarity with a theoretical formulation containing 0.5% of the oxicam ligand and further confirmed by the preclinical screening outcome. The block copolymer was dispersed in purified water, the amount corresponding toan initial concentration of 25%. The hydrated macromolecular agent was maintained at 4-8°C for 24 hours. The alcoholic dispersion of organometallic complex of copper with oxicam was mixed with hydrated copolymer using a mechanical stirrer (Heidolph RZR 2020, Heidolph GmbH, Germany) at 2000 rpm. The required amount of water was added and the resulting hydrogel was maintained 24 hours at room temperature.

The evaluation of the *in vitro* release profiles

The assembly of 12 serially connected vertical diffusion cells of 12 ml (Hansson Research Inc., United States) was used for the assessment of the in-vitro release profiles. The receptor media was a mixture of ethanol:water (50:50, v:v). The selection of the composition was performed based on a preliminary screening of the solubility profile for the four analytes and on the physicochemical characteristics of the organometallic complexes of copper with oxicams, in order to provide the sink conditions. Adequate degassing of the medium was achieved by filtration under vacuum (800 mBar) through 0.45 μm hydrophilic membranes (cellulose acetate filters, Sartorius Stedim Biotech GmbH, Germany).

Hydrophilic polysulphone membranes (Tuffryn®, Pall Life Sciences HT-450; 25 mm diameter, 0.45μm mean pore size) were soaked into receptor medium for at least 30 minutes and used as inert support of the hydrogels. All the experiments were performed in triplicates. The application of the experimental formulations into occluded dosage wafers was performed after gentle removal of the superficial receptor media. Each static cell was equipped with magnetic stirrers with mounted stainless-steel helix. The temperature was maintained at 32±0.5°C. The sampling schedule was: 15, 30, 60, 90, 120, 150 and 180 minutes after the starting point. The agitation was stopped during the sampling procedure. 1mL of receptor media was injected during 1 minute, in order to avoid supplementary pressure onto the membrane and further diffusion of the hydro-alcoholic mixture into the donor. In a closed circuit, during the manual injection of the replacing medium the first 0.5mL was discarded, whereas the second 0.5mL were collected for quantitative analysis.

The quantitative determination of organometallic complexes of copper with oxicams

The quantitative determination of organometallic complexes of copperand cobalt with piroxicam and meloxicam was performed based on a spectrophotometric method, using an Agilent 8453spectrophotometer (Agilent Instruments, Germany) with a series of 8 quartz cells (10.00 mm path) and a diode-array-detector. The analysis was performedusing the specific maximum absorption wavelength. The calibration samples were prepared starting from a stock solution in dimethyl-sulfoxide (400 μg/mL), by sequential dilution with the receptor medium. The samples collected during the in vitro release studies were subject to adequate dilution.

The analysis of experimental data

The diffusion coefficient was calculated as the slope of the regression line obtained by plotting the quantity of the oxicam per surface unit(μg/cm^2) vs. the square root of time (Higuchi model):

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

where Q is the amount of substance (organometallic complex of copper with oxicams) diffused per surface unit, C_0 is the initial concentration in the donor compartment (pharmaceutical formulation), D_m is the diffusion coefficient through the semisolid vehicle and t is time.

The evaluation of the rheological behavior

A rotational viscometer equipped with the SV - DIN cylinder, Haake ViscoTester VT550 (Thermo Electron GmbH, Germany) was used for the evaluation of the structural characteristics of the topical semisolid formulations. The interval of shear rates applied in two consecutive rumps was 0 - 25 1/s (n=6) and the sample volume was 10 ml. The deformation profile was recorded at the storage temperature and fitted with the Ostwald de Waele model: $\tau = K\cdot(\gamma)^n$, whereτ is the

shear stress and $\dot{\gamma}$ is the shear rate. The flow (n) and consistency (K) indexes for each run were calculated automatically for the rump up segment, using the Haake RheoWin Data Manager version 4.63 (Thermo Fisher Scientific, Germany).

RESULTS AND DISCUSSION

Quantitative determination of the organometallic complexes

The overlaid absorption spectra for the calibration samples of the four compounds in the hydro-alcoholic mixture used as receptor media are presented in figure 1.

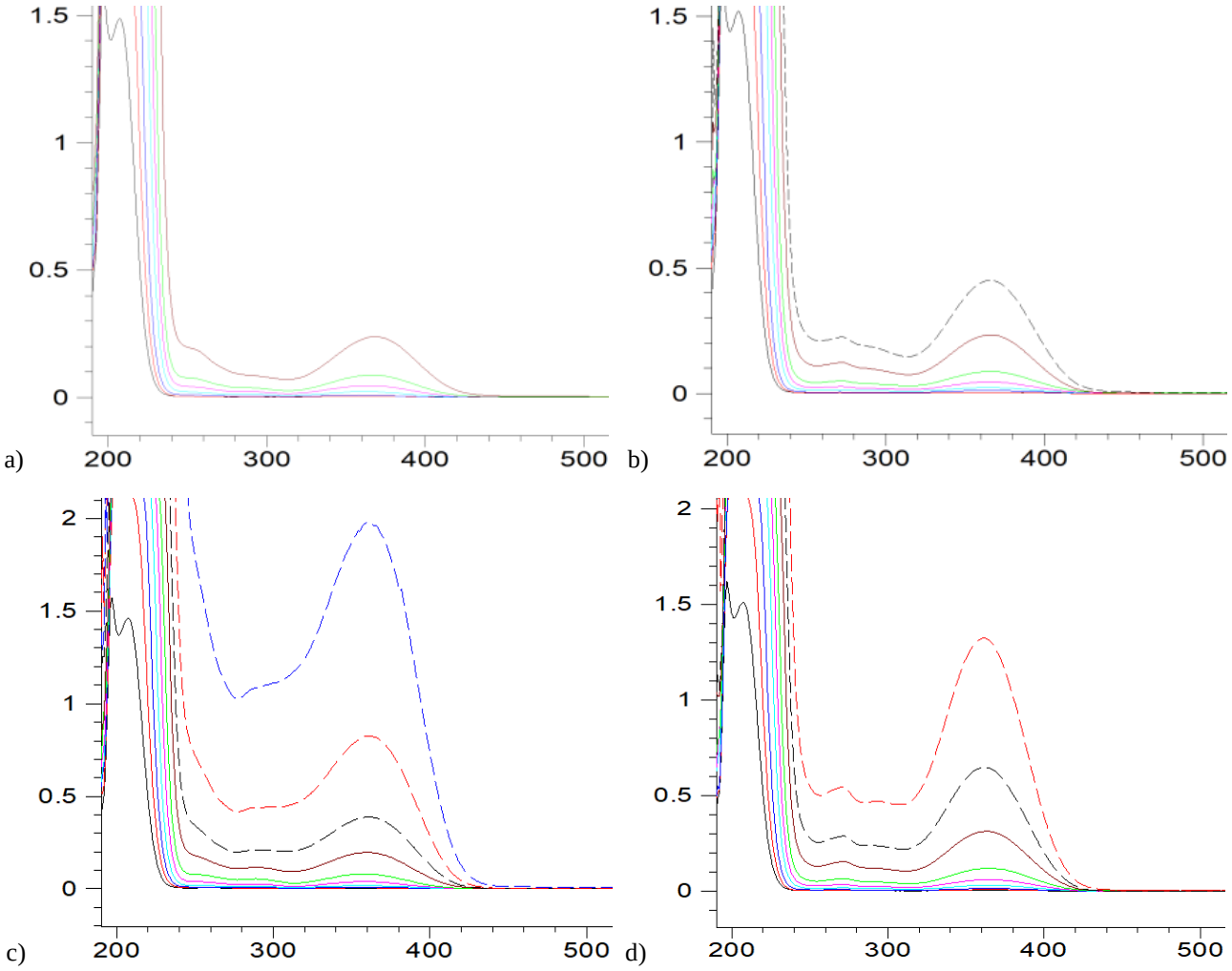

Fig.1. Overlaid absorption spectra of the organometallic complexes in the hydro-alcoholic mixture: a) piroxicam-copper (λ_{max}=366 nm); b) meloxicam-copper (λ_{max}=366 nm); c) piroxicam-cobalt (λ_{max}=361 nm); d) meloxicam-cobalt (λ_{max}=362 nm).

Analysis of the mean *in vitro* release profiles

The *in vitro* release profiles presented significantly kinetic differences due to variable composition and structural factors. The equilibrium of transmembrane diffusion process was attained slowly. The highly hydrophobic characteristics of the organometallic complex determined very low quantities of active compound dissolved in semisolid vehicle. The hydroxyl groups of oxicams were blocked and didn't exhibit theirweak acidic behavior.

In case of F4 formula for copper complexes, containing a mixture of non-ionic tensioactive substances, PEG-7 cocoate glyceryl and PEG-200 palmitate glyceryl, the observed lag-time were influenced by the nature of the organic ligand. The partition of the two complexes for each metal between the lipophylic internal phase and the hydrophilic external phase was comparable (figure 2).

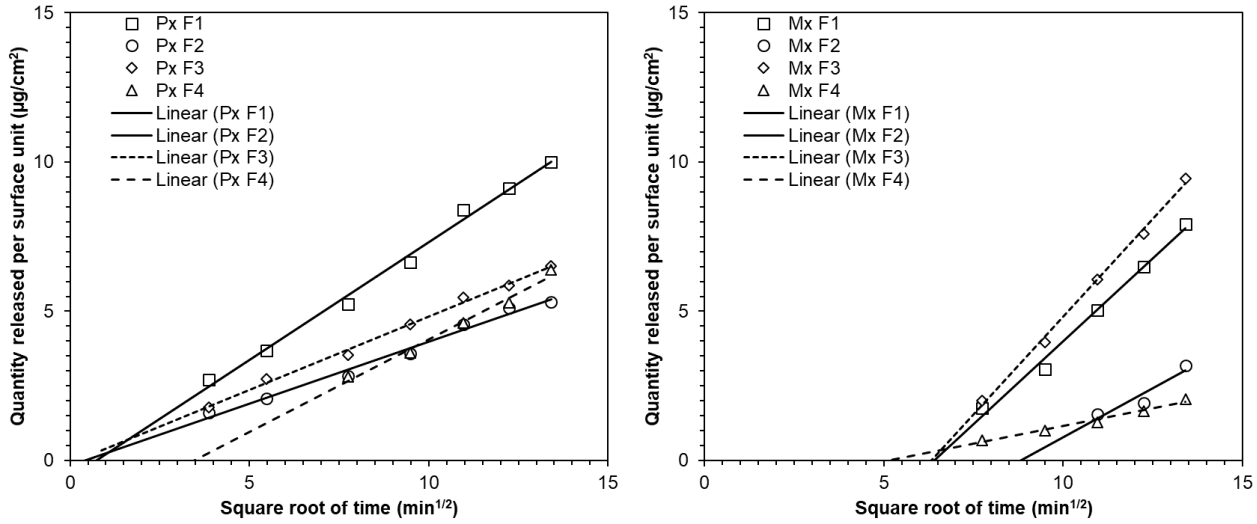

Fig.2. The mean in-vitro release profiles of copper organometallic complexes with oxicam ligand from the experimental formulations (n=3; standard deviations not included for clarity of the graphs)

Probably due to a distinct interaction within the organometallic complex of cobalt, the exposed moiety of the ligand provided a higher thermodynamic activity, transposed into higher in vitro release rates (up to 12.09 $\mu g/cm^2/min^{0.5}$; figure 3). Moreover, the variability of the experimental data was decreased, but preserving the high values of the lag time (above the usual threshold of 10% of the total test duration). This is probably due to slow equilibration of the transfer, but also to the initial microstructural changes that occur within the semisolid donor (transition from 25 to 32°C).

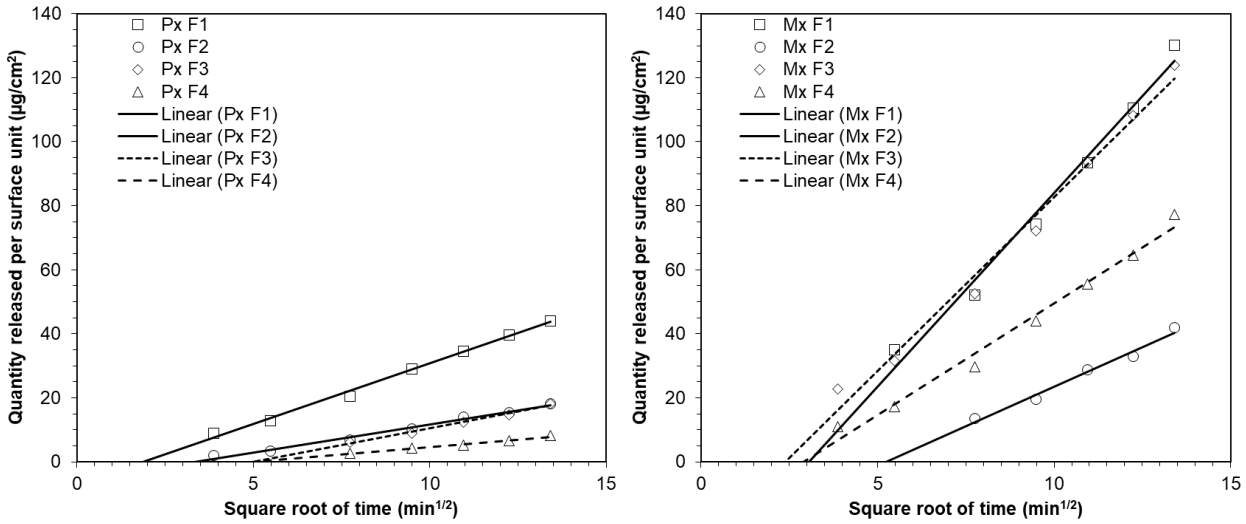

Fig. 3. The mean in-vitro release profiles of cobalt organometallic complexes with oxicam ligand from the experimental formulations (n=3; standard deviations not included for clarity of the graphs)

In some cases, the correlation coefficient for the linear regression performed according to the Higuchi model indicated that the steady state release occurred only after 60 minutes, meaning that delivery across the artificial membranes is biphasic (Table 2).

Tab. 2.
The parameters of the Higuchi model applied to the mean in vitro release profiles

Metal	Ligand	Formulation	Diffusion coefficient ($\mu g/cm^2/min^{1/2}$)	Lag time ($min^{1/2}$)	R^2
Copper	Piroxicam	F1	0.79	0.80	0.9966
		F2	0.41	0.52	0.9927
		F3	0.49	0.22	0.9978
		F4	0.62	3.56	0.9926
	Meloxicam	F1	1.11	6.44	0.9957
		F2	0.66	9.17	0.9479
		F3	1.31	6.35	0.9985
		F4	0.24	5.25	0.9914
Cobalt	Piroxicam	F1	3.79	1.92	0.9974
		F2	1.75	3.39	0.9941
		F3	2.10	5.11	0.9932
		F4	0.93	5.03	0.9950
	Meloxicam	F1	12.09	3.16	0.9925
		F2	4.93	5.35	0.9910
		F3	10.87	2.48	0.9932
		F4	6.97	2.99	0.9927

Evaluation of the rheological profiles

The rheological profiles are presented comparatively in figure 4. Overall, the influence of the two complexes on the microarrangement of the hydrogels is distinct, probably due to their particular physicochemical properties. For the same shear rate, the viscosity was higher in case of copper complexes, which correlates with higher values of the consistency index and decreased in vitro release rates.

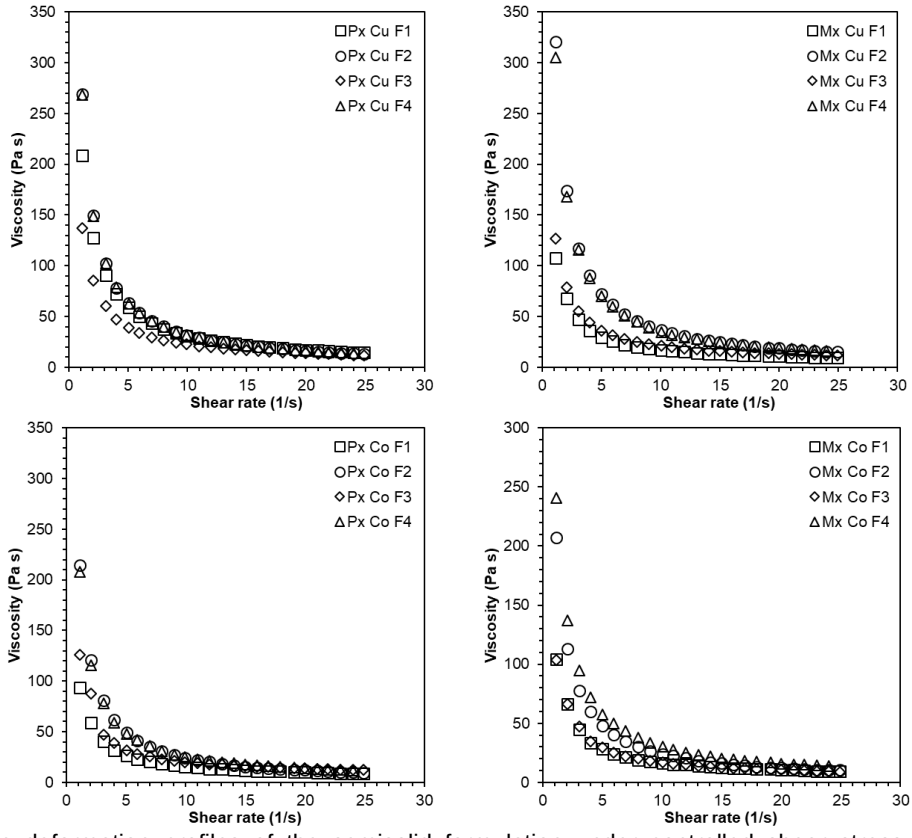

Fig. 4. The mean deformation profiles of the semisolid formulation under controlled shear stress (rump up of the hysteresis loop test)

The deformation profiles revealed the pseudoplastic behavior. The Ostwald de Waele model was applied and all the values calculated for the flow index were lower than 1 (table III).

Tab. 3
The parameters of the Ostwald de Waele model applied to the mean rheological profiles

Metal	Ligand	Formulation	K (Pa/s)	n (s^{-1})
Copper	**Piroxicam**	**F1**	230.48	0.1438
		F2	297.67	0.0357
		F3	149.22	0.1868
		F4	297.38	0.0334
	Meloxicam	**F1**	230.48	0.1438
		F2	297.67	0.0357
		F3	149.22	0.1868
		F4	297.38	0.0334
Cobalt	**Piroxicam**	**F1**	101.86	101.86
		F2	253.43	253.43
		F3	134.52	134.52
		F4	229.12	229.12
	Meloxicam	**F1**	45.08	0.6120
		F2	18.49	0.4198
		F3	50.16	0.6094
		F4	19.24	0.4087

The nature of the tensioactive agent significantly influenced the internal structure of the hydrogels. A possible explanation could be the interaction with the forming structure copolymer. The poloxamer exhibits good tensioactive properties favorable to the dissolution and dispersion of the active substance, but also may change the orientation and, consequently, the relative arrangement of other components.

CONCLUSIONS:

The organometallic complexes of copper and cobalt with oxicam ligands were conditioned as hydrophilic gels for topical administration based on polyoxyethylene-polyoxypropylene copolymer. The minimum amount of cosolvents was used, including 10% of ethanol and 5% of non-ionic tensioactiv agent or standardized mixture of tensioactive substances. The in vitro diffusion profiles revealed very low release rates, depending upon the composition and the viscosity of the semisolid matrix. The lag time of the in vitro release processes was correlated with the reduced solubility of the organometallic complexes both in the formulation and in the receptor media. All the formulations displayed pseudoplastic behavior. The Ostwald de Waele model described accurately the rheological profiles and all the values calculated for the flow index were lower than 1.

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