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CORRELATED EVALUATIONS OF *IN VITRO* RELEASE PROFILES AND STRUCTURAL PARAMETERS FOR TOPICAL SEMISOLID DOSAGE FORMS CONTAINING METRONIDAZOLE

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ABSTRACT: The *in-vivo* performance of topical semisolid dosage forms is determined by a series of factors, related to the thermodynamic activity of the active pharmaceutical ingredient, the history of the formulation, as well as the complexity of interaction between various components of the vehicle and the complex biological barrier. The paper presents the role of correlated *in vitro* release (IVR) tests and structural analysis in the comparative quality assessment of topical formulations containing metronidazole in various concentrations. A simple *in vitro* evaluation protocol was implemented, based on previous reports relating the release rate with *in vivo* dermatopharmacokinetics. The similarity was assessed using a compendial nonparametric statistical method for log slopes (Wilcoxon Rank Sum/Mann Whitney rank test). The experimental data confirmed that IVR tests were discriminatory for the composition and manufacturing factors. In some cases, scaling the value of the diffusion rate with the label claimed dose strength led to similarity. The results of hysteresis loop test revealed a significant impact of structural parameters on the IVR, induced by composition variables.

Keywords: metronidazole, *in vitro* release, topical semisolids, vertical diffusion cells, hysteresis loop test.

INTRODUCTION:

The *in vitro* release (IVR) tests have been initially adopted by the U.S. Food and Drug Administration for the evaluation of composition and manufacturing changes occurring in the lifecycle of a topical semisolid product (SUPAC-SS, 1997). The modifications having a possible impact on the *in vivo* performance were classified as level 2 and are routinely assessed by this type of methodologies, as well as the site-to-site transfer of the technological process. Although not directly predictive of bioavailability or bioequivalence (USP37/NF32, 2014), the experimental data generated on diffusion cells may provide valuable informations on the role of various factors, including particle size of the active entity or droplet size of the internal phase, solubility of the drug in the vehicle and the structure of the matrix etc.

Based on the lack of sensitivity of clinical end-point studies for the formulation factors, the regulatory attitude is to replace this approach for demonstration of bioequivalence for topical semisolids with a battery of tests providing complementary data with respect to quality and / or performance (Yacobi *et al.*, 2014). A specific classification system was proposed recently, based on IVR as a key decision tool (Shah *et al.*, 2015). Moreover, our group demonstrated previously the utility of IVR for understanding apparently discrepant results obtained using various *in vivo* pharmacokinetic protocols (Miron *et al*., 2014). Several questions have been raised on the sensitivity of this simple, reliable and robust technique for the factors likely to interfere with the distribution of a specific drug into the deeper layers of the skin. The aim of the current paper was to investigate the relationship between manufacturing (composition and process) variables of registered topical semisolid dosage forms, the structural consequences and IVR profiles, for topical semisolid dosage forms containing metronidazole.

MATERIALS AND METHODS:

In vitro release testing procedure

Five topical hydrogels were purchased from commercial sources. All of them were registered on the European market, details on the qualitative composition and assigned code being presented in table 1. R1 and R2 represented two batches of the reference product, presumably manufactured in different sites. The generic formulations T1, T2 and T3 presented various degrees on non-similarity with regard to the nature of excipients. No information was available on the quantitative composition, beside the concentration of metronidazole (0.75%, except for T1, 1%).

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Table 1.
The qualitative composition of the evaluated topical dosage forms of metronidazole

Assigned code	**R1, R2**	**T1**	**T2**	**T3**
Metronidazole (%)	***0.75***	***1.00***	***0.75***	***0.75***
Carbomer	V	V	V	-
Hydroxypropyl methylcellulose	-	-	-	V
Octyldodecanol	-	V	-	-
Caprylocaproyl macrogol-8 glycerides	-	V	-	-
Propylene glycol	V	-	V	V
Sodium hydroxide	V	V	V	-
Disodium edetate	V	V	V	-
Phenoxyethanol	-	-	-	V
Methyl parahydroxybenzoate	V	V	V	-
Propyl parahydroxybenzoate	V	-	V	-
Purified water	V	V	V	V

The IVR methodology used in the present study was the same as previously reported (Miron *et al.*, 2014). Briefly, a system composed of vertical diffusion cells (12 mL nominal volume, Hanson Microette, Hanson Research Inc., US) was used in combination with 0.45 μm polysulfone membranes (Tuffryn®, Pall Life Sciences) and hydroalcoholic mixture as receiver (30% absolute ethanol, degassed by filtration). The tests were performed at 32±0.5°C, on 6 individual cells for each product and using occluded conditions. Manual sampling was performed for 360 minutes after application of the drug product and initiation of stirring. The quantitative assessment of the metronidazole amounts recovered in the receiver was performed using a spectrophotometric method, following appropriate dilution (Agilent 8453 spectrophotometer, Agilent Instruments, Germany). The analytical standard of metronidazole and the absolute ethanol were purchased from Sigma Aldrich. The purified water was obtained by reverse osmosis and had a conductivity lower than 0.2 μS/cm.

Analysis of the *in vitro* release profiles

The release rate was calculated according to the Higuchi model, using the guidance-recommended approach (SUPAC-SS, 1997). The R1 coded batch was used as a reference. The selection of time interval for release rate calculation was based on the values of the corresponding correlation coefficient. The *in vitro* similarity assessment was based upon the compendial nonparametric statistical method for log slopes (Wilcoxon Rank Sum/Mann Whitney rank test; USP37/NF32). For the higher strength T1 generic formulation, the procedure was performed with or without dose scaling.

Evaluation of the structural parameters

A volume of approximately 10 mL of each hydrogel was subject to a hysteresis loop test at 25°C (shear rate interval 0 - 25 1/sec; Thermo Haake VT550 rotational viscometer, Thermo GmbH, Germany). Each deformation profile (n=6) was determined using a 250 sec rump-up and rump-down, separated by a 10 sec constant shear at the highest rate. The formulations were allowed to rest for at least 5 minutes between consecutive tests.

RESULTS AND DISCUSSION:

The characteristics of the *in vitro* release profiles

The *in vitro* release profiles obtained using static vertical diffusion cells were typical for semisolid formulations containing a hydrophilic drugs. The rate of the diffusional process was high, with variable lag times. Independently on the composition variables, the steady state transfer across the artificial membrane was observed after less than 10% of the total test duration, which clearly proved that the membrane didn't oppose a significant resistance, i.e. it was not the rate limiting element. Initially, the release process occurred according to the Higuchi model. The amounts of metronidazole diffused across the membrane per unit area increased linearly with the square root of time (correlation coefficients higher than 0.99). In the following time interval, the kinetics were different. This observation corresponded to an amount of drug released overpassing a 60% threshold from the content of the occluded donor compartment (figure 1). The advanced depletion justified the selection of a shorter time interval for the evaluation of the release rates, with sufficient sampling points available. A plateau region was visible after 180 minutes for the two generic products containing 0.75% metronidazole (T2 and T3), whereas for the 1% dosage form the diffusion was much slower. The two batches of the reference product had a similar performance, although the slightly lower amounts of metronidazole were recovered in the receiver for R2.

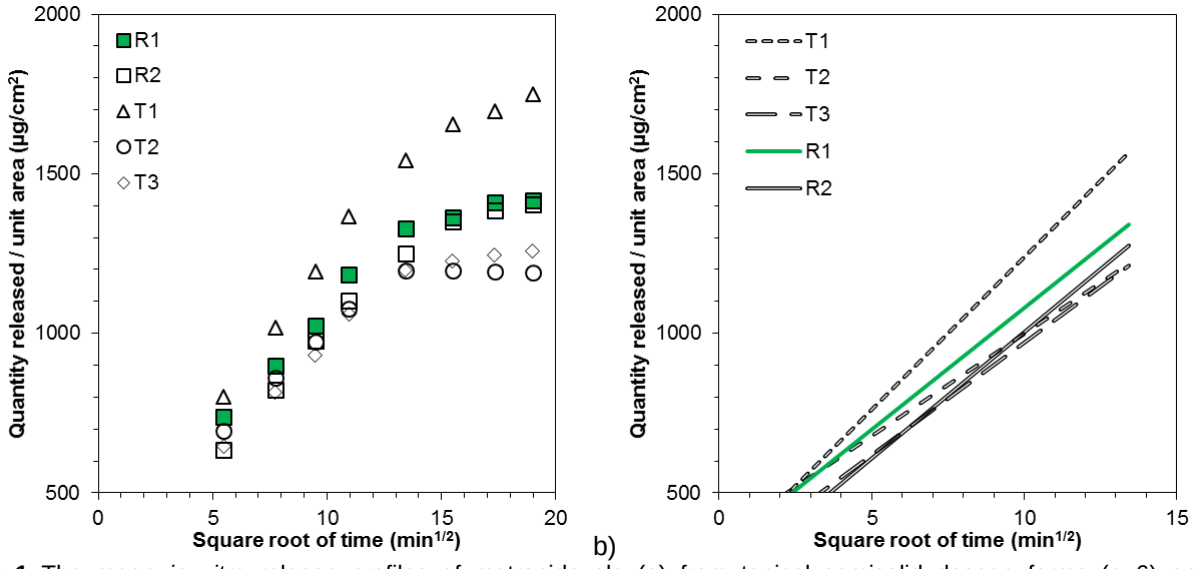

Fig.1 The mean *in vitro* release profiles of metronidazole (a) from topical semisolid dosage forms (n=6) and the regression lines (b) calculated for the first 180 minutes of the experiments (standard deviation not displayed, for the clarity of the graphs)

The structural parameters

The rheological evaluation performed using the hysteresis loop test revealed structural differences. All the evaluated products displayed a pseudoplastic behavior, except for the T3. For the composition including fatty alcohols and glycerides, the changes induced by the controlled mechanical stress applied during the rump-up segment were considerable. The viscosity decreased proportionally with the shear rate in the 1.11 to 5.03 1/s interval, with reduced changes in the following deformation regimens (figure 2). In this case, the variability of the experimental data was high, with coefficients of variations above 14%. Considering the formation of an inner phase within the semisolid matrix, the shearing forced probably determined an alteration of the size of lipid droplets, with delayed and variable recovery of the initial structure. For the two products representing the reference listed drug, minor differences in consistency were noticed, preserved throughout the profile. Despite the similarity of the qualitative composition, a distinct deformation pattern was recorded for the generic T2 formulation, especially at low shear rates.

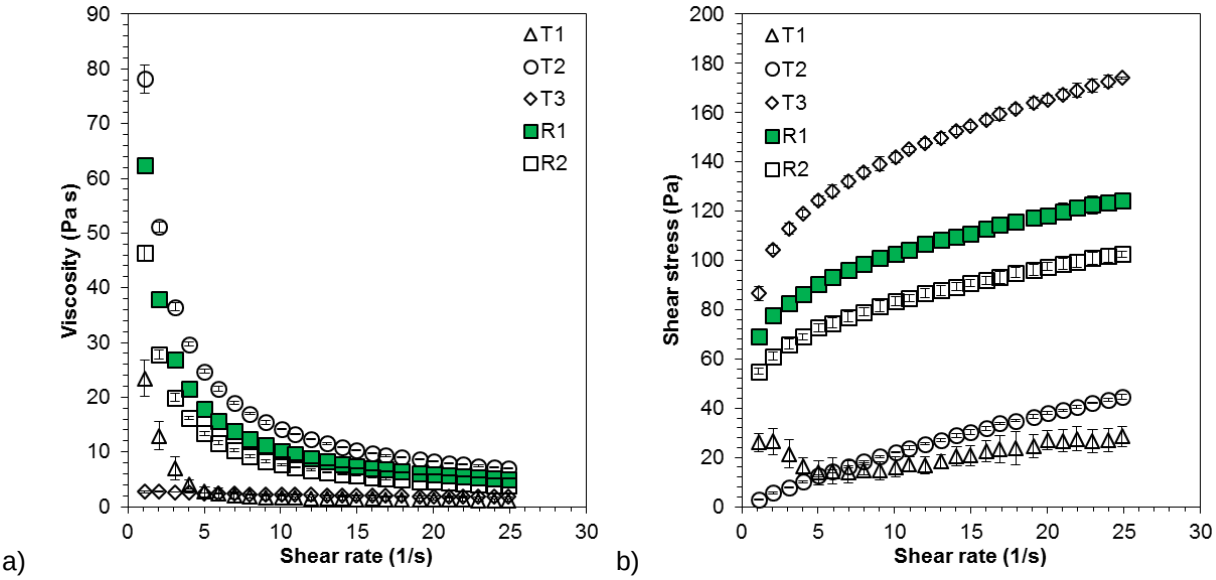

Fig. 2 - The variation of the viscosity (a) and of the shear stress (b) as a function of shear rate, for the rump up procedure at 25±0.5°C (n=6, mean ± standard deviation)

The evaluation of *in vitro* similarity and the relationship with structural or composition particularities

The release rates, calculated according to the non-parametrical SUPAC-SS (1997) methodology, varied between 63.85 and 95.69 ($\mu g/cm^2/min^{1/2}$). The most significant differences were reported for the lag-time, a parameter which is related to the initial diffusional resistance displayed by a particular arrangement of the matter, but also to the solubility of the active pharmaceutical ingredient in the vehicle. The longest delay in achievement of the steady state transfer between the two compartments separated by the inert hydrophilic membrane was observed for the T2 product, having the highest consistency within the group of analyzed formulations. This also corresponded to the lowest value of the release rate, therefore demonstrated that the implemented *in vitro* release test can be discriminative with respect to variation of quantitative composition and/or manufacturing parameters. For the high strength T1 product, the lag time of the diffusion was almost similar to the R2 reference batch, but the values of the release rate were more than 21% higher (25.6% compared to R1, respectively). The difference was correlated with ratio of the label-claimed content and suggested that for the evaluation of the influence of excipients on the thermodynamic activity of the drug, dose scaling should be applied prior to *in vitro* similarity assessment.

The 90% confidence intervals calculated for test to reference slope ratios are presented in table 2. The *in vitro* profile of R1 batch was considered as a reference, based on the expiry date (shorter time interval from the manufacturing). The similarity concluded between R1 and R2 demonstrated the consistency of the pharmaceutical quality. The 33% increase in the concentration of the drug for the T1 product generated a proportionally higher concentration gradient across the membrane, further leading to higher release rates. The non-similarity concluded with respect to R1 product can be regarded as a signal of the discriminatory character of the *in vitro* testing conditions in relation to the strength. This is a key element of the methodological validation. Nevertheless, the similarity obtained after scaling the individual slopes with content ratio (1.33) reveled that, despite the differences in qualitative composition, the drug content is the driving force of the release process

Table 2

The release parameters of metronidazole from the semisolid dosage forms (mean ± standard deviation; n=6) and the evaluation of *in vitro* similarity

Product	Release rate ($\mu g/cm^2/min^{1/2}$)	Lag time ($min^{1/2}$)	90% confidence interval[1]	
			Lower limit (%)	Upper limit (%)
R1	76.19 ± 11.32	16.61 ± 2.45	-	-
R2	78.99 ± 6.53	6.94 ± 0.99	91.82	118.64
T1	95.69 ± 2.79	8.35 ± 0.28	107.23 (80.42)[3]	142.55 (106.91)[3]
T2	63.85 ± 7.74	30.71 ± 1.31	72.59	100.90
T3	70.46 ± 5.45	13.94 ± 0.65	82.38	106.95

[1] R1 was considered as reference drug product, for calculation of individual test to reference slopes ratios, using the compendial acceptance interval of 75 - 133.3% for concluding the *in vitro* similarity;
[3] calculated after scaling of the individual slopes with the ratio of the labeled claimed content.

The absence of significant difference in release rates obtained for T3 cannot be used as a direct indicator of bioequivalence. In this case, the presence of a different macromolecular agent, i.e. a cellulose derivative with specific rheology and water-binding properties, represents a critical change compared to the composition of reference listed drug. The *in vitro* similarity is a complex resultant between the solubility of metronidazole in the semisolid vehicle and the reduced diffusional resistance. When the Ostwald de Waele (power low) model was applied to the mean deformation profiles, the values of the consistency index were more than 33 times lower, demonstrating an obviously distinct arrangement of the matter. For the products with similar qualitative compositions (R1, R2 and T2), the release rate varied inversely proportional with this rheological parameter.

Table 3

The mean values (n=6) of the Ostwald de Waele model, applied to the rheological profiles of hydrophilic gels

Product	Consistency index, K (Pa/s)	Flow behaviour index, n
R1	67.82 ± 1.41	0.1814 ± 0.071
R2	53.66 ± 1.81	0.1903 ± 0.0053
T2	85.88 ± 2.46	0.2255 ± 0.0135
T3	3.02 ± 0.13	0.8538 ± 0.0133

Based on previous reports, for topical semisolid products acting in deeper layers of the skin, such as the dermis in case metronidazole (Holmgaard *et al.*, 2010), the relevance of *in vitro* release tests is revealed only when combined with other *in vivo* approaches such as dermatopharmacokinetics or dermal microdialysis (Miron *et al.*, 2014). The results should be used as signals of similarity or difference in performance during the first stages of the highly complex process of skin penetration. For example, the lipophilic or tensioactive excipients of T1 generic formulations may act as permeability enhancers. As for other hydrophylic entities, polyols may promote their delivery through the stratum corneum. The impact of propylene glycol on the in vivo performance of acyclovir 5% cream formulations has been mentioned previously as a potential source for the lack of bioequivalence between the reference listed drug and the generics (Díez-Sales *et al.*, 2005; Trottet *et al.*, 2005). For metronidazole, propylene glycol added in semisolid dosage forms generated a maximal enhancement of delivery (Wotton *et al.*, 1985). Similarly, the quantitative variations of this critical excipient in the composition of the T2 product may explain both structural and diffusion non-similarity, compared to R2.

The current results support the idea of a more pronounced effect of the vehicle than the concentration of the active entity, in the local delivery of metronidazole (Elewski, 2007). The excipients may be also involved in the clinical outcome. Wagner *et al* (1998) evoked the role of hydration at the level of stratum corneum, a key factor which depends upon the water content of the drug product. This could explain the preference for hydrophilic vehicle for 0.75% products, whereas the initially approved reference formulation (Flagyl®, Aventis) is a 1% lipophilic cream.

The relevance of *in vitro* release comparison is limited by two factors, the degree of non-similarity existing between the evaluated products and the intended site of action for metronidazole. As stated in the 21 CFR 314.94 (2015), the generic manufacturer may perform changes in the type and / or quantity of several excipients, compared to the reference formula, but proofs should be provided that the resulting difference do not impact the therapeutic effect (Shah *et al.*, 2015). The present report illustrates the outcome of two kinds of research and development approaches, i.e. the qualitative and quantitative differences for excipients, some playing an important role in the delivery across the skin. *In vivo* evaluations are mandatory in both instances for establishing bioequivalence.

CONCLUSIONS:

The structural and *in vitro* release parameters were evaluated for topical semisolid dosage forms containing metronidazole, including two batches of the reference listed drug. The products presented various differences it concerns the qualitative compositions. The mean diffusion profiles displayed an increased dependence on the nature of vehicle. The deformation profiles induced by controlled mechanical stress (hysteresis loop test) revealed a distinct arrangement of the matter, with a pseudoplastic behavior in most cases. The *in vitro* methodology was able to reflect the cumulative effect of the structure and solubility of the drug into the semisolid matrix. The relevance of the experimental data for the *in vivo* performance is prospectively limited by the direct influence of excipients onto the permeability of the stratum corneum and by the depth of the site of action.

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