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Equivalence assessment of creams with quali-quantitative differences in light of the EMA and FDA regulatory framework☆,☆☆

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ABSTRACT

EMA and FDA are upgrading guidelines on assessing the quality and the equivalence of topically applied drug products for developing copies of originator products and supporting post-marketing variations. For topical products having remarkably similar composition, both EMA and FDA accept the equivalence on the bases of the comparison of rheological properties and in vitro drug release constant (k) and skin permeation flux (J) values, instead of clinical studies. This work aims to evaluate the feasibility to expand this approach to variations of the composition of complex semi-solid preparations. Ibuprofen (IB) creams at two different strengths (i.e., 1 % and 10 %) were used as a model formulation. Two formulative changes were performed: (a) the addition of the humectant to simulate a minor post-marketing variation; (b) the substitution of the emulsifying system to simulate a major one. These variations impacted only in 1 % IB formulations where both the equivalences of rheological data and J-values failed. At the highest concentration, the presence of IB crystals broke down the differences in rheological patterns and lead the IB thermodynamic activity at the maximum figuring out an overlapping of the J-values. Such data suggest the combination of these studies, which are thought mainly for the development of copies, could be also applied to the management of post-marketing variations that involve product composition.

1. Introduction

The development of a new locally acting product should take into consideration technological and regulatory constraints to ensure the final quality, efficacy, and safety. To speed up pharmaceutical development and prevent quality failures during the product shelf-life, there is a growing interest in the pharmaceutical industry to identify, earlier in the development phases, critical attributes able to affect the manufacturing process and, therefore, the product quality profile. This information contributes to the definition of the Quality Target Product Profile (QTPP), which is defined by the ICH Q8 (R2) guideline as "a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product" (EMA, 2017). Critical Quality Attributes (CQA) of a locally acting semi-solid product may vary based on the features of the semi-solid preparation, and the physical state of the drug substance (e.g., drug-dissolved preparations versus drug-suspended ones) (Chang et al., 2013). In this context, a correct balance between the rheological characteristics of the semi-solid preparation, in terms of usability and physical stability, and the other functionality-related properties should be achieved to reach the target efficacy and safety profiles. Indeed, rheological properties impact not only the product texture properties, but also the drug release from the semi-solid matrix and, consequently, the drug absorption through the skin (Krishnaiah et al., 2014).

These aspects are particularly relevant in the development of generic products or when post-marketing variations of an authorized medicinal product are needed. The novel formulation (i.e., generic product, modified formula) should conform to the same QTPP of the reference product; therefore, product quality metrics are needed to prove

Abbreviations: IB, ibuprofen; QTPP, quality target product profile; CQA, critical quality attributes; IVPT, in vitro permeation tests; IVRT, in vitro release tests; MAHs, marketing authorization holders; LVER, linear viscoelastic region; SC, stratum corneum; Thyst%, thixotropy index; GMR, geometric mean ratio.

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therapeutic equivalence and interchangeability with their reference product (Krishnaiah et al., 2014; Minghetti et al., 2020). However, the in vivo quantification of drug concentration at the local level (e.g., skin) may require sophisticated techniques (Pudney et al., 2007); on the other side, after a local application, the plasmatic drug concentration results negligible in most cases. Therefore, the assessment of bioequivalence for locally acting products and/or follow-on products is challenging and may require clinical endpoint trials (Minghetti et al., 2020). To overcome such gaps, huge efforts have been made in develop and validating minimal invasive methodologies, such as microdialysis and open flow microperfusion, able to determine drug concentration in vivo directly in dermal layer (Birngruber et al., 2022). A parallel approach to waive clinical trials pursued by regulatory authorities is the release of guidance in which surrogate in vitro, and in vivo methods are detailed to assess equivalence (CFR, 2022; EMA, 2018; Miranda et al., 2018). For example, in vitro release tests (IVRT), permeation kinetic studies [e.g., in vitro permeation tests (IVPT), in vivo stratum corneum sampling (i.e. tape stripping)] or, if available, pharmacodynamic tests (e.g., skin blanching for glucocorticoids) have been accepted (CFR, 2022; EMA, 2018; FDA, 1997; Minghetti et al., 2020; Miranda et al., 2018). In particular, IVRT has been recognized as an excellent tool to evaluate the quality of a product formulation and to identify those differences between products that can impact on the drug release profile. On the contrary, IVPT allow an estimation of the impact of drug thermodynamic activity on skin absorption. Therefore, IVRT discriminate formulations based on their qualitative, quantitative, and microstructure attributes (Alves et al., 2021; Krishnaiah et al., 2014; Miron et al., 2021; Tiffner et al., 2021).

In this light, the SUPAC-SS guidance released by the FDA in 1997 remained for a long time the regulatory standard for assessing product equivalence in scaling-up and post-approval changes (FDA, 1997). However, the increased awareness of regulatory communities on relevance of microstructure/rheological patterns in assessing the therapeutic equivalence of semi-solid products (Al-Ghabeish et al., 2015; Krishnaiah et al., 2014; Miron et al., 2021; Navarro-Pujol et al., 2021; Xu et al., 2020) pushed regulatory authorities towards novel orthogonal regulatory approaches. In 2018, the EMA proposes to use so-called extended pharmaceutical equivalence for comparing simpler formulations (e.g., ointments, and gels in which the drug is solubilized) (EMA, 2018). Based on such an approach, the therapeutic equivalence between two products should be based on the sameness of the pharmaceutical form, method of administration, qualitative and quantitative composition, the microstructure/physical properties, and the product performance (e.g., IVRT). For more complex preparations (e.g., multiphase systems such as creams), a permeation kinetic study, or a pharmacodynamic one should be performed, at least, by the applicant. In parallel, the provisions of SUPAC-SS guidance (FDA, 1997) were also expanded by the FDA with a draft guidance in 2022 (FDA, 2022). The current FDA approach for equivalence assessment is built on three pillars: same qualitative (Q1), and quantitative (Q2) compositions, and structurally/functionally equivalence (Q3). The latter one involves not only IVRT, but also the rheological pattern at least. Such an approach has been supported by the extensive literature on Q1/Q2 equivalent formulations (Alves et al., 2021; García-Arieta et al., 2023; Ilić et al., 2021; Krishnaiah et al., 2014; Miranda et al., 2018; Xu et al., 2015). However, after SUPAC-SS exceeding, little is written about novel regulatory approaches that should be applied for assessing equivalence between products having significant differences in quali-quantitative composition, especially in excipients that might affect drug bioavailability and performance (e.g., surfactants, permeation enhancers). Such regulatory uncertainty appears particularly critical for marketing authorization holders (MAHs) that must introduce minor and major post-marketing variations in authorized semi-solid products to meliorate their quality patterns (e.g., better physical stability in real-world conditions) or to ensure the availability of product on the market. This last aspect is particularly true for severe shortages of raw materials or closure of suppliers, in which MAHs are forced to apply for minor or major post-marketing changes to minimize the negative ripple effects on their manufacturing and, consequently, on the supply of drug products on the market (Musazzi et al., 2020). For these conditions, it is evident that the availability of regulatory approaches to waive clinical trials for assessing products' equivalence is extremely critical for the resilience of MAHs and the continuity of care.

This article aims to assess the structural and functional similarity of semi-solid preparations that are not equivalent in terms of qualitative and quantitative composition. Starting from a cetomacrogol base cream reported in the Italian Pharmacopeia (FU XII ed.), two formulative changes were made to simulate: (i) a minor post-marketing variation by addition of the humectant (EMA, 2018) and (ii) a major variation by substituting the emulsifying system. Such variations were adopted in two series of creams that differ for the thermodynamic activity of the loaded drug [i.e., ibuprofen (IB)]: unknown, when IB is solubilized in the semi-solid matrix, and maximal, when it is partially suspended. The final composition of each formulation was adjusted by varying the water content to obtain within the same series of creams the same value of viscosity determined by a Brookfield apparatus. The formulations were then compared in terms of IVRT, IVPT, and rheological pattern. In this latter case, each formulation was characterized by determining the complete flow curve of viscosity versus shear rate, yield stress and linear viscoelastic response.

2. Materials and methods

2.1. Materials

Ibuprofen (IB) was provided by Farmalabor (Italy), Cetostearyl Alcohol, Cetomacrogol 1000, White Soft Paraffin BP-USP were supplied by A.C.E.F. (Italy). Sepineo™ SE68 (Cetearyl alcohol/Cetearyl glucoside) was acquired from SEPPIC (France), and Paraffin oil was purchased from Carlo Erba Reagents (Italy). Glycerol, Polyethylene glycol 400, HPLC grade acetonitrile, cellulose acetate hydrophilic 0.45 μm membrane and nylon 0.22 μm filter were provided by VWR Chemicals (Belgium). The PVDF hydrophilic membrane was supplied by GVS (USA).

2.2. Preparation of o/w creams

The composition of o/w creams prepared simulating a minor and a major post-marketing variations is reported in the Table 1 (placebo

Table 1
Qualitative and quantitative composition (%, w/w) of o/w creams loaded with IB.

Series	Form.	Composition (%)							
		Cetomacrogol 1000	Sepineo™ *SE68*	*Cetostearyl alcohol*	*White petroleum*	*Paraffin oil*	*Glycerin*	*Water*	*IB*
1 %-IB	*1*	1.8	–	7.1	14.9	5.9	–	69.3	1.0
	2	1.7	–	6.8	14.1	5.7	9.9	60.8	1.0
	3	–	8.9	–	14.9	5.9	–	69.3	1.0
10 %-IB	*4*	1.6	–	6.5	13.5	5.4	–	63.0	10.0
	5	1.5	–	6.2	12.9	5.1	9.0	55.3	10.0
	6	–	8.1	–	13.5	5.4	–	63.0	10.0

Table 2
Rheological patterns of formulations loaded with 1 % and 10 % IB (Mean ± St.Dev., $n = 6$).

Form.	Complete flow curve		Amplitude Sweep	Frequency Sweep		Brookfield Apparatus
	η at 100 s^{-1} (Pa.s)	*Thyst%*	*Yield Point (Pa)*	*G' at 1 Hz (Pa)*	*G'' at 1 Hz (Pa)*	*η (Pa.s)*
1	1.47 ± 0.05	10.5 ± 1.2	30.8 ± 0.7	2953 ± 34	1000 ± 10	36,764 ± 798
2	1.56 ± 0.03	10.5 ± 0.6	31.1 ± 1.0	3060 ± 21	931 ± 20	35,197 ± 1765
3	1.52 ± 0.06	15.5 ± 2.2	23.5 ± 1.8	4326 ± 596	1648 ± 211	36,407 ± 476
4	2.39 ± 0.03	32.7 ± 2.6	37.7 ± 1.8	15,697 ± 504	7699 ± 268	36,834 ± 118
5	2.35 ± 0.05	31.7 ± 0.9	38.8 ± 1.5	16,008 ± 579	7844 ± 360	36,808 ± 682
6	2.40 ± 0.16	32.9 ± 2.2	39.1 ± 1.6	16,689 ± 443	8015 ± 642	36,458 ± 455

Formulations are reported in Table S1.1). Preliminarily, the composition of the semi-solid formulations (1 %-IB, and 10 %-IB series) were optimized to reach a similar viscosity between them (p-value > 0.05) when measured by Brookfield apparatus (Tables 2 and S1.2). The o/w creams were prepared using ultrasound technique to obtain a homogeneous system, with a narrow size distribution of droplets of oil phase (≈ 3 μm). Briefly, the aqueous and oil phase were weighed in two separate beakers and incubated in a water bath at 65 ± 1 °C until the complete melting of all components was achieved. Then, the oil phase was rapidly added into the aqueous phase and the system was sonicated using an ultrasonic processor (UP200St, 200 W - 26 kHz Hielscher, Germany) equipped with 2 mm sonotrode (S26d2) at a 70 % amplitude. Each formulation was subjected to four 20-*sec* cycles of sonication, interspersed by three 5-sec cycles off. The formed emulsion was allowed to cool to room temperature under a constant stirring. In the case of drug loaded formulations, IB was added, when the temperature was cooled down at 55 ± 5 °C. All formulations were stored at 25 °C in a plastic container for at least 24 h before use.

2.3. Rheological studies

Brookfield apparatus. The study was performed by using a rotational viscometer (Viscometer Rotavisc me-vi, version: 1.02.004/1.11.004, IKA, Germany). The viscosity of the formulations was determined at a stable temperature of 32 ± 1 °C, which is assumed to be human skin temperature (Lee et al., 2019). The VOL-SP-11 spindle was able to read the viscosity of the complete set of emulsions. The viscosity readings were taken for 60 sec at 10.00 rpm rotation speed. The results were expressed as the average of three different sample.

Rheological properties. The study was performed by using an oscillating dynamic rheometer (Modular Compact Rheometer MCR 302, Anton Paar, Austria) equipped with a cone plate with a diameter of 50 mm and a cone angle of 1°. All measurements were done at 25 ± 1 °C and 32 ± 1 °C and samples were analysed after 24 h storage. About 3 g of each formulation were carefully placed on the instrument's lower plate and the upper plate was freely lowered until reaching a minimum gap between plates of 103 μm: the following experiments were performed according to the draft of the guideline. First, a complete flow curve of viscosity versus shear rate (0.01–100 s^{-1} range) in non-oscillatory regimen was performed to understand the pattern of each formulation and its eventual thixotropic behaviour. Secondly, an amplitude sweep analysis was performed at the shear strain ranging from 0.01 to 100 % at the constant angular frequency of 10 rad/s (1.59 Hz) to identify the linear viscoelastic region (LVER) and the upper limit of this region (yield point), as well as the sample's behaviour when this limit is exceeded. Finally, a frequency sweep test was conducted at a Shear Strain of 0.1 % and the oscillation frequency was gradually decreased from 30 to 0.01 Hz. All the experiments were performed on different aliquots of the same cream batch.

2.4. In vitro drug release studies

IVRT were performed in occlusive conditions using 3.0 mL Franz Vertical Diffusion Cell Apparatus (PermeGear, Hellertown, USA) and a cellulose acetate membrane (0.45 μm, Sartorius Stedim Biotech GmbH, Germany). Such membrane was selected based on preliminary studies to validate the IVRT protocol (Supplementary Materials, Section S2). Before starting experiments, the membranes were hydrated in HPLC-grade water for 1 h and mounted on the Franz Cell, whose receptor compartment was filled with PEG 400 solution (30 %, w/V). Particular care was taken to avoid air bubbles between the medium and the membrane in the receptor compartment. At the beginning of the experiment, semi-solid preparations (≈ 100 mg) were applied to the membrane surface in a 1-mm-thick layer using polyethylene-disk support. The membrane surface temperature was kept at 32 ± 1 °C throughout the experiments. At predetermined times (1, 2, 4, 6 h), 200 μL samples were withdrawn from the receptor compartment and replaced with a fresh receptor phase. Sink conditions were maintained throughout the experiments. At the end of each set of experiments, the samples were analyzed according to the method described below. The results were expressed as the mean ± standard deviation of 12 replicas.

2.5. In vitro permeation studies

The permeation of IB loaded in the o/w creams was determined through pig-ear skin, using Franz's diffusion cells. The porcine ears were provided by a local slaughterhouse. All experiments were performed on frozen and thawed skin, stored at −20 °C and used for the experiments within 6 months. The part of skin required for the experiments was removed from the outer side of the pig's ear, cut and dermatomized (de Soutter Medical, Netherlands). The thickness of the skin piece was measured with an external digital electronic micrometer (model MI-1000, ChemInstruments, USA) and samples considered for the permeation experiments had a thickness between 0.630 and 0.770 mm. The integrity of each human epidermis sample was assessed measuring the electrical resistance (voltage: 100 mV, frequency: 100 Hz; Agilent 4263B LCR Meter, Milan, Italy), following the procedure validated by Cilurzo et al. (2018). The specimen was used in the permeation experiment if the value was higher than 10 kΩ cm^2. Then, the skin samples were mounted on the receptor compartment of each Franz Diffusion Cell with the stratum corneum (SC) side facing upwards in the donor compartment. At the beginning of the experiment, semi-solid preparations (≈ 15 mg) were applied to the membrane surface in a 1-mm-thick layer using polyethylene-disk support. The membrane surface temperature was kept at 32 ± 1 °C throughout the experiments. At predetermined times (1, 3, 5, 7, 24 h), 200 μL samples were withdrawn from the receptor compartment and replaced with a fresh receptor phase. Other methods parameters (e.g., receptor phase) are the same as the IVRT protocol described above. Sink conditions were maintained throughout the experiments. Samples were analyzed by HPLC according to the method described below. The results were expressed as the mean ± standard deviation of 6 replicas.

2.6. HPLC analyses

The amount of IB was determined by high performance liquid chromatography (HPLC; HP 1100 ChemStations, Agilent Technologies, USA), equipped with ultraviolet detector at 225 nm. Water acidified

with orthophosphoric acid (pH 2.6)/acetonitrile/ (40/60, v/v) was used as mobile phase at a flow rate of 1.5 mL/min and the analysis temperature was fixed at 25 °C. The compound separation was carried out using reverse-phase column (HyperClone™ 5 μm BDS C18 130, 150 mm X 4.6 mm, Phenomenex, USA) and the injection volume was set at 20 μL. The retention time of IB was 3.0 min and two calibration curves were constructed in the overall range of 0.1–100 μg/mL ($R^2 = 0.999$)

2.7. Data analysis

Rheology theoretical considerations. For the draft guidance of the EMA, the quality assessment of two semi-solid formulations should include the determination of: (a) the complete flow curve of shear stress (or viscosity) versus shear rate, (b) yield stress and creep testing; (c) the linear viscoelastic response (storage and loss modulus vs frequency).

The complete flow curve allows for the determination of the degree of destructuration of the semi-solid product when exposed to constant shear rate (Ghica et al., 2016). The results of this type of rotational test can be displayed as a flow curve diagram by plotting viscosity as a function of shear rate. Most semi-solid products show thixotropic behaviour: a decrease of apparent viscosity over time when under a shear force, due to the gradual deterioration of the inner structure, and a return to the original viscosity after the subsequent cessation of the shear force. Thixotropy is determined by these two parameters, i.e., the difference between initial and final viscosity when a shear force is applied, and the time required to come back to the initial viscosity as shear force is no longer applied. An ideal product should be able to return each time to its original apparent viscosity within a certain time to ensure physical stability over time. Differences between formulations may result in non-equivalent spreadability and/or physical stability, especially when the product is subjected to repeated efforts to be removed from the container (Simões et al., 2020). In this work, the thixotropic behaviour of tested formulations is expressed in terms of thixotropy index (*Thyst%*), which is expressed as a percentage of the thixotropy area. The thixotropy index (*Thyst%*) is useful for comparing different samples and is obtained using the following Eq. (1):

$$\text{Thyst}\% = \frac{S_{fwd} - S_{bw(t)}}{S_{fwd}} \cdot 100 \quad (1)$$

where S_{fwd} is the area corresponding to the maximum forward curve and $S_{bw(t)}$ is the area under the backward curve at moment "t" of stirring (Ghica et al., 2016).

The yield point is the shear stress value at which the external force acting on the sample exceeds the product's cohesive force, inducing its flow. It is dependent on the pre-treatment of the sample and the measurement method used, so it is not to be regarded as a material constant. In this light, the yield stress is meaningful for predicting the handling behavior of structured systems such as o/w creams (e.g., when a force is applied to remove the formulation from the container, as well as the application and sensory properties of the product). The measuring results of amplitude sweeps are presented as a diagram with shear strain (%) plotted on the x-axis and shear stress (Pa) on the y-axis; both axes are on a logarithmic scale. Many ways for measuring yield stress are known; in this work this parameter was determined as flexion point of the curve plotting data of shear stress versus shear strain obtained in amplitude sweep tests.

The linear viscoelastic response (LVER) is the stress range in which the Storage (G') and Loss (G'') moduli are independent of strain and only related to the internal microstructure. Beyond this region, the sample may experience significant structural failure (Hamed et al., 2016; Pisal et al., 2013). Once the LVER is identified by amplitude sweep test, the time-dependent rheological behavior of o/w creams can be investigated by applying frequency sweep test. Indeed, such tests are generally performed in non-destructive deformation range (e.g., LVER) to obtain the fingerprint of a semi-solid product. On the one hand, its behavior when stressed at low-frequency loads can reveal information useful for predicting its long-term physical stability and creep behavior. On the other hand, high frequencies are useful to study the viscoelastic behavior of semi-solids, which is relevant for appreciating differences during squeezing inside the primary containers or spreading onto the skin.

In vitro *release study*. The release rate constant (k) is defined by a mathematical model derived from a simplification of the Higuchi equation. It is calculated as the slope of released fraction of active substance versus the square root of time for the linear portion of the drug release profile (EMA, 2018):

$$\frac{M_t}{M_\infty} = k\sqrt{t} \quad (2)$$

where M_∞ refers to the amount of active drug contained in the dosage unit, instead M_t refers to the amount of IB released over time.

In vitro *permeation study*. The following parameters were calculated starting from the concentrations withdrawn from the receptor compartment of the Franz's diffusion cell: a) the cumulative amount permeated through the skin per unit of area was calculated according to the Eq. (3):

$$Q_t\left(\frac{\mu g}{cm^2}\right) = \frac{C_t\left(\frac{\mu g}{mL}\right) \times V(mL) + C_{t-1}\left(\frac{\mu g}{mL}\right) \times 0.2(mL) + C_{t-2}\left(\frac{\mu g}{mL}\right) \times 0.2(mL) + \ldots}{0.636\, cm^2} \quad (3)$$

where Q_t is the permeated amount at the time t, C_t is the drug concentration at the same time point, V is the volume of the Franz cell, 0.2 mL is the volume withdrawn from the receptor compartment at each time point and 0.636 cm^2 is the surface area of the Franz cell. The Q_t values were plotted as a function of time. Such plot should yield a straight line. b) the permeation flux, J ($\frac{\mu g \times h}{cm^2}$), of the drug permeated through the skin was calculated as the slope of the linear portion of the curve obtained by plotting the cumulative amount of drug permeated per unit area (μg/cm^2) as function of time.

Statistical analyses. The possible outliners within each series were checked by Dixon's Q test and eventually discarded. Tests for significant differences were performed by the one-way ANOVA followed by Dunnet and Turkey-Kramer post-analyses (JMP® 14, SAS Institute Inc., USA). Differences were considered significant at the p-value lower than 0.05. The comparison of release constants (k), permeation flux (J) and rheological parameters of the two modified formulations versus reference were performed on provisions reported in the EMA's draft guideline on quality and equivalence of topical products (EMA, 2018). In particular, the statistical analysis was performed, upon a null hypothesis of non-equivalence considering the treatment of parallel experiments, adapting the statistical procedures described by Cilurzo et al. (2018). All analyses were performed applying a Two One-Sided Test Procedure (TOST; $\alpha = 0.05$) by SAS 9.4 TS Level 1M3 (SAS Institute Inc., USA). To be considered equivalent, the 90 % confidence interval for the difference of means of test and reference formulations should be contained within the acceptance criteria defined by the EMA's draft guideline. In general, the acceptability interval in the log scale is 0.8–1.25. However, in the case of data presenting a high variability, it could be computed using the total between-experimental unit variance. Thus, the U and L of the acceptability interval were therefore determined according to the following equations (4,5):

$$U = e^{K\ CV} \quad (4)$$

$$L = e^{-K\ CV} \quad (5)$$

where K is constant set to 0.760 and CV is the coefficient of variation of the log-transformed values of parameter values of the reference product.

3. Results

3.1. Rheological studies

1 %-IB loaded formulations. The complete flow curve of viscosity versus shear rate is the first rheological test required in the EMA draft guideline. It is able not only to measure the breaking strength of the microstructure, but also to predict the spreadability and formulation residence time at the application site, and thus patient compliance (Suñer-Carbó et al., 2019). Moreover, it allows for the identification of formulation changes that can potentially influence drug release and, therefore, drug bioavailability (Binder et al., 2019; Simões et al., 2020). The obtained results showed a shear-thinning behaviour for all 1 %-IB loaded formulations: as the shear rate increased, the apparent viscosity decreased due to a reorganisation/break-up of the internal microstructure (Fig. 1). However, differences in apparent viscosity were observable as a function of the formulative change adopted: Sepineo™ SE68 (Formulation 3) reduced the apparent viscosity compared to both base (Formulation 1) and glycerine-addition (Formulation 2; Fig. 1A). Such a trend was consistent with placebo formulations (**Table S1.2**). These results agree with the known relevance of the emulsifier in determining the cream microstructure (Forster et al., 1990; Ribeiro et al., 2004) even if the surfactants in each pair are similar: cetomacrogol 1000/cetostearyl alcohol and cetearyl glucoside/cetearyl alcohol pair for Formulations 1 and 3, respectively. As a matter of fact, Sepineo™ SE68 can promote the formation of lamellar bilayers at oil/water interface, creating a stable shell preventing coalescence (Terescenco et al., 2018); cetostearyl alcohol generates a multi-lamellar structure as well, but it also creates a liquid crystalline network structure in which free water and the internal phase droplets are immobilised (Ilić et al., 2017; Ribeiro et al., 2004).

This different structure is reflected in the different thixotropic behaviour, which is lower in the case of Formulation 1 compared to Formulation 3 (Table 2), owing to the different organization of the two surfactant pairs. Indeed, cetomacrogol 1000/cetostearyl alcohol system appeared to be more structured. This high degree of structuration (gel phase), which is due to the presence of cetostearyl alcohol, also explains why the addition of glycerin did not affect the system's viscosity despite its ability to form hydrogen bonds (Fig. 1A, Table 2).

Regarding amplitude sweep experiments, the presence of Sepineo™ SE68 (Formulation 3) had a negative effect on the yield point (Fig. 2A, Table 2). This agrees with previous rheological evidence of a looser, less rigid microstructure than creams containing cetomacrogol 1000/cetostearyl alcohol (Formulations 1 and 2). It is known that the yield point, at which there is a strong reduction in the modulus of accumulation, increases with the volume fraction of the dispersed phase (Rajinder et al., 1999). However, the obtained results clearly highlighted how yield point is also affected by formulative changes (e.g., surfactant substitution) which influence the internal structure of the o/w cream (Ilić et al., 2017). Thus, under the same skin application, Formulation 3 started flowing earlier than both Formulations 1 and 2, even though these had lower modulus values. In all formulations, the yield point fell within the range of 10 to 100 Pa, suggesting that they should be easy to apply and spread on the skin, as the force required to cause creep is 1–10 mN force over 1 cm^2 area (Krishnaiah et al., 2014). Moreover, referring to LVER, in Formulation 3 the destruction of the microstructure occurred earlier than in other formulations. Thus, the alteration of the internal microstructure induced by surfactant substitution impacted not only on the resistance to flow of the product, but also on its physical stability in static conditions. In contrast, the addition of glycerin to the Formulation 1 affected neither yield point nor LVER. As for the frequency sweep experiments performed in the linear region, G' modulus always remained higher than G'' modulus regardless of the applied changes,

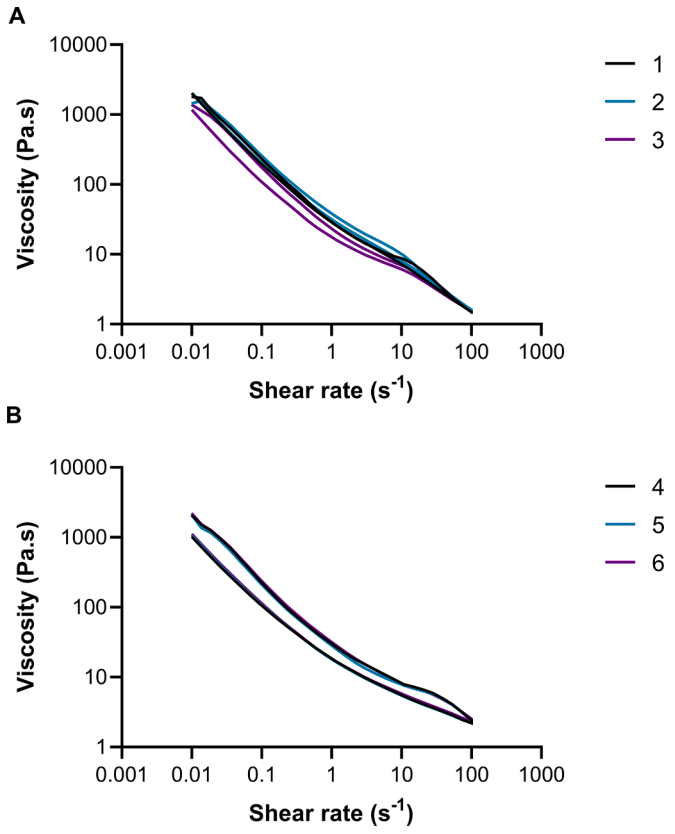

Fig. 1. Complete flow curve of viscosity versus shear rate of o/w creams loaded with 1 % (a) and with 10 % (b) w/w of IB.

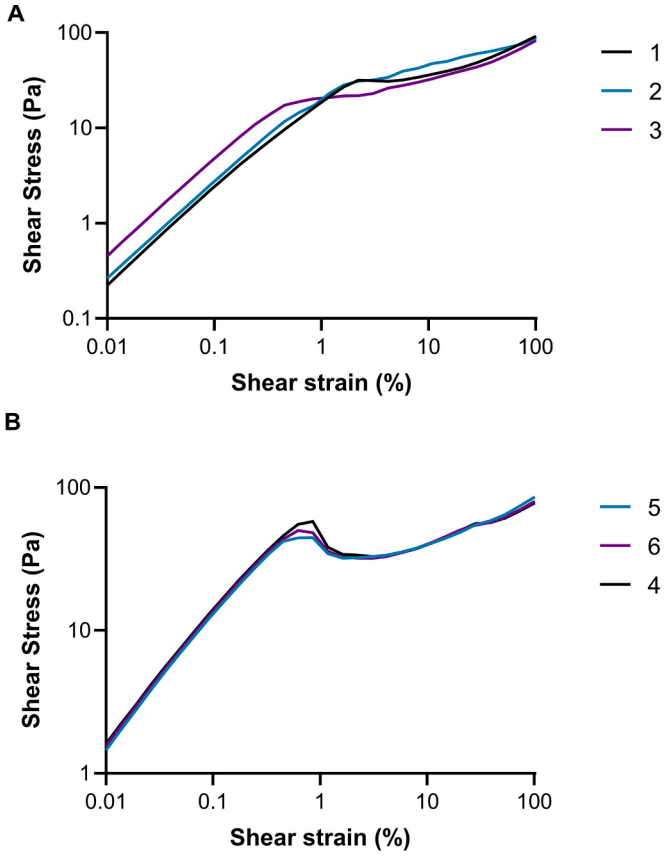

Fig. 2. Results of amplitude sweep tests, performed at 25 °C on o/w creams loaded with 1 % (a) and with 10 % (b) w/w of IB.

demonstrating that the formulations showed a predominantly elastic behavior (Fig. 3A). Consequently, all formulations seemed suitable for being applied onto the human skin and persist at the application site (Jones et al., 2009). However, the replacement of surfactants with Sepineo™ SE68 (Formulation 3) induced an increase in the value of both modules, indicating a more elastic structure. Regarding the comparison between base and glycerin addition, the trend of the G'' modulus of Formulations 1 and 2 was superimposable, whereas that of the G' modulus at low frequencies was slightly discordant but became superimposable for higher frequencies. In this case, the greater number of hydrogen bonds due to the glycerin probably creates a cross-linked structure that results in greater physical stability of Formulation 2 versus Formulation 1 during storage (Fig. 3A).

10 %-IB loaded formulations. When the drug was suspended in the semi-solid matrix, the impact of formulative changes as discussed for the 1 %-IB series became negligible in comparison to the influence of drug crystals. Hence, dispersed material had a greater impact on the internal microstructure than the structural differences of the o/w creams, so the profile became overlapping (Fig. 1B). Also, in amplitude sweep test, the presence of crystals prevailed over the effect of excipient changes, suggesting a similar spreading behavior of all drug-suspended creams on the skin. The LVER increases as the G' modulus values do, so the crystals interfered by strengthening the internal microstructure (Fig. 3B). In terms of viscoelastic behavior and stability, the profiles of the different formulations are also superimposable.

3.2. In vitro release and permeation studies

Unlike rheological results, the IVRT did not reveal significant differences among formulations (p-value > 0.05) within the 1 %-IB and 10 %-IB series, as shown in Table 3 and Fig. 4. As expectable, the higher the drug concentration (10 %-IB series) in the semi-solid preparation, the higher the drug release rate. However, neither the addition of a humectant nor the substitution of the emulsifying system had a significant impact on the release profile.

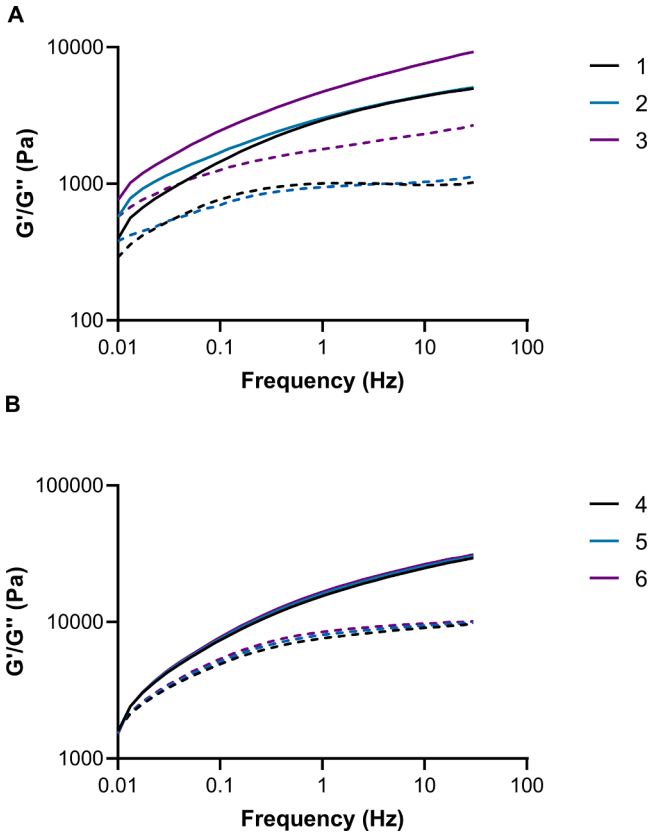

Fig. 3. Storage modulus (G'; solid lines) and loss modulus (G''; dotted lines) versus frequency obtained by frequency sweep tests (within LVER) on o/w creams loaded with 1 % (a) and with 10 % (b) w/w of IB.

Table 3
Release constants (k, $\sqrt{h}$), and permeation flux (J, μg/cm^{2}h) of formulations loaded with 1 % and 10 % IB (Mean ± St.Dev., $n = 12$). Geometric Mean Ratio (GMR) were calculated referring to J-values.

Formulations	k ($\sqrt{h}$)	J (μg/cm^{2}h)	GMR (%)
1 %-IB series			
1	0.052 ± 0.008	1.99 ± 0.66	–
2	0.047 ± 0.006	1.73 ± 0.80	86.93
3	0.052 ± 0.007	1.39 ± 0.22	69.85
10 %-IB series			
4	0.013 ± 0.004	4.34 ± 1.44	–
5	0.011 ± 0.001	4.29 ± 2.38	98.85
6	0.011 ± 0.001	4.50 ± 0.89	103.69

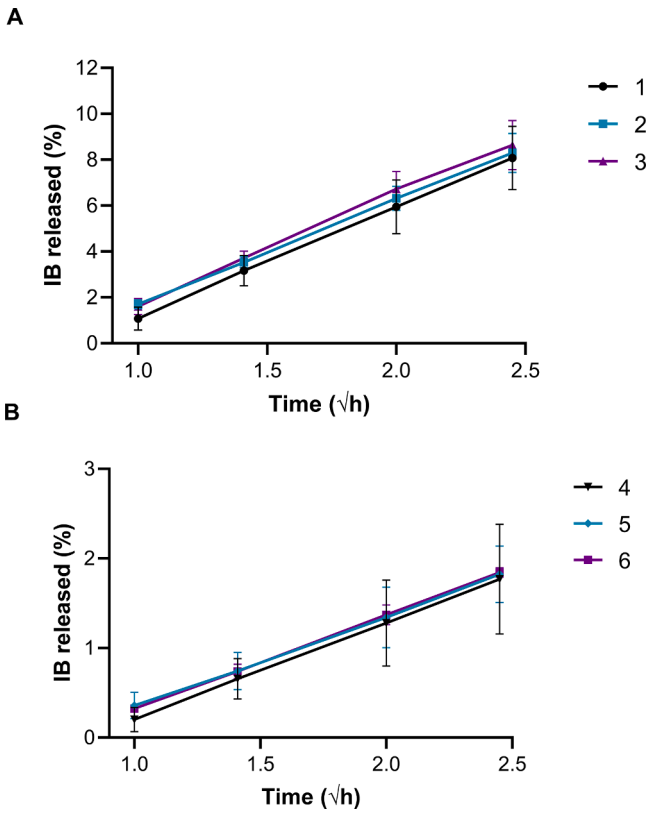

Fig. 4. In vitro release profiles of o/w creams loaded with 1 % (a) and with 10 % (b) w/w of IB.

In vitro permeability studies revealed a trend more coherent with the rheological results obtained. Concerning 1 %-IB series, Formulations 1 and 2 had an overlapping trend while Formulation 3 deviated. This trend mirrored that of the rheological behaviour. In more detail, the formulation with the surfactant variation had a narrower linear viscoelastic region, thus a tendency to undergo irreversible structural change more rapidly than Formulation 1. Furthermore, Formulation 3 had a higher G' and G'' modulus value than Formulation 1, which was reflected in a higher viscosity, corresponding to a lower skin permeability (Fig. 5A).

Conversely of the previous case, the presence of the drug at the solid-state (10 %-IB series) lead to, not only, superimposable in vitro drug release, but also skin permeability profiles, in agreement with the rheological pattern (Fig. 5B).

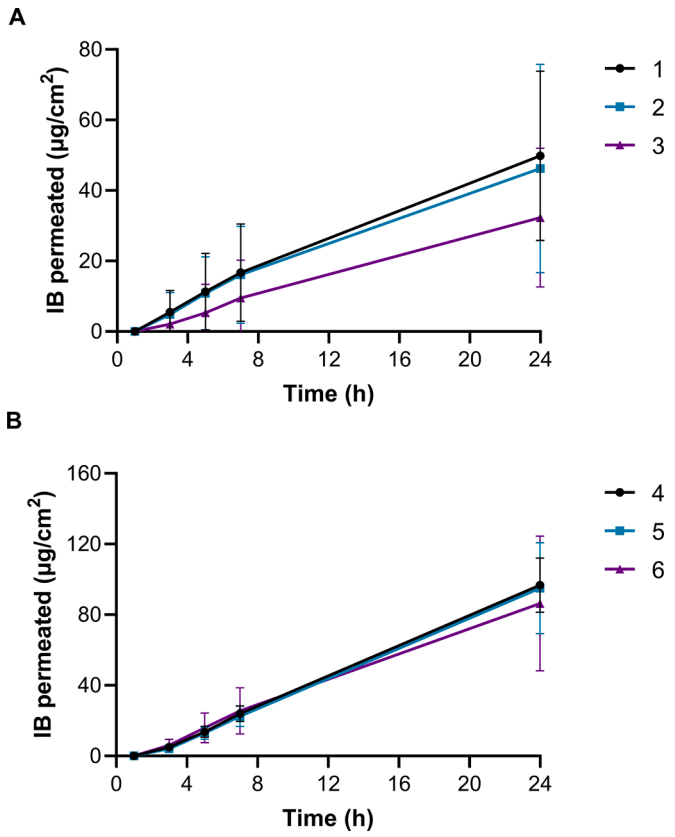

Fig. 5. In vitro permeability profiles of o/w creams loaded with 1 % (a) and with 10 % (b) w/w of IB.

3.3. Equivalence assessment of semi-solid preparations

From a regulatory perspective, the assessment of equivalence between formulations could be assessed only if the 90 % confidence interval of tested parameters is contained within the acceptance intervals reported in the EMA draft guideline (EMA, 2018). For quantitative analysis (e.g., rheological tests) and in vitro release studies, the guideline fixed the acceptance interval at 90 %−111 %. On the contrary, for in vitro permeation studies, the EMA draft guideline has widened the acceptance interval considering the higher within-subject or within-donor variability of drug permeation. In this context, the acceptance interval ranged from 80.00 to 125.00 % to a maximum of 69.84–143.19 %. As shown in Table 3, the fluxes of reference formulations (i.e., Formulations 1 and 4) showed a wide variability since the coefficients of variation were almost 33 %. In agreement with the EMA draft guideline, the acceptance range can be enlarged up to 77.23–129.48 if the Geometric Mean Ratio (GMR) between test and reference flux values was within 80.00–125.00 % of reference ones. For the 10 %-IB series, the GMR is within the acceptance range established by the guidelines. Consequently, the widening of the acceptance interval is applicable. For the 1 %-IB series, the requirements are met only in the case of minor variations (i.e., Formulations 1 versus 2).

Given these premises, the results reported in Table 4 evidence that, for minor formulation changes (e.g., humectant addition), all rheological parameters and release rate constants resulted equivalent to those of reference product, regardless of the thermodynamic activity of the drug in the semi-solid matrix. Such findings are confirmed by the results of IVPT. Consequently, the Q3 assessment seems sufficient for demonstrating the equivalence of two similar semi-solid products, even if Q1 and Q2 are not fulfilled (e.g., Formulations 1 versus 2, or 4 versus 6). On the contrary, as previously discussed, when the drug thermodynamic activity is not maximal (i.e., 1 %-IB series), the major variation introduced to the reference product has a so significant impact on physicochemical properties that the modified formulation resulted not equivalent to the reference one both in terms of rheological patterns and as permeation profile.

Although further such findings should be confirmed by results obtained from other semi-solid bases and drug substances, the following remarks can be withdrawn. First, due to the intrinsic complexity of topically applied semi-solid products, an orthogonal approach is mandatory for assessing the equivalence between test and reference products. Although IVRT or Brookfield viscosimeter are a sensitive method for quality-control and batch-release purposes, the present case study clearly highlights that they might not be appropriate for the in vitro equivalence assessment for waiving clinical data. Indeed, although both IVRT and Brookfield viscosimeter provided superimposable results for both cream series, differences in spreadability and drug permeation have been recorded as shown in Tables 2 and 3. Secondly, the design of equivalence assessment of semi-solid products should take into consideration more the peculiarity of quality target product profiles of the reference product. The overall results highlighted the impact of the drug's physical state on the relevance of performed tests and obtained data. On the one side, in-depth rheological characterization and IVPT were essential for highlighting differences between minor and major variations in the 1 %-IB series. On the other side, the Q3 assessment was sufficiently sensitive for demonstrating the equivalence of modified formulations of the 10 %-IB series, in which the impact of formulative changes appears less due to the maximal thermodynamic activity of the drug substance.

4. Discussion

The importance of an in-depth physicochemical and structural characterization of semi-solid preparations was clearly underlined by the overall data and an extensive literature on the topic. Although they

Table 4
Ninety percent confidence intervals for the ratio of means of the test and comparator products for the rheological, release and permeation parameters. Intervals outside specification of EMA draft guidelines are in bold in the table.

Pairs	η at 100 s^{-1} (Pa.s)	Thyst% (%)	Yield Point (Pa)	G' at 1 Hz (Pa)	G'' at 1 Hz (Pa)	k (√h)	J (µg/cm²h)
1 %-IB series							
1 vs 2	91.3–96.3	91.1–109.2	95.8–102.2	95.6–97.5	105.5–109.3	94.9–108.9	81.7–120.7 (1)
1 vs 3	93.3–100.5	**59.7–77.2**	**129.4–154.4**	**62.4–75.8**	**55.7–66.9**	99.4–110.8	**102.3–137.5** (1)
2 vs 3	100.0–106.6	**61.2–75.7**	**130.8–156.2**	**64.7–78.6**	**51.8–62.4**	93.9–106.0	**99.8–143.1** (2)
10 %-IB series							
4 vs 5	100.1–103.4	96.7–109.4	96.8–105.5	94.7–101.5	94.2–102.3	96.2–110.6	86.1–114.7 (1)
4 vs 6	94.9–104.7	92.0–107.3	93.3–99.0	91.3–96.9	90.3–102.6	94.6–109.4	93.0–116.5 (1)
5 vs 6	93.1–103.0	91.4–102.0	91.0–99.3	92.8–99.0	91.7–104.8	91.7–102.8	89.5–122.6 (1)
EMA acceptance intervals	90–111	90–111	90–111	90–111	90–111	90–111	(1) 77.2–129.8 (2) 80.0–125.0

Note: In agreement with EMA draft guideline (EMA, 2018), the acceptance interval for permeation fluxes: (1) 80.00- 125.00 %; (2) can be enlarged based on intra-subject variability of the reference products following the procedure reported on "Section 4.1.10 Highly variable drugs or drug products" of the EMA Guideline on Investigation of Bioequivalence (EMA, 2010).

are well-established formulations, both in terms of manufacturing processes and clinical uses, the development of a stable and robust semi-solid formulation remains challenging. Due to the native complexity of the delivery route, slight changes in formulation components may have an enormous impact on the QTPP. Such aspects should be carefully evaluated during the pharmaceutical development by comprising in-depth the product design space and the relative CQAs (EMA, 2017). Consequently, both the EMA and the FDA guidelines stressed the importance of "handling carefully" components that, when introduced in the formulation, may influence the API bioavailability or, more in general, product performance (EMA, 2018; FDA, 2022). However, it is also noteworthy that such an assessment cannot be performed *a priori*, strictly basing on the physicochemical properties of the components, but should also take in consideration the peculiarities of semi-solid product in which they are added and evaluating their possible effects in light of their interaction with other components in the heterogeneous and complex semi-solid matrix. The results obtained in this work provide a clear example. Although it is known that changing the emulsifiers' pair impacts on the performance on a semi-solid product (Badruddoza et al., 2023; Casiraghi et al., 2017; Franzol et al., 2021; Ilić et al., 2017; Kumar Sharma et al., 2021; Opawale et al., 1998), the overall obtained results highlighted clearly how the impact of such a formulative changes on product rheological properties, release, permeation profiles may vary significantly considering the QTPP of the semi-solid products. Such formulative change is strongly relevant for creams having drug solubilized in the semi-solid matrix, whereas it is negligible when drug reached the maximal thermodynamic activity. In addition to such findings, it is noteworthy that semi-solid performance can be altered by apparently negligible changes in semi-solid compositions. It is the case of antimicrobials: literature evidence demonstrated that parabens or alkanediols influence the equilibrium distribution of drugs in the phases of different semi-solid product (Casiraghi et al., 2016; Sigg and Daniels, 2021a, 2021b). Therefore, a quali-quantitative variation of antimicrobials may influence drug release and/or permeation from both simple and complex formulations. Although this evidence may appear obvious in the light of current scientific knowledge, it is not from a regulatory point of view. Indeed, unlike the approaches proposed by both the major Regulatory authorities (EMA, 2018; FDA, 2022), it cannot be stated *a priori* that two semi-solid products, which differ in terms of the qualitative (Q1), quantitative (Q2) compositions of excipients, are also different in terms technological performances (Q3). As well, it cannot be excluded *a priori* that a qualitative/quantitative changes in antimicrobials may not impact on the technological performances. On the contrary, no reasonable scientific doubts remain on the therapeutic equivalence of such products, if the equivalence of technological performances (Q3) is properly demonstrated. This change of a regulatory paradigm is particularly relevant in supporting post-marketing variations of a marketed product. Based on the current regulatory guidelines, the benefit/risk balance of formulative changes should pass through the availability of clinical evidence demonstrating the maintenance of efficacy/safety patterns. However, such approach seems not reasonable in presence of validated in vitro methodologies for both economic and ethical reasons. Resorting to clinical trials for post-marketing variations may be too cost-expensive for well-established medicinal products, such as most of topically applied products on the market. Indeed, they are lengthy and expensive due to the greater variability that requires the enrollment of a substantial number of subjects to achieve sufficient statistical power (Krishnaiah et al., 2014). Without a clear advantage, affecting the economic sustainability of such products may result in an increased risk of shortages or market withdrawals (Musazzi et al., 2020). If the failure of a generic product in obtaining marketing authorization may have only consequences on the economic sustainability of healthcare systems, undermining the economic sustainability of an already-marketed products poses significant ethical concerns since it affects patients' access to effective treatments. Whenever possible, the equivalence assessment of post-marketing changes should be based on in vitro data. Existing literature and obtained results underline the importance of providing the equivalence assessment between two semi-solid products based on an integrated approach. Unlike findings of Krishnaiah et al. (2014), a single test (e.g., IVRT) is not sensitive enough to assess equivalence between two semi-solid products. This supports the efforts made by the EMA and the FDA to overcome IVRT-based equivalence assessments (e.g., SUPAC-SS guidance) towards a more orthogonal comparability exercise, including the evaluation of rheological properties, in vitro release, and permeation performances (EMA, 2018; FDA, 2022). In this context, the availability of compendial Pharmacopoeia tests and/or validated protocols is strategic for supporting manufacturers in maintaining high quality standards. It is the case for IVRT and IVPT, for which monographs and/or guidelines have been released both in the USA and the EU (EMA, 2018; FDA, 1997; USP, 2023). On the contrary, the estimation of rheological properties of semi-solid products lacks tests validated by regulatory authorities. Both the EMA and the FDA guidelines limit to provide indications on how products should be characterized. Moreover, the current draft guidelines are not totally harmonized. As an example, for the EMA, the yield point determination is a key-parameter for characterizing all type of semi-solid products, whereas FDA requires it only for semi-solid products having a plastic flow behaviour (García-Arieta et al., 2023). This condition leaves manufactures in a "regulatory uncertainty", and plenty of room for establishing in-house methods and specifications for their own products. The comparison of obtained results and, consequently, the equivalence assessments resulted particularly difficult in presence of semi-solid products with an intrinsic inter-batch variability (Mangas-Sanjuán et al., 2019).

Finally, it is worthy observing that outcomes of such in vitro equivalence assessment should be evaluated on the basis of CQAs of the semi-solid products. For an example, for simple dosage forms (e.g., ointments) or when drug thermodynamic activity is maximum, rheological and IVRT studies may be sufficient to assess the equivalence between two products, even if they are different for quali-quantitative composition. On the contrary, when changes concern complex formulation (e.g., cream) in which the drug is solubilized, changes in quali-quantitative composition may have a significant impact on the drug thermodynamic activity and microstructure. However, conflicting results that may arise from Q3 assessment should be evaluated based on in vitro permeation kinetic studies and on their therapeutic relevance. In this light, the dermal microdialysis/microperfusion methodologies may be useful to provide supportive data to study borderlines situation to waive clinical endpoint trials (Birngruber et al., 2022; Handler et al., 2021). They are also valuable to validate in vitro permeation methods (e.g., IVPT) as pivotal equivalence tests in all cases in which an in vitro/in vivo correlation has been not already established.

5. Conclusion

Regardless of the regulatory framework of reference, the development of generic locally acting semi-solid products should be based on the demonstration of its qualitative (Q1) and quantitative (Q2) equivalence to the reference. In this context, regulatory authorities accept in vitro methods to demonstrate that generic and reference products are structurally and functionally similar (Q3). However, a proper in vitro comparability studies to assess changes of formulation composition may be necessary during pharmaceutical development. In this light, overall results clearly highlighted the importance of approaching equivalence assessment by a comprehensive approach. Unlike some findings in literature, the equivalence between two products cannot be assessed based only on results of IVRT, but a more in-depth evaluation of rheological and permeation performances is needed. However, it is noteworthy that the type and entity of tests to be performed is linked to the physical state of drug substance after loading in the semi-solid matrix. Indeed, the obtained results clearly evidenced that, when the drug thermodynamic activity is maximum, rheological and IVRT studies are

sufficient to assess the equivalence between two products even if they are different for quali-quantitative composition. On the contrary, when changes concern complex formulation (e.g., cream) in which the drug is solubilized, changes in quali-quantitative composition may have a significant impact on the drug thermodynamic activity and microstructure. However, conflicting results that may result from the physically and functionally assessment should be evaluated based on in vitro permeation kinetic studies and on their therapeutic relevance. Otherwise, for post-marketing variations, the chance of requiring a proper clinical trial in lieu of a biowaiver should be carefully assessed by regulatory authorities, taking in consideration the availability of in vitro methodologies, the impact on the product economic sustainability, and patients assess to treatment.

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CRediT authorship contribution statement

Paola Volontè: Investigation, Visualization, Writing – original draft. **Umberto M. Musazzi:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Luca Arnaboldi:** Data curation, Investigation. **Marco A. Ortenzi:** Validation. **Antonella Casiraghi:** Methodology, Validation. **Francesco Cilurzo:** Conceptualization, Writing – review & editing. **Paola Minghetti:** Supervision.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2024.106726.

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