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RESEARCH ARTICLE

Quality by design enabled the development of stable and effective oil-in-water emulsions at compounding pharmacy: the case of a sunscreen formulation

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ABSTRACT

It is widely accepted that the use of topical sunscreens has medical importance with potential to prevent skin damage by protecting from solar ultraviolet radiation (UVR) effects. Pharmaceutical emulsions require an optimal qualitative and quantitative combination of emollients, emulsifiers and others compounds such as softening agents and, for sunscreens, a combination of chemical and physical UV filters. Herein, we applied the quality by design (QbD) concept to achieve stable and effective compounded sunscreen emulsions. By using the statistical tool of design of experiments, it was possible to identify the influence of emulsifier type (with low and high Hydrophile-Lipophile Balance) and concentrations of emollient and softening agent on the achievement of formulations with suitable organoleptic and physicochemical features. Compounded emulsions with pleasant macroscopic aspects were obtained. Three formulations with physicochemical properties in targeted ranges were selected, namely pH ~6.0, conductivity > 0.0 μS/cm^2, spreadability factor ~1–1.5 g/mm^2, viscosity ~12000 mPa.s and sunscreen protection factor ~30. Freeze-thaw cycle and accelerated stability study under different storage conditions allowed selecting a stable emulsion that ensured photoprotection in biological assays. The QbD approach was essential to select the best, low-cost compounded sunscreen emulsion, with targeted physicochemical parameters.

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1. Introduction

It has widely been accepted that protection from ultraviolet radiation (UVR) spectrum reduces the risk of acute and chronic skin damage in humans (Diffey et al. 2000). In addition to preventing sunburn and photoaging, sunscreens exert beneficial effects against skin cancer, and their regular use did not increase mortality (Lindstrom et al. 2019). Originally, sunscreens were developed to minimize erythema from sunburn, because their action spectrum is about 1000 times per unit dose (J/m^2) more effective against UVB than UVA (Young 2017). Sunscreen formulation protection is defined as the efficacy in preventing UV-induced erythema. It is expressed as sun protection factor, which is directly measured by a standard *in vivo* procedure, with erythema as the end point (Binks et al. 2017).

Despite such a role of sunscreens, the poor compliance is still considered a drawback (Weig et al. 2020). Compliance with use in a specific brand or in sunscreen manufactured in compounding pharmacies depends on the technology employed for the development of cosmetically pleasing sunscreen formulations, for example in terms of sensorial properties (suitable spreadability leading to easy application), non-whitening appearance of the skin after application (Matts et al. 2010; Apolinário et al. 2013) and water resistance (Lionetti & Rigano 2017).

So, as for drug delivery systems, the pharmaceutical development framework for sunscreen formulations includes pre-formulation assays exploring a plethora of qualitative and quantitative factors that interfere with colloidal stability, especially in the case of topical emulsions (Praça et al. 2020; Mancuso et al. 2021). Currently, a promising approach to enhance performance of pharmaceutical processes and products at compounding pharmacies is the quality by design (QbD) approach. The goals of such a systematic concept are often achieved by linking the product quality to the desired biological performance, and then designing a robust formulation and manufacturing process aiming to obtain the desired product quality consistently (Yu et al. 2014).

QbD application has been stimulated by Food and Drug Administration (FDA) and European Medicines Agency (EMA) (European Medicines Agency (EMA) 2013) for large scale production in the pharmaceutical industry; however, to the best of our knowledge, there are no reports concerning QbD approaches for compounded formulations. In the case of sunscreen emulsions, formulations produced at industrial scale and compounding pharmacies have a similar quality target product profile (e.g. dosage form as semisolid emulsion and topical route of administration), so the critical quality attributes (e.g. colloidal stability and photoprotective action) are also similar (Peres et al. 2017; Namjoshi et al. 2020). However, it is quite complicated to apply QbD in these pharmacies because techniques for measuring and establishing critical quality attributes require rather expensive instrumentation such as High-Performance Liquid Chromatograph (HPLC) or light

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Supplemental data for this article can be accessed here.

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scattering methods, which are feasible only in industry, large research centres and universities.

In reason of this scenario, herein we aim to employ an innovative QbD approach to produce a sunscreen with macroscopic and physicochemical characteristics suitable for colloidal emulsions to be prepared at laboratory scale in compounding pharmacy using simple, fast, and accessible techniques. Statistical tools allowed achieving a targeted profile in terms of physicochemical parameters that are useful to increase compliance with the usage as well as colloidal stability.

2. Materials and methods

2.1. Materials

The polymer Carbopol® 940 (polyacrylic acid) and the polymeric emulsifier Pemulen™ TR2 (acrylates/C10-30 alkyl acrylate crosspolymer) were acquired from Lubrizol (São Paulo, SP, Brazil). The chelating agent EDTA (disodium ethylenediaminetetraacetate), the antioxidant BHT (butylated hydroxytoluene), and triethanolamine (2,2′,2″-nitrilotriethanol), acting as buffer, chelating agent and surfactant, were purchased from Synth (Diadema, SP, Brazil). The broad spectrum antimicrobial agent Phenonip™, made up of methylparaben, propylparaben, ethylparaben and phenoxyethanol, was acquired from Clariant (Jacareí, SP, Brazil). The non-ionic emulsifier Eumulgin® B2 (Ceteareth-20) used to prepare O/W emulsions was obtained from Croda do Brasil Ltda (Campinas, SP, Brazil). The oil-soluble film former Antaron™ V 216 (VP/hexadecene Copolymer) was purchased from Ashland (Covington, KY, USA). The humectant propylene glycol and the base fluids cyclomethicone and silicone elastomer were acquired from Dow Corning (Hortolândia, SP, Brazil). The O/W emulsifier Emulium® 22 (Tribehenin PEG-20 Esters), organic UV filter, solubilizer and emollient Cocoate™ BG (butylene glycol cocoate) were acquired from Gattefosse (Saint-Priest, France). The co-emulsifier Olivem® 900 (Sorbitan olivate) was obtained from Biovital (São Carlos, SP, Brazil), while the modified natural polymer Dry Flo® (Aluminium Starch Octenylsuccinate), that acts as softening agent, was obtained from Via Farma (São Paulo, SP, Brazil).

The UVA/UVB filter Eusolex® 4360 (benzophenone-3) was acquired from Infinity Pharma (Campinas, SP, Brazil). Tinosorb®S (bis-ethylhexyloxyphenol methoxyphenyl triazin) was purchased from Biovital. The UVB filter Uvinul® MC80 (octyl methoxycinnamate) was acquired from Valdequímica (São Paulo, SP, Brazil), while the UVB filters octyl salicylate (ethylhexyl salicylate), 4-methylbenzylidene-camphor (4-MBC) and UVA I absorber Neo Heliopan® 357 (avobenzone) were purchased from Biovital.

2.2. Quality by design framework

The quality target product profile (QTPP) allows identifying the critical quality attributes (CQAs) of a product and depends on the process design. These concepts were released in the International Conference of Harmonisation (ICH) guidelines Q8(R2)–Pharmaceutical Development (Food and Drug Administration (FDA), 2009a), Q9–Quality Risk Management (Food and Drug Administration (FDA), 2006) and Q10–Pharmaceutical Quality System (Food and Drug Administration (FDA), 2009b). Here, the QTPP was defined according to scientific and regulatory assessment. CQAs with impact on the final product quality were identified from QTPP (Chang et al. 2013; Veiga et al. 2018).

Table 1. Selected independent variables and their respective levels used in the 2^3-full factorial design.

	Levels	
Factor	–	+
Type of emulsifier	Emulium®22[a]	Olivem® 900[b]
Concentration of emollient (Cocoate™ BG)	2 % (w/v)	4 % (w/v)
Concentration of softening agent (Dry Flo®)	2 % (w/v)	4 % (w/v)

[a]Hydrophile-Lipophile Balance (HLB) = 10.5.
[b]HLB = 4-5.

2.3. Design of experiments

A design of experiments elaborated with a full three-factor and two-level 2^3-factorial design (Table 1) was applied to identify the effects of the three selected independent variables, namely the type of emulsifier (Emulium® 22 or Olivem® 900), the concentrations of the emollient (Cocoate™ BG, 2 or 4% w/v) and softening agent (Dry Flo®, 2 or 4% w/v). The dependent variables (CQAs) were pH, conductivity, spreadability factor, sun protection factor, apparent viscosity, and droplet size.

The experimental data were analysed using the Statistica software version 7.0 and program Assistat version 7.7. to perform analysis of variance (ANOVA) and Tukey test ($p < 0.05$).

2.4. Emulsions preparation

Sunscreen emulsions were prepared in triplicate by the phase inversion method. In summary, both phases were heated at 60 °C, and then the aqueous phase was added to the oil phase under mechanical stirring at 2000 rpm for 15 min. Assays were randomized to eliminate bias (Table S1 in the Supplementary Material). The full composition of formulations is listed in Table 2.

2.5. Macroscopic analysis

All formulations were briefly submitted to organoleptic (colour, odour) and physical (phase separation, creaming) analyses. Then, the formulations were classified, according to the criteria from the Guide for the Quality Control of Cosmetic Formulations of the Brazilian National Health Surveillance Agency (ANVISA), as: a) Normal (N), i.e., without any visual change such as phase separation, precipitation of filters, colour modification compared to the formulation manufactured on the first day; b) Slightly modified (SM); c) Modified (M); d) Intensely modified (IM) (Brazilian Health Regulatory Agency (ANVISA)), 2004).

2.6. Physicochemical characterization of emulsions

2.6.1. Microscopic analysis

Droplets size was measured according to Apolinário et al. 2013 after 1:10 (m/v) dilution of all emulsions into a 1:1 (v/v) mixture of propylene glycol in water until complete homogenization under slight magnetic stirring. Samples (~50–100 μL) were placed on a slide and covered with a cover slip and observed with magnification of 700x using an optical microscope, model KH-7700 (Hirox, Tokyo, Japan). One hundred droplets were counted in different fields of slide, and three measurements were performed for each replica of formulations.

2.6.2. pH and conductivity

The pH of formulations was measured with a pHmeter, model PG 1800 (Gehaka, São Paulo, SP, Brazil), in samples dispersed in purified water obtained by reverse osmosis at concentration of 10% (w/v) at 25 °C. The electrode (FC09) was previously calibrated with

Table 2. Full composition (% w/v) of emulsions based on the 2^3-factorial design presented previously on Table 1, in which the positive level corresponds to the use of Olivem® 900 as emulsifier and the concentration of 4% (w/v) of Dry Flo® and Cocoate™ BG, while the negative one to the use of Emulium® 22 and the concentration of 2% (w/v) Dry Flo® and Cocoate™ BG.

Component	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol® 940	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Pemulen™ TR2	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Tinosorb® S	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Uvinul® MC80	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Eusolex® 4360	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Octyl Salicylate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Neo Heliopan® 357	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
4-Methylbenzylidene-camphor	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BHT	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Phenonip™	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Eumulgin® B2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Antaron™ V 216	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Propylene glycol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cyclomethicone	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Silicone elastomer	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Emulium® 22	2.0	2.0	0.0	0.0	2.0	2.0	0.0	0.0
Olivem® 900	0.0	0.0	2.0	2.0	0.0	0.0	2.0	2.0
Cocoate™ BG	2.0	2.0	2.0	2.0	4.0	4.0	4.0	4.0
Dry Flo®	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0
Purified water q.s.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

pH 4.0 and 7.0 standard solutions. For conductivity measurements, the same samples were analyzed with a conductivity meter, model PWT (Hanna, Barueri, SP, Brazil), provided with an electrode previously calibrated with a standard solution with conductivity of 146.9 μS/cm^2. All assays were performed in triplicate.

2.6.3. Spreadability

The spreadability factor was determined according to Garg et al. (2002). Briefly, a round glass plate (diameter = 20 cm; thickness = 0.2 cm), with a central hole of 1.2 cm in diameter was placed on a glass support plate (20 cm x 20 cm), which was put on a printable graph paper. Samples (about 1 g) were carefully inserted inside the central hole in the support plate to fit the whole space. The support plate was carefully removed, and another glass plate of known weight was placed on the sample. After one minute, the diameters were measured in two opposite positions. Afterward, the mean diameter was calculated. This procedure was repeated by successively adding other plates at one-minute intervals. The results were expressed in terms of the spreading area (S_i, mm^2) resulting from the applied mass (g) according to the equation:

$$S_i = d^2 x \frac{\pi}{4} \quad (1)$$

in which: d (mm) is the mean diameter of the sample.

The spreading area was plotted against the plate weight to obtain the spreading profiles. The spreadability factor (S_f, mm^2/g), which represents the spread that a semisolid formulation can reach on a smooth horizontal surface when one gram of weight is added on its top, was calculated according to the equation:

$$S_f = \frac{A}{W} \quad (2)$$

in which: A (mm^2) is the maximum spread area after the addition of the series of weights used in the experiment and W (g) the total weight added.

2.6.4. Viscosity

The apparent viscosity of formulations was determined at 25 ± 1 °C using a Viscolead rotational viscometer with spindle R7 (Fungilab, Hauppauge, NY, USA). The rotation speed was set at 100 rpm, and the measurements were performed in three positions of the emulsion placed in a becker. The results, corresponding to the average of three determinations, were expressed in mPa.s. The values were standardized at 30% of the torque value.

2.6.5. Sun protection factor

The sun protection factor (SPF) was determined *in vitro* according to the methodology proposed by Mansur et al. (1986), using a UV-Vis spectrophotometer, model UV-1650 PC (Shimadzu, Kyoto, Japan). The formulations were diluted in ethanol to a final concentration of 0.2 mg/mL. The absorption spectra of samples were acquired in the wavelength range from 290 to 320 nm every 5 nm using a 1-cm quartz cell. Three determinations were made at each point using ethanol as a blank. SPF was calculated by the equation:

$$SPF = CF \sum_{290}^{320} EE_\lambda \; x \; I_\lambda \; x \; Abs_\lambda \quad (3)$$

in which: $CF = 10$ is the correction factor, while EE_λ, I_λ and Abs_λ are the erythemogenic effect of radiation, the sunlight intensity, and the formulation absorbance at the wavelength λ, respectively.

2.7. Formulation stability

Formulations were submitted to stability tests, and at the end of each step they were analyzed for all CQAs described previously.

2.7.1. Preliminary stability

2.7.1.1 Centrifugation. Centrifugation tests were performed on formulations directly after preparation. The emulsions were submitted to centrifugation at 1000 *g* for 15 min. At the end, the formulations were classified, as previously mentioned in section 2.5, as follows: a) N; b) SM; c) M; d) IM (Baby et al. 2008; Pianovski et al. 2008).

2.7.1.2 Freeze – thaw cycling. To test the effect of freeze and thaw conditions on stability of the emulsions, the samples were stored in alternated six cycles for 24 h in each cycle, being either

at 40 ± 2° C or at 4 ± 2 °C. The entire study lasted 12 days (Brazilian Health Regulatory Agency (ANVISA) 2004).

2.7.2. Accelerated stability testing

Long-term accelerated stability tests were carried out for 180 days according to the recommendations of guideline for Q1A Stability Testing of New Drug Substances and Products from FDA (Food and Drug Administration (FDA), 2003) and Guideline on Stability Testing: Stability Testing of Existing Active Substances and Related Finished Products from EMA (European Medicines Agency (EMA) 2003) under the following conditions: incubation at 40 ± 2 °C, cooling in fridge at 4 ± 2 °C, and exposure to UV radiation at room temperature (25 ± 2 °C). All formulations were hermetically sealed in tubes to prevent moisture.

2.7.3. Rheological assessment of the selected emulsion

To assess the rheological behaviour of the selected sunscreen, a cone and plate rheometer (RST-CPS Cone/Plate, Brookfield, Middleborough, USA) equipped with Rheo3000 software was used gradually increasing and decreasing the shear rate in the range of 0–200 s^{-1} at 32 °C. The correlation between shear rate and viscosity was plotted, and the power law equation (Equation 4) was assessed as previously reported (Junqueira Garcia et al. 2018):

$$\tau = k\gamma^{n} \quad (4)$$

in which: τ is the shear stress, γ the shear rate, k the consistency index and *n* the flow index.

2.7.4. Determination of particle size and polydispersity index of the selected emulsion

Size distribution and polydispersity index (PDI) of the selected sunscreen were measured at 25 °C using a Zetasizer Nano ZS90 instrument (Malvern Instruments Ltd., Malvern, UK) with a detector set at an angle of 90°. Sunscreen was diluted with filtered ultrapure water at 1:1000 (m/v). Polystyrene cuvette was used to measure the hydrodynamic diameter. To obtain PDI values, a correlation function by using a cumulative analysis was employed. The hydrodynamic diameter (D_f) was obtained through the Stokes-Einstein equation:

$$Df = \frac{k_B T}{6\pi\eta R_h} \quad (5)$$

in which: k_B is the Boltzmann constant (1.3806×10^{-23} J/K), T the temperature, η the absolute viscosity of water and R_h the emulsion's droplets hydrodynamic radius.

For zeta potential measurement of the dispersed emulsion, a glass cuvette with a dip cell was used (Malvern Instruments Ltd.). Results were presented as the average of three measurements.

3. Results and discussion

3.1. Quality target product profile (QTPP), critical quality attributes (CQAs) and risk analysis

The early risk assessment (Figure 1) was determined by Failure mode and effects analysis (FMEA), aiming to remove or reduce the potential failures in order of priority. The interdependence rating was performed between categorized critical quality attributes (CQAs) and critical material attributes (CMAs), CQAs and critical process parameters (CPPs), and the factors were prioritized for risk management according to three aspects: 1) Severity, 2) Occurrence and 3) Detectability. A risk priority number (RPN) was calculated following the ranking: 3, serious; 2, minor; and 1 negligible (Ha et al. 2017). This approach allowed to identify and rank process parameters and materials as: 1–29 low risk; 30–59 medium risk and 60–125 high risk.

An Ishikawa diagram was created to show the CQAs that are more likely to cause product failure regarding proper properties for compounded sunscreen emulsions (Figure 2). Ishikawa plot allowed to identify risks concerning analytical methods, raw materials and process parameters that can trigger effects and influence the sunscreen emulsion quality. The Quality Target Product Profile (QTPP) and CQAs are listed in Table 3.

Critical variables and their levels used in the Design of Experiments (DoE) were selected based on the literature as

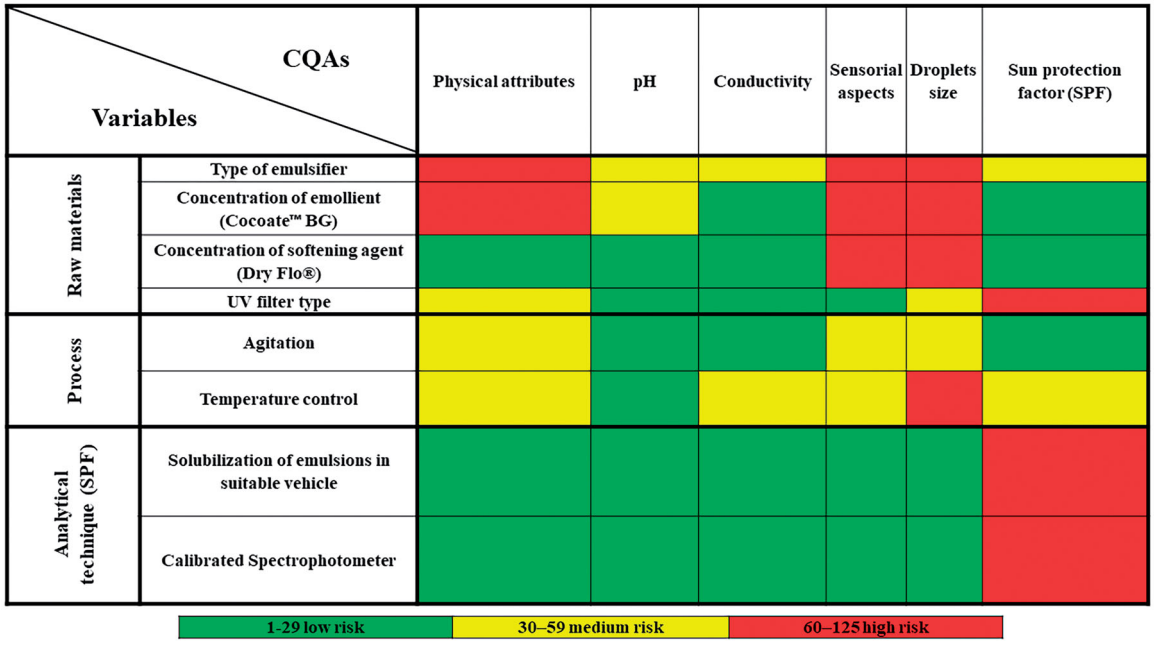

Figure 1. Initial risk assessment of emulsions sunscreens based on the estimated interdependence between the CQAs and CMAs/CPPs. The risk management is designed according to three aspects: 1) Severity 2) Occurrence and 3) Detectability, following the ranking: 3, serious (red); 2, minor (yellow); and 1, and negligible (green).

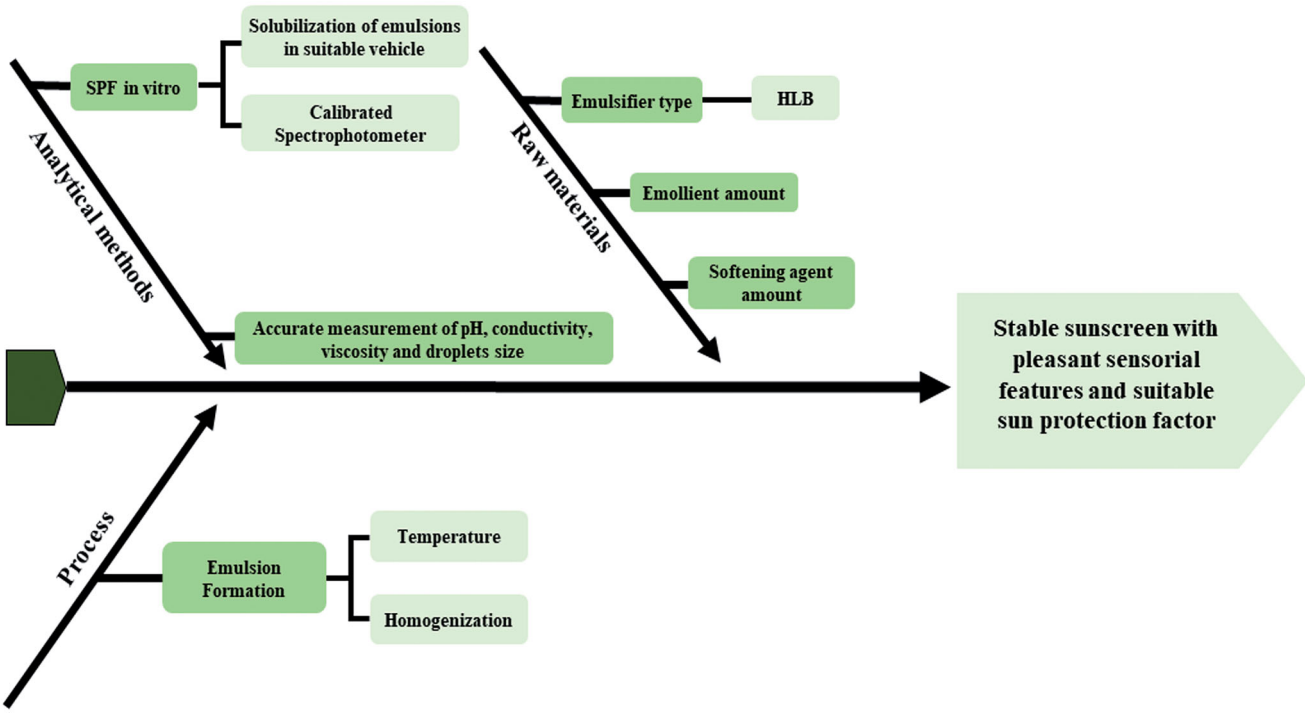

Figure 2. Ishikawa diagram illustrating factors that might have an impact on pleasant sensorial properties, stability, *in vitro* protection effect against UVB radiation of a compounded sunscreen emulsion. Factors were divided into the following categories: process of emulsion formation, analytical methods for physicochemical characterization of formulations and raw materials/critical quality materials (CQM). CQMs encompass type and concentrations of materials employed for emulsification and formation of an oil-in-water emulsion.

reported in this table. Considering that the analytical methods are already calibrated and/or standardized and that the process of phase inversion for emulsions production is classical, we decided to focus on modifications in raw materials or CMAs of the emulsions, which could either enhance performance, protection factor and/or sensorial features of the final product or reduce its costs.

3.2. Macroscopic characteristics

Visual examination of formulations from F1 to F6 carried out 24 h after their preparation suggested that no formation of visible aggregates occurred over 24 h. These formulations were slightly yellowish white, had pleasant odour and homogeneous appearance and showed no sign of phase separation. On the other hand, the formulations F7 and F8, which contained Olivem® 900 instead of Emulium® 22 as emulsifier and a higher concentration of Cocoate™ BG as emollient, showed yellowish colour and phase separation. Such separation was observed as a coalescing liquid region in semisolid formulations. It is possible that the presence of larger droplets in these formulations may have enhanced their creaming rate, leading to phase separation (Mancuso et al. 2021). Some formulations are prepared extemporaneously at compounding pharmacies, so it is necessary to aim at an initial stability of these emulsions, which could be immediately sold to customers.

Such a change in behaviour and appearance of the latter formulations may be ascribed to the fact that Emulium® 22, which is a complex mixture formed by transesterification of tribehenin and PEG-20 (Tribehenin Esters PEG-20), is more hydrophilic than Olivem® 900, a surfactant based on olive oil and sorbitol, considering their Hydrophile-Lipophile Balance (HLB) as being 10.5 and 4-5, respectively.

HLB is a key parameter to balance the interfacial tension between the two immiscible phases of emulsions. In fact, it is known that HLB values in the range 4-8 usually stabilize water-in-oil (W/O) emulsions, with values ≤ 6 resulting in the formation of poor emulsions, whereas HLB values from 8 to 18 stabilize oil-in-water (O/W) emulsions (Alam et al. 2020). This may have been the reason of Olivem® 900 inability to emulsify Cocoate™ BG when used in concentration up to 2% at a low surfactant-to-oil ratio (SOR). It has been accepted that an increase in SOR and HLB could lead to improved stability of emulsions (Nejadmansouri et al. 2016).

3.3. Physicochemical characterization of sunscreen emulsions

The significance of factor coefficients, i.e., the variation of the CQAs when the coded level of an independent variable is changed from -1 to $+1$, was evaluated by the Analysis of Variance (ANOVA), which together with Response Surface Methodology (RSM) is one of the most widely used statistical tools in emulsion optimization studies. The most significant factors for physicochemical properties of the formulations were assessed from the results collected under the conditions of the selected 2^3-full factorial design (Table S1).

The DoE methodology has proven useful in optimizing formation and formula of emulsions (Marto et al. 2015; Marto et al. 2016; Veiga et al. 2018). As expected, the interactions among compounds had significant effects on pH, conductivity and viscosity, thus confirming the importance of understanding not only the individual properties of materials but also the possible interactions among ingredients (Cizauskaite et al. 2017; Julian and Mahdi 2018; Terescenco et al. 2018). Conversely, variations in spreadability, sun protection factor (SPF) and droplet size were not statistically significant according to the one-way ANOVA ($p > 0.05$).

The effects of pH, conductivity and viscosity are commonly investigated for sunscreen development at compounding pharmacies, because pH and conductivity meters as well as viscosimeter are equipment easily accessible to them.

Table 3. Quality Target Product Profile (QTPP) and Critical Quality Attribute (CQA) profile for compounded sunscreen emulsions.

QTPP	Target	Reason
Dosage form	Oil-in-water (O/W) emulsion allowing solubilization of sunscreen agents into oil phase	O/W emulsions allow incorporating hydrophobic molecules, it is feasible to apply on extensive body surface and different body parts such as the face (Herzog et al. 2020)
Route of administration	Topical	Easy application and direct skin protection from sunlight (Jansen et al. 2013)
Appearance	Semisolid O/W emulsion with pleasant colour and odour	Commercial features lead to compliance and may be related to sunscreen performance (Granemann et al. 2013)
Sensorial features	Easy application and efficacy	Consumer-friendly features allow regular sunscreen application and effective sunscreen thickness on skin (Sambandan and Ratner 2011; Jansen et al. 2013)
Properties on the skin	Water resistance, protection, no irritation, and no whitening of skin	Formulation must have high sun protection factor according to its label and not trigger skin irritation during use or skin whitening (Diffey et al. 2001; Tanner 2006)
Stability	Shelf stability	No caking or creaming and no phase separation (Tanner 2006)
CQA	**Target**	**Reason**
Physical attributes	An emulsion without phase separation (caking or creaming) No unpleasant odour Soft yellowish white	These features can affect customer acceptance and product efficiency (Tanner 2006; Marto et al. 2016)
pH	4.0–5.8	pH < 4.0 causes tissue damage (Angelova-Fischer et al. 2018; Proksch 2018)
Conductivity	Constant over time and >0 μS/cm^2 for O/W emulsions	Conductivity is related to colloidal stability (Hao 2016; Mohamed et al. 2017)
Sensorial aspects	Rheological behaviour, viscosity and spreadability must be measured and be constant over time	Easy application and adhesion of the formulation should be allowed to maintain sufficient thickness of emulsion on the skin (Adeyeye et al. 2002; Savary et al. 2019). It is essential to avoid penetration of sunscreen agents through the epidermal membrane
Droplets size	Constant and uniform size distribution (>1 μm) over time	Size < 1 μm is related to nanoemulsions, and larger size distribution is a sign of further phase separation (Vlachou et al. 2020)
Sun protection factor (SPF)	Quantitative protective effect against UVB radiation according to the concentration of sunscreen agents used in the formulation	Sunscreens should provide the expected photoprotection to skin tissues (Yang et al. 2018)

The significance of pH as a CQA of formulations is also due to its influence on the acid barrier of the *stratum corneum* and cutaneous antimicrobial defence, which involves several pH-dependent enzymes. Overall, healthy skin is mildly acidic with pH varying from 4 to 6, except in the axillae, anal region and interdigital area which are alkaline (Schmid-Wendtner and Korting 2006; Bliss et al. 2017; Gustin et al. 2020).

Variations in pH were significant, with determination coefficient of 0.99, $F_{calculated}$ (183.25) > $F_{tabulated}$ (3.37), and an excellent correlation between predicted and observed values (Figure S1A). A design space for pH of emulsions is relevant because sunscreen performance relies on an enough thickness of emulsion that should be applied on extensive areas of skin, for long time, with reapplication when needed and daily use.

Whereas the individual independent variables did not statistically influence this response (Figure S2A), the interactions between emulsifier type and emollient or softening agent concentration had significant negative effects on it, i.e., the use of Olivem® 900 instead of Emulium® 22 as emulsifier combined to an increase in emollient or softening agent concentration led to a pH reduction in formulations. On the contrary, a statistically significant positive effect was exerted by the interaction between concentrations of softening agent and emollient, whose simultaneous rise resulted in an increase in pH. These effects are better evidenced in the response surfaces generated by RSM (Figure 3(A–C)), where pH is plotted as a function of the type of emulsifier and concentration of emollient and/or softening agent. In particular, the formulations produced using Emulium® 22 as emulsifier at the lower concentration either of emollient (2% w/v Cocoate™ BG) (F1 and F2) or of softening agent (2% w/v Dry Flo®) (F1 and F5) achieved pH values close to the target required for QTPP and CQA (4.0 < pH < 6.0). This goal was ensured by the simultaneous use of the lower concentration of emollient and higher concentration of softening agent (F2 and F4), regardless of the type of emulsifier. These findings are in agreement with previous studies that demonstrated significant interactions between the surfactant molecules and other ingredients of emulsions, specially emollients (Calixto et al. 2018; Terescenco et al. 2018; Terescenco et al. 2018).

The model of conductivity, as well as that of pH, has proven statistically significant, with determination coefficient of 0.99 and $F_{calculated}$ (13.40) > $F_{tabulated}$ (3.37), and suitable for predictive purposes (Figure S1B). Conductivity is not only considered the most sensitive property to detect physical alterations in emulsions (Masmoudi et al. 2005), but also indicates emulsion type or phase inversion under instability (Tan et al. 2014). The mean values of conductivity were in the range of 98.77 to 133.54 μS/cm^2, indicating that emulsions were O/W type (Masmoudi et al. 2005).

The Pareto chart describing the significance of the effects of independent variables on conductivity (Figure S2B) shows that it was individually influenced only by the softening agent concentration. Moreover, similarly to what was observed for pH, the interactions between the emulsifier type and the concentration of emollient or softening agent resulted in negative effects on such a CQA, while that between the concentrations of emollient and softening agent in a positive one. As expected, the respective response surfaces (Figure 3(D–F)) show that the emulsions with lowest conductivity values were those prepared using Emulium® 22 as emulsifier and the lower concentration of Cocoate™ BG or Dry Flo® as well as those using the lower Dry Flo® and the higher Cocoate™ BG concentrations.

As for viscosity, the model presented a coefficient of determination as high as 0.99, a $F_{calculated}$ (5.75) > $F_{tabulated}$ (3.37), meaning statistical significance, and an excellent correlation between predicted and observed values (Figure S1C).

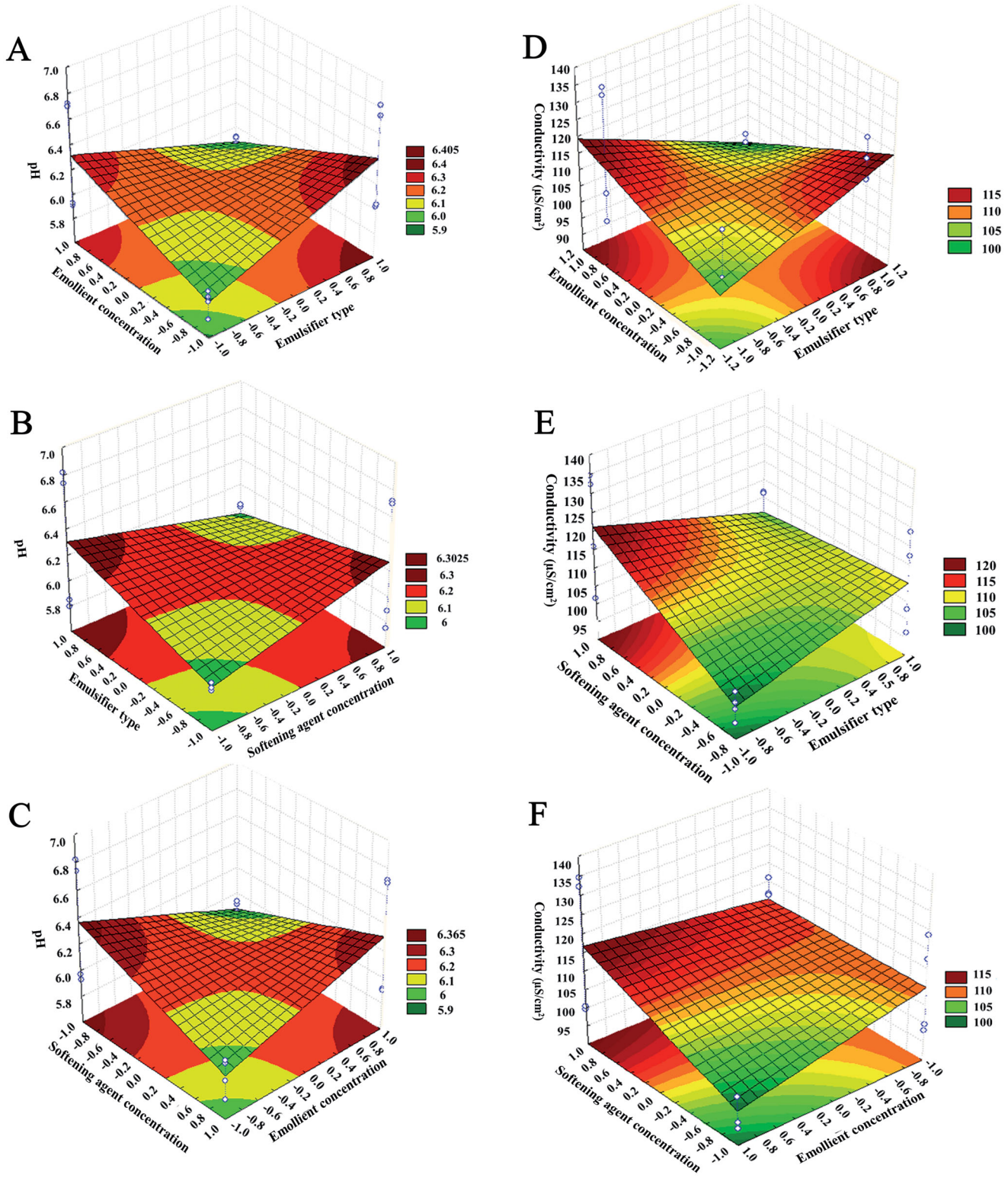

Figure 3. Response surfaces illustrating the simultaneous effects of the emulsifier type (Olivem® 900 or Emulium® 22) and concentration of emollient (Cocoate™ BG) or softening agent (Dry Flo®) on pH (A, B, C) and conductivity (D, E, F) selected as critical quality attributes. Values reported for the independent variables do refer to their coded levels according to the 2^3-factorial design of Table 1.

The corresponding Pareto chart (Figure S2C) shows that both type of emulsifier and concentration of Cocoate™ BG had negative, statistically significant influence on viscosity either individually or in combination. Herein, we are considering that the target could be either higher or lower values of viscosity depending on an infinite (very thick) or finite dose application of sunscreen, but we rather prefer lower viscosity values considering a greater spreadability and user preference. Reports demonstrated that thickening agents like benzophenone-3, if applied in infinite dose, could delay cutaneous penetration of sunscreens due to diffusional resistance in the formulation. In contrast, the flux could grow with increasing viscosity in finite or "in use" (very thin) dose

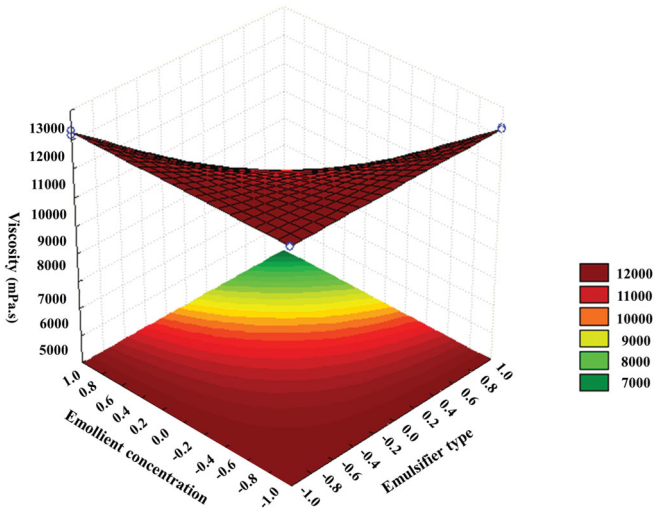

Figure 4. Response surface illustrating the simultaneous effects of emulsifier type (Olivem® 900 or Emulium® 22) and concentration of emollient concentration (Cocoate™ BG) on viscosity (mPa.s) selected as critical quality attribute. Values reported for the independent variables do refer to their coded levels according to the 2^3-factorial design of Table 1.

(Cross et al. 2001). As is evident in the response surface of this CQA (Figure 4), the lowest viscosity values were obtained at the lower concentration of Cocoate™ BG (2% w/v) using Emulium® 22 as emulsifier.

Resuming, the sunscreen emulsions F1, F2, F4 and F5 were appropriate in terms of pH, while for conductivity, since the target for O/W emulsions are values > 0, we selected the same formulations because the effects of the independent variables were qualitatively the same. Finally, F1 and F2 were the best formulations in terms of viscosity. Based on these results, the formulations capable of simultaneously satisfying all the three responses were F1, F2 and F5, which were all prepared using Emulium 22® as an emulsifier, regardless of the concentrations of the emollient and the softening agent.

3.4. Stability of sunscreen emulsions

3.4.1. Centrifugation

The centrifugation test, which simulates an increase in the force of gravity, increasing the mobility of particles is able to anticipate possible instabilities that could lead to phase separation (Baby et al. 2008; Pianovski et al. 2008); it can therefore be considered a forced coalescence (Gri and Daigle 2020). This preliminary test is widely used for emulsions and is aimed at evaluating the occurrence of phase separation or not, which makes it possible to identify formulations suitable for further characterization tests and to evaluate, in a short period of time, their eventual physicochemical stability (Baby et al. 2008; Pianovski et al. 2008). The formulations F1, F2 and F5 remained stable after the centrifugation test, not presenting macroscopic changes.

3.4.2. Freeze-thaw stress response

After freeze-thaw testing, it was not observed any phase separation or visual change in F1, F2 and F5 physical appearance such as turbidity or creaming, thereby confirming their stability (Daudt et al. 2015; Bhuptani and Patravale 2019). All CQAs analyzed kept in the targeted values (Table 4) and showed no statistically significant differences before and after freeze-thaw tests.

3.4.3. Accelerated stability testing

Since we have considered the preparation of formulations available for sale not just in an extemporary way, their shelf stability would be essential. Macroscopic analyses of formulations exposed to different storage conditions and times indicated absence of any spontaneous phase coalescence, flocculation, and phase separation in the F1 and F2 formulations, which remained slightly yellowish white in colour and homogeneous in appearance, while F5 showed slight changes and phase separation after 60 days (Table S3).

Conductivity (Table S5), droplet size (Table S6), spreadability factor (Table S7) and SPF (Table S9) have not undergone statistically significant changes under all the accelerated stability conditions. Instead, the pH of F5 changed significantly ($p < 0.05$) after 90 days of exposure to room temperature under UV radiation (Table S4), even if its values remained within the target range. The viscosity of F1 increased significantly after 90 days of exposure to room temperature and that of F2 after only 60 days both at room temperature and at 4 °C (Table S8). However, it should be noticed that such variations, although statistically significant, were rather small (about 0.3%) in a practical perspective.

Overall, these findings demonstrated absence of instability signs for both F1 and F2. Since F1 was prepared with a lower concentration of softening agent, this formulation has been chosen as optimized compounded sunscreen.

3.5. Size and rheological behaviour of the selected emulsion

Microscopic analysis of the selected emulsion submitted to stability assay demonstrated absence of precipitated sunscreen crystals and indicated that the formulation indeed allowed to solubilize the chemical filters employed, which are hydrophobic molecules (Slomberg et al. 2021). Both images showed droplets (Figure 5(A,B)) with size around 1 μm, i.e., macroemulsions. This outcome was confirmed by the analysis of hydrodynamic diameter that indicated that size distribution by number and intensity (Figure 5(C)) agrees with optical microscopy observations. As expected for macroemulsions prepared by low energy method with spontaneous emulsification (phase inversion method), the high polydispersity index (PDI $>$ 0.7-38.6 ± 4.70) indicated a polydisperse system (Slomberg et al. 2021), with presence of large and small droplets, which were also observed in the microscopic images. Meanwhile, the zeta potential value of the droplets in the emulsions (−38.6 ± 2.75 mV) indicates that the stabilization mechanism of the selected emulsion is electrostatic repulsion. In such cases, values of zeta potential above 30 mV (absolute values) imply strong repulsion forces, which lead to a targeted stability (Cacua et al. 2019).

Concerning the rheologic behaviour, we obtained the values of apparent viscosity and flow index. The selected sunscreen emulsion showed pseudoplastic behaviour since viscosity values decreased with increase in the shear rate (Figure 5(D)), which is further supported by a flow index value below 1 (0.0128). The pseudoplastic behaviour of sunscreens is known to lead to a protective film that covers the skin surface. Moreover, this characteristic leads to easy spreading, and the applied formulation can gain viscosity instantaneously to resist running. Differently, formulations with Newtonian behaviour leads to very quick spreading on the skin, reducing the protective film (Gaspar and Campos 2003; Souza and Campos 2017).

Table 4. Critical quality attributes measured in the formulations F1, F2 and F5 at initial time (T0) and after the freeze-thaw tests (F/T).

Formulations	pH	Conductivity ($\mu S/cm^2$)	Spreadability factor (g/mm^2)	Sun protection factor	Viscosity (mPa.s)	Droplet size (μm)
F1 T0	5.95 ± 0.02	99.10 ± 1.23	1.75 ± 0.28	26.0 ± 0.0	12328 ± 10	1.100 ± 0.030
F1 F/T	5.93 ± 0.04	73.07 ± 1.23	1.91 ± 0.16	25.5 ± 0.7	12326 ± 19	1.000 ± 0.003
F2 T0	5.82 ± 0.01	108.79 ± 10.01	1.79 ± 0.07	26.0 ± 1.4	12306 ± 1	1.078 ± 0.007
F2 F/T	5.92 ± 0.01	87.29 ± 19.25	1.85 ± 0.20	25.5 ± 0.7	12356 ± 37	1.008 ± 0.035
F5 T0	5.92 ± 0.01	98.77 ± 5.98	1.62 ± 0.41	27.0 ± 0.0	12242 ± 136	1.099 ± 0.035
F5 F/T	6.29 ± 0.41	75.92 ± 19.21	1.71 ± 0.22	26.5 ± 0.7	12362 ± 6	0.986 ± 0.010

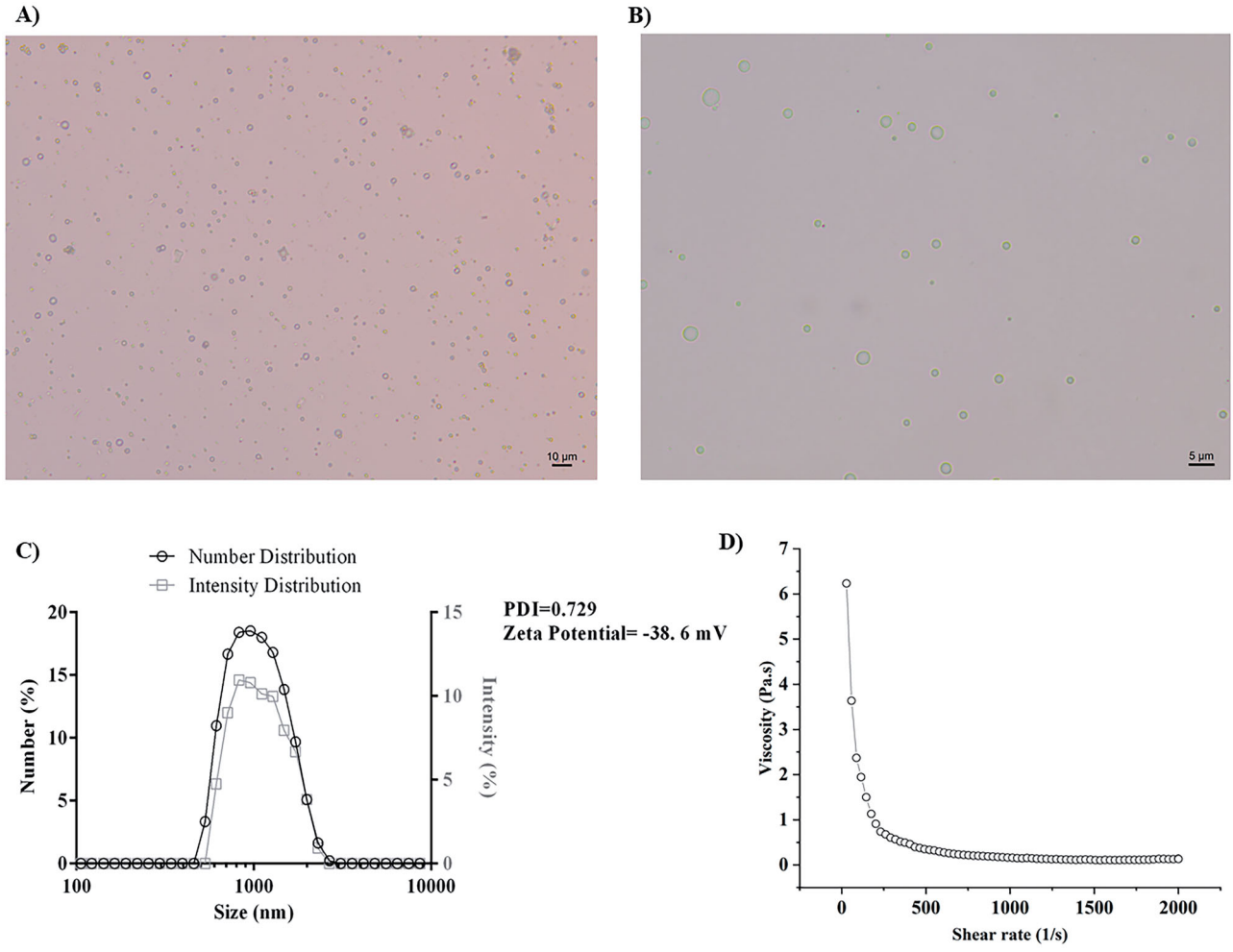

Figure 5. Size distribution and rheological behaviour of the selected formulation. Panels A and B show the images obtained by optical microscopy (Leica Microsystems DMC 2900, SP, Brazil) using 40 x (4 A) and 100 x (4B) lens with immersion oil for the selected emulsion 1:10 (m/v) diluted in a (1:1 v/v) mixture of water:propylene glycol. Panel C shows the size distribution of the selected emulsion by number and intensity with PDI of 0.729 ± 0.470 and zeta potential of −38.6 ± 2.75 mV. Panel D shows the rheological behaviour of the emulsion at 32 °C, which highlights a decrease of viscosity with increasing the shear rate.

4. Conclusions

Sunscreen emulsions have attracted studies both in the field of colloids and surfaces and in the biomedical area. In the present work, we applied, in an innovative way, a quality by design approach to develop sunscreen emulsions at compounding pharmacy by using statistical tools such as design of experiments and very simple and fast techniques. Compounded emulsions were prepared with critical quality attributes considered suitable to guarantee efficacy, stability, and usage compliance of sunscreen emulsion. The QbD approach applied to compounding pharmacy allowed to control variables concerning emulsions composition. Outcomes indicated the possibility of producing compounded sunscreen emulsions with targeted physicochemical properties, which are fundamental to ensure the quality of these products, such as pH and viscosity. The most promising emulsion did not show any changes in pH, droplets size, conductivity, and sun protection factor over time, demonstrating stability under storage. The zeta potential value above 30 mV (absolute value) after stability assay indicated the electrostatic mechanism of stabilization for the final formulation. Such selected compounded formulation presented pseudo-plastic behavior, which is attractive for sunscreen performance on skin after application, by allowing a film formation. Therefore, the QbD approach enabled the development of stable and effective oil-in-water emulsions at compounding pharmacy, which are expected to be more effective than commercial formulations. Furthermore, the *in vitro* sun protection factor of the emulsions was

demonstrated, indicating active protection against damages from UV irradiation according to the amount of chemical filters employed.

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