BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e21217

Article

PVP solid dispersions containing Poloxamer 407 or TPGS for the improvement of ursolic acid release

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Solid dispersions (SDs) of ursolic acid (UA) were developed using polyvinylpyrrolidone K30 (PVP K30) in combination with non-ionic surfactants, such as D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) or poloxamer 407 (P407) with the aim of enhancing solubility and in vitro release of the UA. SDs were investigated using a 2^4 full factorial design, subsequently the selected formulations were characterized for water solubility, X-ray diffractometry (XRD), differential scanning calorimetry (DSC), particle diameter, scanning electron microscopy, drug content, physical-chemical stability and in vitro release profile. SDs showed higher UA water-solubility than physical mixtures (PMs), which was attributed by transition of the drug from crystalline to amorphous or molecular state in the SDs, as indicated by XRD and DSC analyses. SD1 (with P407) and SD2 (with TPGS) were chosen for further investigation because they had higher drug load. SD1 proved to be more stable than SD2, revealing that P407 contributed to ensure the stability of the UA. Furthermore, SD1 and SD2 increased UA release by diffusion and swelling-controlled transport, following the Weibull model. Thus, solid dispersions obtained with PVP k-30 and P407 proved to be advantageous to enhance aqueous solubility and stability of UA.

Keywords: Ursolic acid. Solid dispersions. Solvent method. Water-solubility.

INTRODUCTION

Ursolic acid ($C_{30}H_{48}O_3$) is a pentacyclic triterpene carboxylic acid with molecular weight of 456.70 g/mol, log P value of the 6.43 and aqueous solubility of 0.102 µg/L at 25º C (US EPA, 2009; Zhang *et al*., 2013). This drug can be extracted from various plants and has demonstrated several pharmacological properties, including anti-inflammatory (Wang *et al*., 2020), cardioprotective (Chakraborty *et al*., 2016), antitumor (Feng, Su, 2019), anti-diabetic (Guzmán-Ávila *et al*., 2018), neuroprotective (Salau *et al*., 2021) and trypanocide (Eloy *et al*., 2015).

Although it has excellent pharmacological properties, UA is a class IV drug in the Biopharmaceutics Classification System (low solubility and permeability). The high lipophilicity of this acid makes it difficult to rapidly pass through biological membranes. Thus, it is partitioned and retained in the membranes contributing to its low permeability, which decreases the amount of the administered dose reaching the bloodstream (low bioavailability) (Gudoityte *et al*., 2021). Therefore, to enable the therapeutic efficacy of UA there is a need to develop a formulation to improve its biopharmaceutical properties. In this context, transdermal drug delivery systems have important applications in treating varied diseases and clinical conditions, because they offer some advantages, including the bypass of gastrointestinal disturbances, diminished renal toxicity, reduced dose frequency and high patient compliance (Petrilli *et al*., 2016).

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In the Solid Dispersions (SD) technology, one or more pharmacologically active ingredients are distributed in an inert carrier. These carriers are generally formed by high molecular weight polymers with hydrophilic properties that favor the solubility of hydrophobic drugs. In addition, the drug in SD can be present in the molecular state, amorphous particles or crystalline particles, resulting in lower energy barrier required to dissolve the molecules, with higher surface area leading to higher drug solubility and dissolution rate (Vo *et al*., 2013). Thus, the solid dispersion approach becomes an attractive and efficient method to improve the solubility of poorly water-soluble drugs.

There are two major methods used to produce solid dispersions, the solvent-evaporation method and the melting method. In the melting method, formulations are prepared by melting of drug and vehicles at a temperature above the melting point of the mixture. Then, the melt product is cooled and solidified (Tran *et al*., 2019). Although a simple and economical method, the drug and carrier need to be miscible in the molten state and its application is limited for thermostable drugs (Leuner, Dressman, 2000). Solid dispersion obtained by solvent-evaporation method is formed from of a solution of both drug and polymer in a single solvent followed by solvent evaporation. This technique generally enables the dispersion of drug in molecular level or in amorphous state in the matrix, which is preferred to increase the solubility of crystalline drug (Baghel *et al*., 2016). Mixture of drugs and carriers in one solvent can be difficult if they have significant polarity differences. In this case, solvent mixtures can be used as a strategy to obtain SDs by solvent-evaporation method (Baghel *et al*., 2016). In addition, the solvent-evaporation method enables loading thermosensitive drugs into SD, using carriers with high melting point. However a disadvantage of this method is the employment of organic solvents (Leuner, Dressman, 2000).

According to their composition, SDs can be classified into four generations. Carriers and drugs are in a crystalline state in the first generation of SDs. In this case, high thermodynamic stability of the carrier results at lower dissolution rates compared with amorphous SDs (Panizzon *et al*., 2019). Second-generation SDs are prepared with amorphous polymers and the drug may be molecularly dissolved or dispersed. Although the second generation promotes higher drug dissolution rates compared to first generation, drug can recrystallize from SDs during obtaining process or storage, and further can precipitate at supersaturation state in vivo (Vasconcelos *et al*., 2007; Vo *et al*., 2013).

Third-generation SDs differ from second-generation only by addition of surfactant to the system, with main objective of reduce problems related to drug precipitation and recrystallization. Therefore, formulations contain mixtures of amorphous polymers and surfactants as carriers (Vasconcelos *et al*., 2007). Fourth-generation of SDs employ insoluble or swellable polymers to sustain the drug release. These SDs are used for drugs with low water solubility and short plasma half-life (Vo *et al*., 2013).

Several polymers can be used in third-generation SDs and their properties improve the solubility of liposoluble drugs. Polyvinylpyrrolidones $(C_6H_9NO)n$ are popular hydrophilic carriers used in SDs because of their amorphous nature, high molecular weight, low melting temperature, high solubility in water, low cost, and biological compatibility (Patel, Patel, 2007). In particular, PVP K-12 to K-30 (molecular weight from 2500–50,000) have been extensively used as vehicles in SDs, since high molecular size of these carriers enables the formation of solid solutions (Sethia, Squillante, 2004a).

The addition of surfactants improves the physical stability of SDs because they increase the drug-polymer miscibility. Besides, surfactants can improve drug wettability and solubility (Tambosi *et al*., 2018). Poloxamers are copolymers consisting of a hydrophobic poly(oxypropylene) (POP) block between two hydrophilic poly(oxyethylene) (POE) blocks, which have been widely employed in several formulations for their ability to solubilize hydrophobic compounds, showing biocompatibility and increased drug stability in SD systems. Moreover, poloxamer 407 is a semi-crystalline surfactant with both crystalline and amorphous domains within its structure. These and other properties make this amphiphilic polymer a suitable vehicle for SDs preparations (Simonazzi *et al*., 2018).

The use of lipid-based vehicles in SDs is interesting due to their solubilizing properties. TPGS (D-α-tocopherol polyethylene glycol 1000 succinate) is a nonionic surfactant formed by polyethylene glycol (polar head) and

tocopherol succinate (apolar tail), giving it amphiphilic characteristics that favor the formation of stable micelles (Sethia, Squillante, 2004b). This water-soluble derivative of vitamin E has a semicrystalline structure due to the presence of polyethylene glycol chains. Several drugs have been developed with TPGS since FDA approved its use as pharmaceutic excipient. Lang *et al.* (2016) investigated the in vitro and in vivo effect of TPGS in the solid dispersions properties of itraconazole and noted that even a low amount of TPGS enabled the increase drug supersaturation in neutral pH conditions, and also showed rapid drug absorption after oral administration.

Although, PVP k30 has been used extensively in SDs, the formulations SDs of PVP k30 demonstrated limited physical stability due drug recrystallization within polymeric matrix upon storage (Nair *et al*., 2020). Therefore, the combination of PVP k30 with surfactants, such as P407 and TPGS can help to stabilize the drug in an amorphous state by increase of the physical miscibility of drug in the polymeric matrix (Chaudhari, Dugar, 2017). The amphiphilic properties of these carriers also can favor the skin permeation of ursolic acid. In addition, intermolecular interaction through hydrogen bonds is desirable to form solid dispersions. From the chemical structures of the molecules, hydrogen bonding could be expected between the hydroxyl groups of P407 and TPGS and the carbonyl functionality of the ursolic acid (Eloy, Marchetti, 2014). The presence of the hydroxyl group in the UA molecule also enables the formation of hydrogen bonding with carbonyl function of the PVP (Nair *et al*., 2020).

There are few studies reporting the preparation of SDs with ursolic acid for transdermal delivery. Therefore, in the present study we addressed the development of solid dispersions of UA using the combination of PVP K-30 with P407 and PVP K-30 with TPGS aimed at enhancing the water-solubility and in vitro release of UA (Figure 1). Solid dispersions were characterized through differential scanning calorimetry (DSC), X-ray diffraction (XRD), stereoscopy and scanning electron microscopy (SEM) to obtain information on the physical state of the drug, particles diameter, melting point and powders morphology. Physical stability of UA-SDs was studied by XRD and drug content analyses. Finally, in vitro permeation of the UA in SDs was evaluated using the Franz cells apparatus.

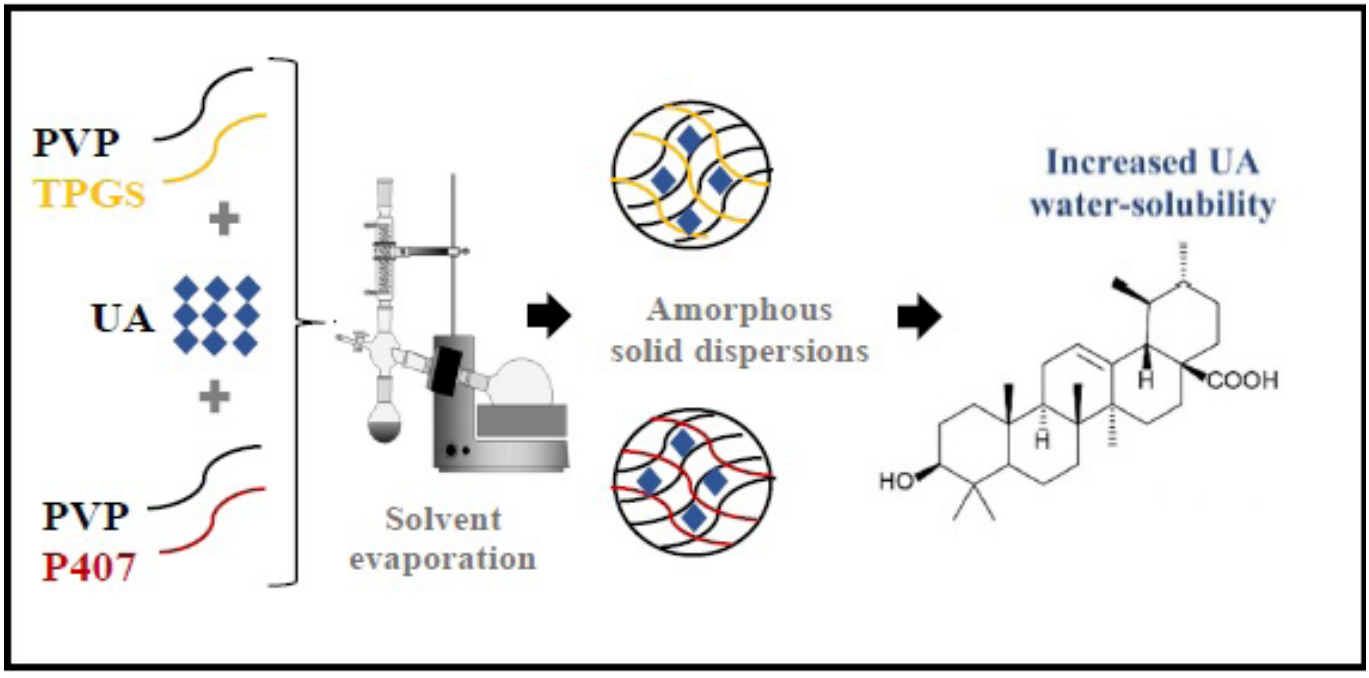

FIGURE 1 - Representative scheme of the preparation of amorphous solid dispersions of ursolic acid by solvent-evaporation method.

MATERIAL AND METHODS

Material

The following reagents were purchased: ursolic acid (Idealfarma, Brazil) polyvinylpyrrolidone K-30, average molecular weight of ~ 40,000 g/mol (Synth, Brazil); glacial acetic acid (Synth, Brazil); HPLC grade acetonitrile (J.T. Baker, USA); dichloromethane (J.T. Baker®, USA); sodium phosphate dibasic anhydrous (Synth®, Brazil); sodium phosphate monobasic (Synth®, Brazil); sodium lauryl sulfate (Synth®, Brazil), polytetrafluoroethylene 0.45 μm membrane (Merck, Germany). Poloxamer 407 (average molecular weight of ~12,600 g/mol) and D-α-tocopherol polyethylene glycol 1000 succinate – TPGS (average molecular weight of ~1,513 g/mol) were obtained from Sigma-Aldrich, USA.

Methods

Experimental design

A 2^4 full factorial design was used for development of the solid dispersion and was conducted in a randomized order. As shown in Table I, three independent variables were evaluated (X_1, ratio of drug:polymer:surfactant, w/w/w; X_2, rotation number per minute during rotary evaporation process; X_3: two different surfactants; X_4: volume of solvent, each at two levels (-1) e (+1), in order to study the influence of their individual and combined effects on the response of the UA solubility (Y, dependent variable).

The effect of a variable is significant when there is a statistically significant change in the response (in this case UA solubility) when migrating from the condition level (-1) to level (1). Then, the following parameters were calculated: variance (s^2) and experimental error (s); variance ($\Sigma a_i^2.s^2$) and error of the effects (Do Nascimento et al., 2014). The t-value was calculated with a 95% confidence interval. The statistical evaluation of the factorial design results was performed using Microsoft Excel. The contour graph and pareto diagram were plotted using Minitab software 19.

TABLE I - Experimental design for the solid dispersions manufacturing

Independent variables (factors)	**Levels**	
	(- 1)	**(+1)**
X_1: ratio of drug:polymer:surfactant, w/w/w	0.5:7.5:2	1:6:3
X_2: rpm during rotary evaporation process	50	100
X_3: volume of solvente (ml)	20	30
X_4: two different surfactants	TPGS	P407
Dependent variable (response)	**Constraints**	
Y: drug solubility (mg/ml)	maximize	

Abbreviations: w/w/w, weight/weight/weight; rpm, rotation per minute; TPGS, D-α-tocopherol polyethylene glycol 1000 succinate; P407, poloxamer 407.

Preparation of solid dispersions and physical mixtures

The SDs containing the drug, polyvinylpyrrolidone K-30 and poloxamer 407 or TPGS were prepared by the solvent method. As shown the Table I, to prepare 500 mg of SD in the drug:polymer:surfactant ratio of 1:6:3 (w/w/w), 50 mg of drug, 300 mg of polymer and 150 mg of surfactant were weighed. While, to obtain the SD in the drug:polymer:surfactant ratio of 0.5:7.5:2 (w/w/w), 25 mg of drug, 375 mg of polymer and 100 mg of surfactant were weighed. Thus, UA and carriers were dissolved in dichloromethane (total solids concentration in the solvent was of 2.5 % w/v) under magnetic stirring (200 rpm) for 30 minutes and at room temperature. Subsequently, the solvent was removed by rotary evaporation at a temperature of 30°C. The formulations were put into air circulation oven at 40°C for 24 hours to allow the complete evaporation of solvent, pulverized using mortar and pestle. After the pulverization, the particles were sieved (65 mesh, 212 μm) and stored in a desiccator at room temperature (Goddeeris & Van den Mooter, 2008). The physical mixtures (PMs) were obtained by simple mixing the drug and carriers at the same ratio of SDs using mortar and pestle and stored in a desiccator.

Solubility Study

The water solubility of pure UA and from PMs and SDs was analyzed with addition of the drug in excess to 2 mL water on flask, under mixing at 50 rpm at 32.5°C by 48 h. The samples were filtered (polytetrafluoroethylene 0.45 μm membrane, Merck) and diluted in acetonitrile. Quantification was performed as previously described in triplicate.

X-ray diffraction

The selected samples of solid dispersions (SD1, SD2, SD3 and SD4), as well as their controls in the form of physical mixtures (PM1, PM2, PM3 and PM4) were analyzed by XRD. Experiments were conducted by powder X-ray diffraction (Rigaku/Rint 2000, Osaka, Japan) equipment using CuK-α radiation (1.5406 A, 40 kV and 30 mA). Diffractograms were obtained over a 2θ range of 4° to 40° (step width 2°C; scan rate 1 deg/minute).

Differential scanning calorimetry

Thermal events were obtained by a DSC Star System (Mettler Toledo®). The selected samples of solid dispersions (SD1, SD2, SD3 and SD4), as well as their controls in the form of physical mixtures (PM1, PM2, PM3 and PM4) were weighed in aluminum pans (5 mg) and then hermetically sealed. An empty pan was used as reference. Thermograms were recorded at a heating rate of 10 °C/min from 25 °C to 300 °C and under nitrogen purge (20 mL/min).

Particles size

The particle size distribution of samples was analyzed on a stereoscope (Leica MZ APO) at 80-fold magnification and diameter of 150 particles of each sample was measured using an imaging software (Motic Advance Images 3.2).

Scanning electron microscopy

Images of surface morphology of UA, carriers, SD1 and SD2 were determined by scanning electron microscope JOEL (JSM-7900F, USA). A graphite double-sided adhesive tape was covered by samples and then were made conductive by coating with gold, using voltage 10 to 25 kV.

Quantification of ursolic acid

Quantification of the drug loaded in formulations, drug solubility and in vitro release assay was performed by high performance liquid chromatography (HPLC) using an analytical method previously validated (Eloy *et al*., 2012). HPLC (LC Infinity 1220 Agilent) system was employed. UA was detected at 203 nm with a flow rate of 1.0 mL/min using acetonitrile: water solution (88:12, v/v) as mobile phase. Separation was performed with a Zorbax Eclipse Plus C18 column (250 mm x 4.6 mm, 5 μm) and the injection volume was 20 μL. All determinations were performed at controlled temperature (25 ± 2.0 °C) under isocratic elution.

Drug content

UA content was evaluated in SDs samples (corresponding to 0.5 mg of drug) solubilized in 2 mL of acetonitrile. The samples were homogenized in a vortex shaker for 10 minutes, filtered (0.45 μm polytetrafluoroethylene membrane) and quantified by HPLC. The analyses were performed in triplicate.

Stability study

For the study of short-term physicochemical stability of the solid dispersions, the SDs samples were placed in sealed containers and stored in controlled temperature at 30±2°C and 60±0.5% relative humidity. The physicochemical properties of these samples were evaluated (drug content and XRD) after 0, 30, 60 and 90 days.

In vitro release study

In vitro release of UA from the SD1 and SD2 was evaluated using a Franz cell apparatus (Hanson Research Corporation, Chatsworth, CA, USA). A

synthetic polyethersulfone membrane was placed between the receptor and donor compartment. The area for diffusion was 1.77 cm^2 and the receptor volume was 7 mL of solution composed of a sodium phosphate buffer 30mM (pH 7.4) and sodium lauryl sulfate 1.25% (w/v), ensuring the sink condition throughout the test. The SDs and UA samples were dispersed in water (with a drug concentration of 3 mg. mL^{-1}) and placed (300 µL) on the membrane surface at the donor compartment. The receptor solution was kept at 32°C and under constant magnetic stirring (300 rpm) throughout the assay. Samples were collected automatically from receptor medium at predetermined time intervals (0.5, 1, 2, 4, 8, 12, 18, and 24 h) and the same volume of pure medium was added to the receptor compartment. All samples were filtered (polyvinylidene fluoride membrane 0.45 µm, Merck) and analyzed by HPLC. The results obtained were presented as an average of six analyses and the error is reported as standard deviation. The kinetics and release mechanism of UA from SDs were evaluated by linear regression using different mathematical models like first order, Weibull model, Korsmeyer-peppas model, Baker & Lonsdale model; Hixon & Crowell model and Higuchi model. The *Sigma Plot* 12 software was used for the fitting the equations of the experimental data. The diffusion parameters of drug through synthetic membrane were calculated by linear regression of the plots.

RESULTS AND DISCUSSION

The Pareto plot for the UA solubility with p-values for the different factors and interaction in rank order demonstrated that the variables X1, X3 and X4 have significant impact on UA solubility in the solid dispersions (Figure 2), since the p-values of each variable X1, X3 and X4 (1.06, 0.69, 1.04) were found to be higher than t-value (± 0.55). The contour plot with respect to UA solubility in the SDs (Figure 3) revealed that the change of drug: polymer: surfactant ratio (w/w/w) from 0.5:7.5:2 to 1: 6: 3 resulted in a reduction in the aqueous solubility of UA in the SDs. This phenomenon could be attributed to a small reduction of ratio of carriers in SD, which can affect the dispersion of the drug in the polymeric matrix and consequently caused lower drug solubility (Vo *et al*., 2013). It can be stated, thus, that the ratio 0.5:7.5:2 (w/w/w) was the best one investigated for increased UA solubility in the SDs. The increase in speed from 50 to 100 rpm caused a significant improvement in UA solubility from SD. These findings suggest that when drying was performed at a speed of 100 rpm, the dispersion of the drug in the polymeric matrix was more homogeneous and thus allowed a more efficient amorphous distribution of UA in the solid system, which resulted in greater drug solubility. A higher solubility of UA was observed for system containing poloxamer 407 compared to the TPGS system. Poloxamer (HLB = 18-23) is more hydrophilic than TPGS (HLB = 13) and therefore can promote greater drug solubility in water (Kolašinac *et al*., 2012). This also can be attributed to the arrangement of ethylene oxide and propylene oxide blocks of the poloxamer that self-assemble into micelles in aqueous solutions, which promote greater drug solubility (Kolašinac *et al*., 2012). Furthermore, improved wettability, absence of drug molecules aggregation, conversion of crystallinity to amorphous forms are other phenomenon can be related to this enhancement of UA water-solubility (El-Badry *et al*., 2009).

Therefore, based on the results of the factorial design it was observed that the best conditions for obtaining the solid dispersions were the following: rotary evaporation at 100 rpm, a concentration of 0.5: 7.5: 2.0 (drug: polymer: surfactant) and the use of poloxamer 407 as surfactant in the formulation.

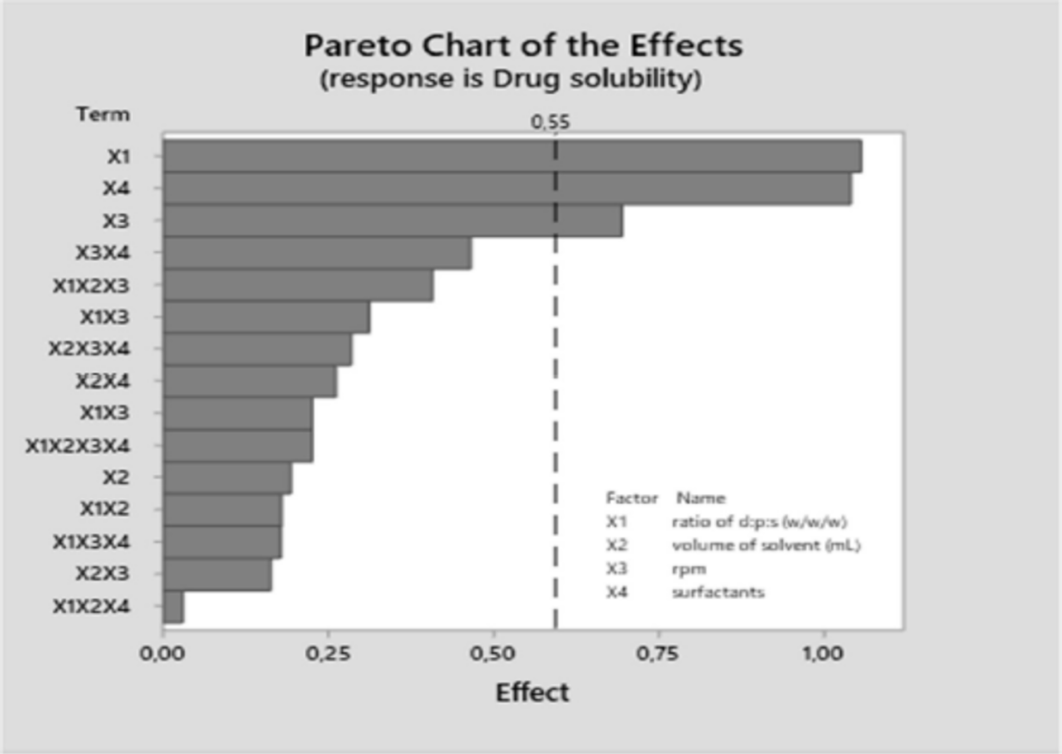

FIGURE 2 - Pareto plot of effects on ursolic acid solubility in solid dispersions.

Although the results of the factorial design indicate that the lower drug concentration is one of the optimal conditions for obtaining of solid dispersions that provide greater UA water-solubility, solid dispersions with higher drug concentration were also selected for further investigation, since they also demonstrated a significant increase of solubility compared to the pure drug. Furthermore, higher drug load could be advantageous for therapeutic application. In addition, with the aim of in future studies investigate the effect of skin penetration of SDs, we selected formulations containing D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) due to its ability to promote drug skin penetration. For better reading, the selected formulations were named as shown the Table II.

TABLE II - Nomenclature for solid dispersions

Name	drug: polymer: surfactant ratio (w/w/w)	surfactant
SD1	1:6:3	P407
SD2	1:6:3	TPGS
SD3	0.5:7.5:2	P407
SD4	0.5:7.5:2	TPGS
PM1	1:6:3	P407
PM2	1:6:3	TPGS
PM3	0.5:7.5:2	P407
PM4	0.5:7.5:2	TPGS

Abbreviations: SD, solid dipersion; PM, physical mixture; TPGS, D-α-tocopherol polyethylene glycol 1000 succinate; P407, poloxamer 407.

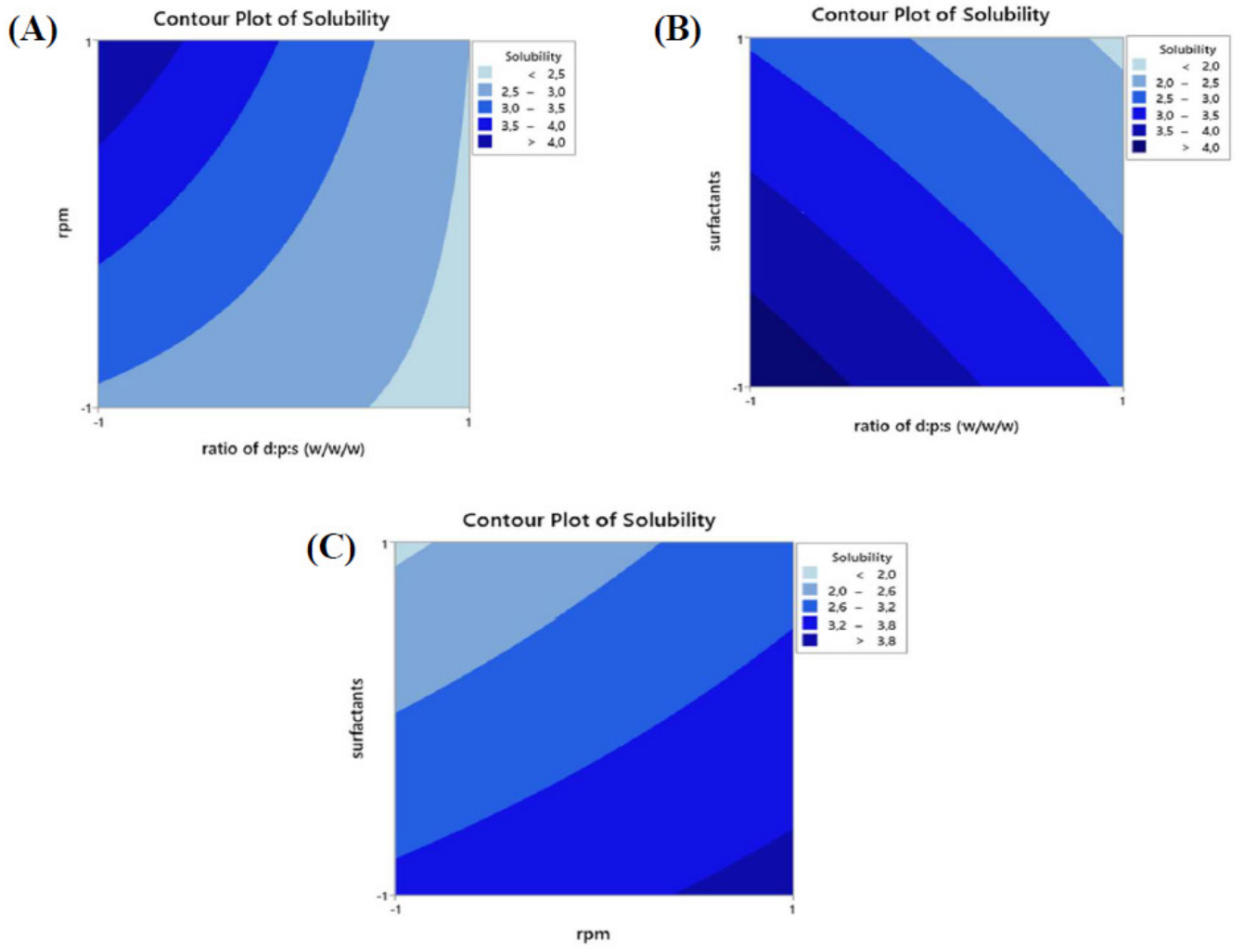

FIGURE 3 - The contour plot of UA solubility in the SDs with respect to the mutual effects of process parameters such as: (A) ratio of drug:polymer:surfactant (w/w/w) versus agitation speed, rpm; (B) ratio of drug:polymer:surfactant, w/w/w versus surfactants and (C) surfactants versus agitation speed, rpm.

UA exhibited a very poor solubility in water due to its high lipophilicity, therefore, the pure drug was not detected (detection limit = 0.37 µg/mL). However, considering the aqueous solubility value of pure UA found in the literature, corresponding to 0.102 µg/L at 25ºC (US EPA, 2009), the drug when present in all the physical mixtures and solid dispersions showed a significant increase ($p < 0.05$) in water-solubility (Figure 4). The solubility in water of UA in the physical mixtures prepared using UA:PVP:P407 in the ratio 1:6:3 and 0.5:7.5:2 was 1.75 ± 0.1 mg/mL (PM1) and 2.12 ± 0.01 mg/mL (PM3), respectively. Physical mixtures composed by UA:PVP:TPGS in the ratio 1:6:3 and 0.5:7.5:2 showed drug solubility values of 1.94 ± 0.05 mg/mL (PM2) and 1.91 ± 0.89 mg/mL (PM4), respectively. The solubility of SD1 and SD3 using UA:PVP:P407 in the ratio 1:6:3 and 0.5:7.5:2 in water was found to be 3.04 ± 0.18 mg/mL and 3.51 ± 0.02 mg/mL respectively. SD2 and SD4 consisting of UA:PVP:TPGS in the ratio 1:6:3 and 0.5:7.5:2 showed drug solubility values of 2.61 ± 0.5 mg/mL and 3.01 ± 0.12 mg/mL, respectively. Comparing the solubility data, it was found that SD3 ($p < 0.05$) showed the highest solubility of the drug, in an increasing order of higher UA solubility in water for solid dispersions SD2 <SD4 = SD1 <SD3. Thus, the best solubility values were obtained from a dispersion composed by a

ratio of 0.5: 7.5: 2 of drug: polymer: surfactant, that is, containing greater proportions of carrier than drug and containing poloxamer 407 as a surfactant. Therefore, although physical mixtures also improved UA solubility, a significantly higher UA water solubility ($p < 0.05$) was observed for the solid dispersions. This showed the efficiency of the solvent-evaporation method for producing solid dispersions.

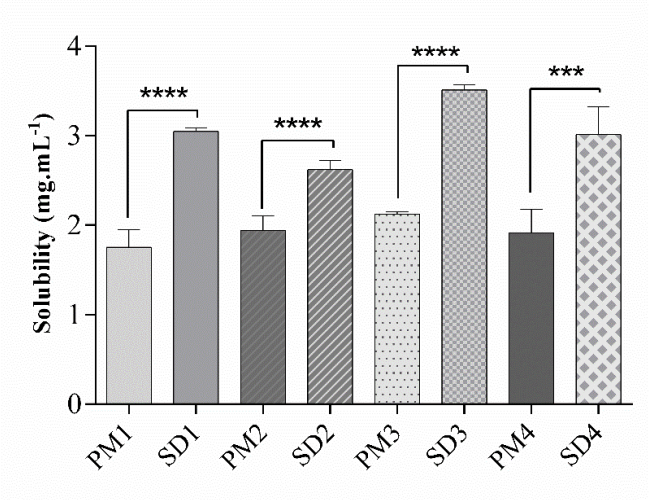

FIGURE 4 - Water solubility of UA from solid dispersions and physical mixtures at temperature of 32.5 °C after 48 h. The asterisks denote statistically significant differences (**** $p<0.0001$, *** $p = 0.0001$) for the indicated samples (Student's T test).

Regarding the compositions of SD employed here, binary or ternary drug-polymer or drug-polymer-surfactant systems to inhibit the drug precipitation in solid dispersion have been previously reported (Feng *et al*., 2018). In this context, poloxamer is composed of hydrophilic side groups (ethylene oxide block) and hydrophobic core (polypropylene oxide block), which gives its amphiphilic properties. In addition, it self-aggregates, so forming micelles allowing the solubilization of drugs with hydrophobic characteristics (Vyas *et al*., 2009). In a previous study, poloxamer 407 was used in SD as a strategy to improve solubility and dissolution rate of benznidazole (BZL). One of the most relevant findings of the study was that the dissolution rate of the BZL SD was about 400-fold faster than the pure BZL, physical mixtures and a commercial formulation, respectively (Simonazzi *et al*., 2018). Szafraniec and collaborators (Szafraniec *et al*., 2019) verified the positive effect of the self-assembly phenomenon of poloxamers on the increased dissolution of bicalutamide, a poorly water-soluble drug. Moreover, results of water solubility of the SD1 and SD3 found herein (containing UA in PVP-30 and P407 in a ratio at 1: 6: 3 or 0.5: 7.5: 2 equivalent to 3.04 mg/mL and 3.51 mg/mL) were higher compared with the solubility values of the SDs loaded with UA reported in a previous publication by Eloy *et al.* (2014), in which the SD were prepared by the solvent-evaporation method using only P407 as a carrier at a 1:10 ratio of drug: carrier (with mean solubility value equal to 0.69 mg/mL). Thus, the SD obtained with the combination of PVP k-30 and P407 shows an increase of 4 to 5-fold in the value of the drug's solubility compared to the SDs prepared only with P407. This may be attributed to the higher drug wettability and dispersibility in ternary systems compared to the effect of single poloxamer 407.

D-tocopherol polyethylene glycol 1000 succinate (TPGS) is a water-soluble derivative of vitamin E, which due to its properties as a non-ionic surfactant, has been extensively employed as carrier for solid dispersion, since it allows the solubilization of lipophilic drugs, in addition, it is able to improve the stability of these formulations because to its antiplasticizing effect (Yuana *et al*., 2013). Song *et al*. (2016) observed that the solubility and dissolution rate of the curcumin from the solid dispersion TPGS-based improved significantly compared with those of pure curcumin and physical mixture of curcumin.

SDs, when in contact with water, are rapidly hydrated resulting in a polymeric dispersion, which promotes the solubilization and release of drug particles (Newa *et al*., 2007). Furthermore, enhanced solubility of UA may be due to a decrease in the crystallinity of UA because of conversion to the molecular or amorphous dispersion in the carrier matrix as evidenced in the XRD and DSC analyses herein addressed. XRD experiments were performed to analyse the physical state of UA molecules in solid dispersions. The diffraction spectra of UA showed characteristic peaks at 2θ in 5.6°, 7.8°, 11.3°,14.4º, 14.8° e 16.8° (Figure 5). These findings confirm the crystalline form I of the UA as previously reported by Zhou (Zhou *et al*., 2015). For the surfactants present in the formulations, P407 and TPGS, two diffraction peaks were observed at 19 and 23°, showing that both have a semi-crystalline

structure (Karolewicz, Owczarek, 2017; Yoon, Cho, 2015). PVP diffractogram revealed two broad halos, which are located around 12 and 22°. Although PVP is an amorphous polymer, this data suggests that enthalpic relaxation might have occurred generating a certain degree of order of the PVP chains (Mendes *et al*., 2010). The XRD patterns of the physical mixtures PM1 and PM3 contain peaks corresponding to the UA, PVP and P407. This suggests that the crystalline arrangement of drug molecules was kept in these PMs. In the diffractograms of SD1 and SD3, crystalline domains of the poloxamer 407 remained present, however, no characteristic peaks of the UA were detected.

On the other hand, diffractograms of PM2 and PM3 showed only the crystalline profile of TPGS and the broad halos of PVP. In this case, the absence of the drug peaks in the PM2 and PM3 indicate that the simple mixing of the carriers with the UA was enough to change the drug crystallinity to an amorphous or molecular state. XRD patterns of the SD2 and SD4 also did not show the UA diffraction peaks. Additionally, the X-ray peaks of TPGS were absent in SD2 and SD4, suggesting that the SDs formation contributed to the amorphization of TPGS.

The disappearance of characteristic peaks of the drug in the developed SDs indicates the conversion of crystalline state to a UA amorphous state, or it could suggest that the UA is dispersed at the molecular level in the SDs (Eloy, Marchetti, 2014; Zhai *et al*., 2017).

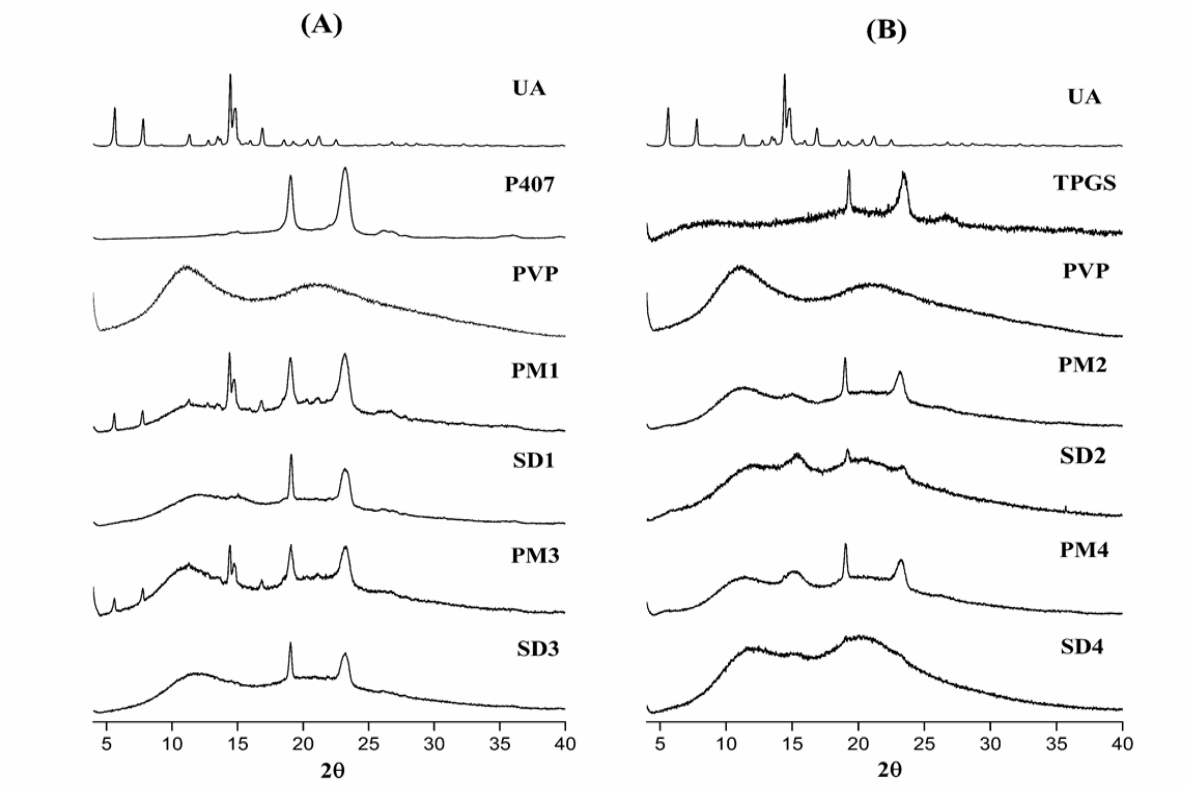

FIGURE 5 - X-ray diffractograms of SDs and PMs containing ursolic acid: polyvinylpyrrolidone k-30: poloxamer 407 (A) and ursolic acid: polyvinylpyrrolidone k-30: D-α-tocopherol polyethylene glycol 1000 succinate (B) in the ratios of 1: 6: 3 (SD1, PM1, SD2 and PM2) and 0.5: 7.5: 2 (SD3, PM3, SD4 and PM4).

DSC curve of the UA revealed two thermal events, a small exothermic peak at temperature Tonset = 199.74 ° C (ΔH =16.30 $J.g^{-1}$), corresponding to the phase transformation and a sharp melting point at temperature Tonset = 282.95 ° C (ΔH = 72.89 $J.g^{-1}$) in the Figure 6. These data corroborate with the results of XRD showing that the UA is in polymorphic form I (Zhou *et al*., 2015). In respect to the carriers, the DSC thermograms for

P407 and TPG revealed a single endothermic melting peak at T onset of 56.03 °C (ΔH = 124.95 $J.g^{-1}$), and 32.37 °C (ΔH = 96.39 $J.g^{-1}$), respectively, corresponding to the melting of ordered polyethylene glycol (PEG) chains present in both excipients. The position of the P407 and TPGS peaks is associated to the degree of folding of the PEG chains, therefore, the extended form shows a highest temperature of melting and a decrease in melting temperature upon folding (Goddeeris, Van den Mooter, 2008). DSC thermogram of PVP K30 showed a glass transition temperature at 166.05 °C and the absence of any melting peak, confirming to its amorphous nature. Besides, a broad endothermic range from 40.11 to 138.87 °C (ΔH = 6.00 $J.g^{-1}$) was observed in thermal profile of the PVP K30 which was attributed to the dehydration of the hygroscopic polymer upon heating (Chan *et al*., 2015).

The folded PEG chains present in P407 perseveres through all investigated formulations, although the melting enthalpy (Table III) decreases as the amount of copolymer present in every formulation is lowered. Therefore, PM1 and SD1 showed a ΔH (34.15 and 25.13 $J.g^{-1}$) greater than PM3 and SD3 (19.59 and 14.25 $J.g^{-1}$). Comparing PM1 and PM3 with SD1 and SD3 it was possible to verify a reduction of ΔH in the SDs. The same thermal effects were observed for the formulations constituted by TPGS (PM2, PM4, SD2 and SD4), suggesting that the SD process contributed to a reduction of the crystallinity of the P407 and TPGS.

The melting peak of UA in both physical mixtures and solid dispersions were absent and only the thermal events corresponding to the carriers used in the formulations were observed. The similarity of physical mixtures and solid dispersions in the DSC thermograms may indicate that the drug was converted from crystalline to amorphous form or that the drug was dissolved in the melted carrier before reaching its fusion temperature. However, this hypothesis has been previously clarified by XRD analysis, which demonstrated the presence of UA diffraction peaks in the in PM1 and PM3. Thus, the disappearance of the drug melting endotherm in these formulations can be explained by the UA dissolution in the melted carrier before reaching its fusion temperature. In contrast, diffraction patterns das PM2 and PM4 revealed the absence of the crystalline peaks corresponding to the UA, therefore, DSC analysis confirms that the drug was converted from crystalline to amorphous form in the PM2 and PM4.

For solid dispersions, thermal and XRD results were compatible and showed that all the solid dispersions developed herein contained the UA molecules arranged in an amorphous state in the solid matrices. In particular, it was possible to observe that SD1 and SD2 despite containing twice the amount of drug they were able to keep the drug in an amorphous state. Furthermore, as discussed earlier, despite SD1 and SD2 showed UA aqueous solubility values statistically lower than SD3 and SD4, these formulations also demonstrated a significant increase in UA solubility that will favor its pharmacological application. Therefore, SD1 and SD2 owing to their ability to carry a greater amount of drug were selected to continue physicochemical studies, because drug loading capacity has an essential role in the therapeutic efficacy.

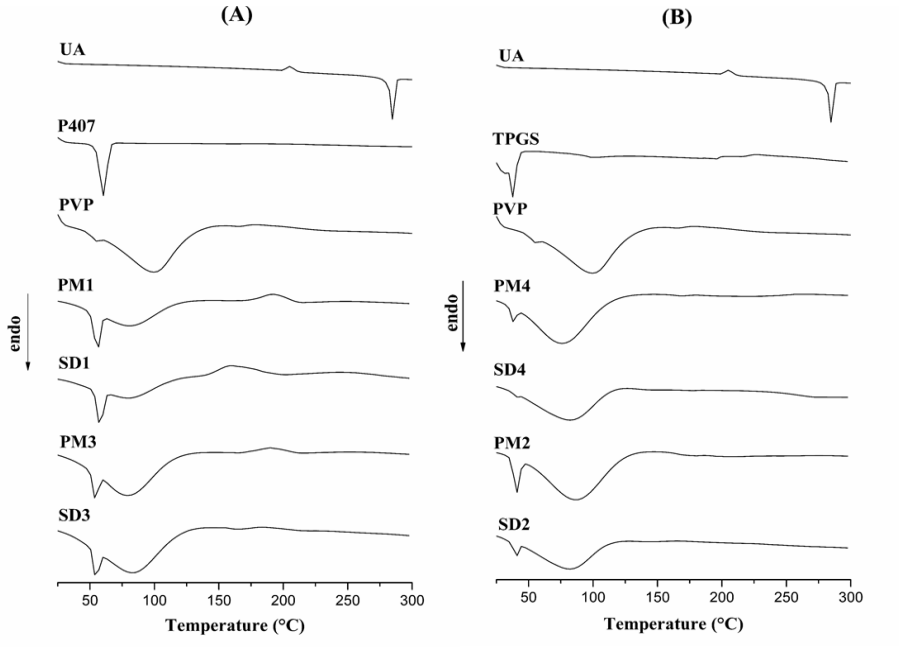

FIGURE 6 - DSC curves of SDs and PMs containing ursolic acid: polyvinylpyrrolidone k-30: poloxamer 407 (A) and ursolic acid: polyvinylpyrrolidone k-30: D-α-tocopherol polyethylene glycol 1000 succinate (B) in the ratios of 1: 6: 3 (SD1, PM1, SD2 and PM2) and 0.5: 7.5: 2 (SD3, PM3, SD4 and PM4).

TABLE III - Melting enthalpy (ΔH), onset melting temperature, offset melting temperature, peak melting temperature of the investigated samples

Samples	T onset (°C)	T peak (°C)	T offset (°C)	ΔH ($J.g^{-1}$)
UA	282.95	284.31	291.87	72.89
PVP	51.11	-	132.67	6.00
P407	56.03	59.36	69.00	124.95
TPGS	32.37	37.88	44.00	96,39
PM1	51.19	55.36	60.00	34.15
PM2	36.04	41.06	49.30	15.62
PM3	50.91	54.42	60.00	19.59
PM4	35.04	38.73	45.71	8.57
SD1	53.29	57.98	62.74	25.13
SD2	37.49	39.93	45.28	3.16
SD3	51.12	55.16	60.00	14.25
SD4	37. 58	39.62	45.00	0.76

Abbreviations: UA, ursolic acid; SD, solid dipersion; PM, physical mixture; PVP, polyvinylpyrrolidone k-30; TPGS, D-α-tocopherol polyethylene glycol 1000 succinate; P407, poloxamer 407.

The particles size distribution for UA, carriers, physical mixtures and solid dispersions are depicted in Figure 7. Although the particle size distribution is unimodal (single peaked) for all samples, the shape of the distribution suggests that several fractions of particles of different sizes were present in the samples. The modal diameter of the UA particles was 92.90 µm (Figure 7, A). The analysis of carriers indicated that PVP k30 and poloxamer 407 exhibited modal diameters of 122 and 195.00 µm (Figure 7, B; C). In the case of the particles of physical mixtures PM1 and PM2 the modal diameters were 164.84 and 121.90 µm (Figure 7, D; E). However, solid dispersions SD1 and SD2 showed smaller diameters that PMs, which were equivalent to 68.66 and 84.09 µm (Figure 7, F; G), respectively. Moreover, both PMs and SDs showed uniform distribution of the UA, with a drug recovery between 100.96 to 101.49% and between 99.30 to 105.00%, which indicates the efficiency of the solvent-evaporation method used in the preparation of the UA solid dispersions (Table IV).

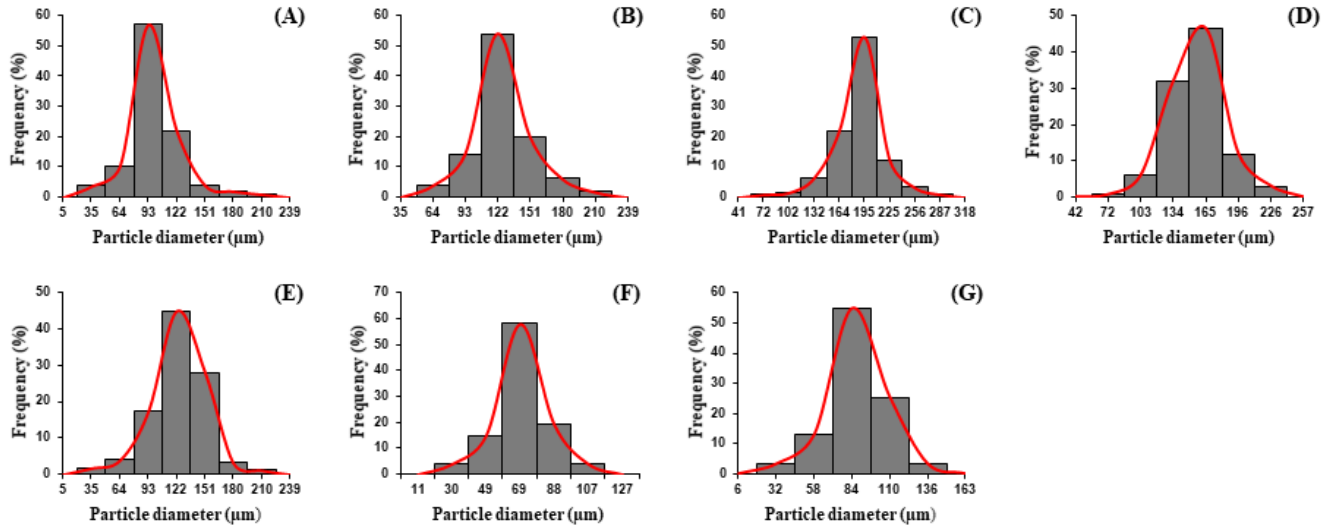

FIGURE 7 - Particle diameter distribution of UA (A), PVP k-30 (B), P407 (C), PM1 (D), PM2 (E), SD1 (F) and SD2 (G).

TABLE IV - Average particles diameter and drug content of solid dispersions and physical mixtures

Sample	Average particle diameter (µm) ± SD	Drug content (%)
UA	77.54 ± 79.26	-
PVP K30	112.86 ± 60.47	-
P407	170.88 ± 75.90	-
PM1	144.98 ± 57.89	101.49 ± 1.03
PM2	96.47 ± 68.81	100.96 ± 0.78
SD1	59.09 ± 32.46	99.30 ± 2.51
SD2	70.28 ± 44.14	105.00 ± 4.70

Abbreviations: UA, ursolic acid; SD, solid dipersion; PM, physical mixture; PVP, polyvinylpyrrolidone k-30; P407, poloxamer 407.

In the scanning electron microscopy analysis (Figure 8), UA appeared as rectangular-shaped crystals, with smooth-surfaced and in different sizes, in agreement with the DSC and XRD data, which confirmed its solid crystalline structure. Regarding carriers, the SEM images revealed some spherical PVP K30 particles with diameters greater than 100 µm and other smaller particles that were broken during the analysis. Poloxamer 407 appeared as smooth surfaced spherical particles with sizes around 200 µm. TPGS appeared as a waxy film in the SEM images.

Both formulations selected with or without UA within their matrices showed to be composed by particles of different sizes with irregular surface. SEM images also demonstrated the absence of UA crystalline particles in the all the SDs evaluated, suggesting that the drug is dispersed within the solid matrix. However, SEM pictures showed that SD1 without drug had a smaller size (<100 µm) than the particles of SD1 with drug, while for the particles of SD2 the size was not changed with the addition of the drug in the systems.

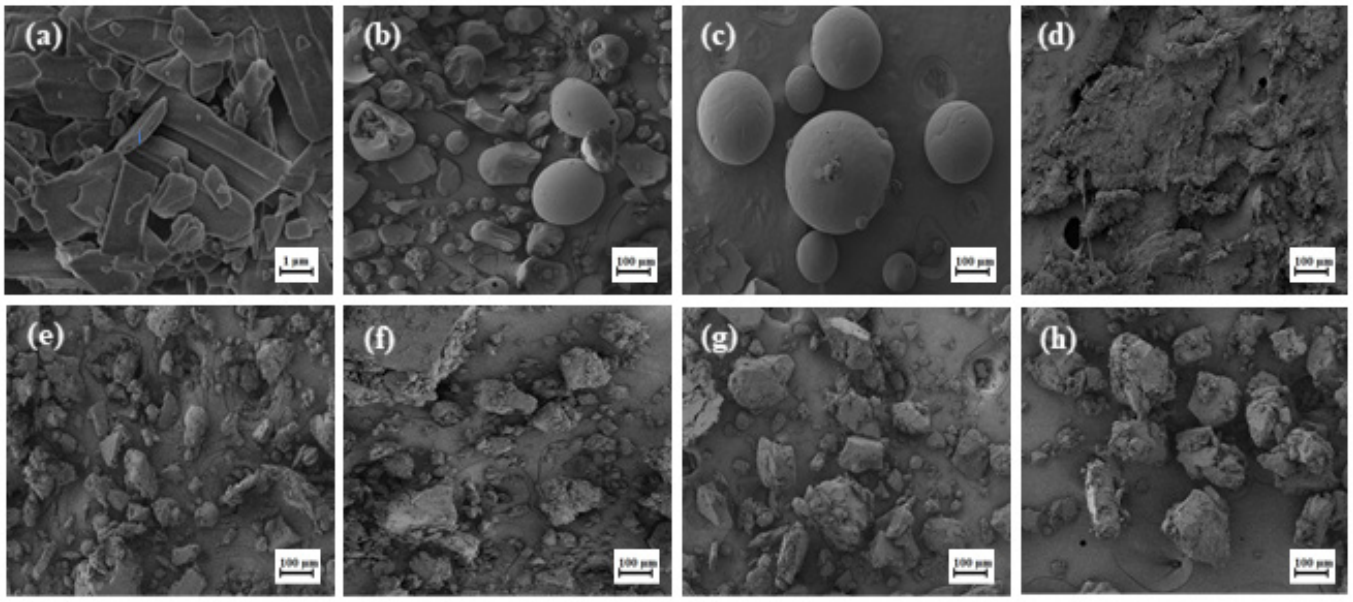

FIGURE 8 - SEM images of UA (a), PVP k-30 (b), P407 (c), TPGS (d), SD1 of PVP k-30 and poloxamer 407 without drug (e), SD1 of UA in PVP k-30 and P407 (f), SD2 of PVP k-30 and D-α-tocopherol polyethylene glycol 1000 succinate without drug (g), SD2 of UA in PVP k-30 and TPGS (h).

Drug stability is one of the critical attributes of quality and must be considered in the pharmaceutical development phase and needs to remain controlled during clinical studies and after marketing. Stability tests allow to evaluate the quality of an active pharmaceutical ingredient under controlled temperature and humidity variation in different time interval (Guo *et al*., 2013). These evidences favor to determine the shelf life and their storage conditions of product. The stability of the SD1 and SD2 was evaluated at a temperature of 30°C under 60% relative humidity during 90 days by x-ray experiments and drug content analysis every 30 days. In addition, after 30 days of experiment, the disappearance of the characteristic peaks of the TPGS at 19 and 23° was observed in the SD2. However, both solid dispersions remained amorphous during the 90 days of stability study, since the diffractograms below (Figure 9) did not reveal any signs of crystallization of UA in the SD1 and SD2. In a study conducted by Chokshi *et al.* 2007 the presence of poloxamer as a carrier in dispersion solid also ensured the stability of the system, because the drug remained dispersed in the amorphous state in the SDs under temperature of 40°C and 75% of RH after 30 days of experiment.

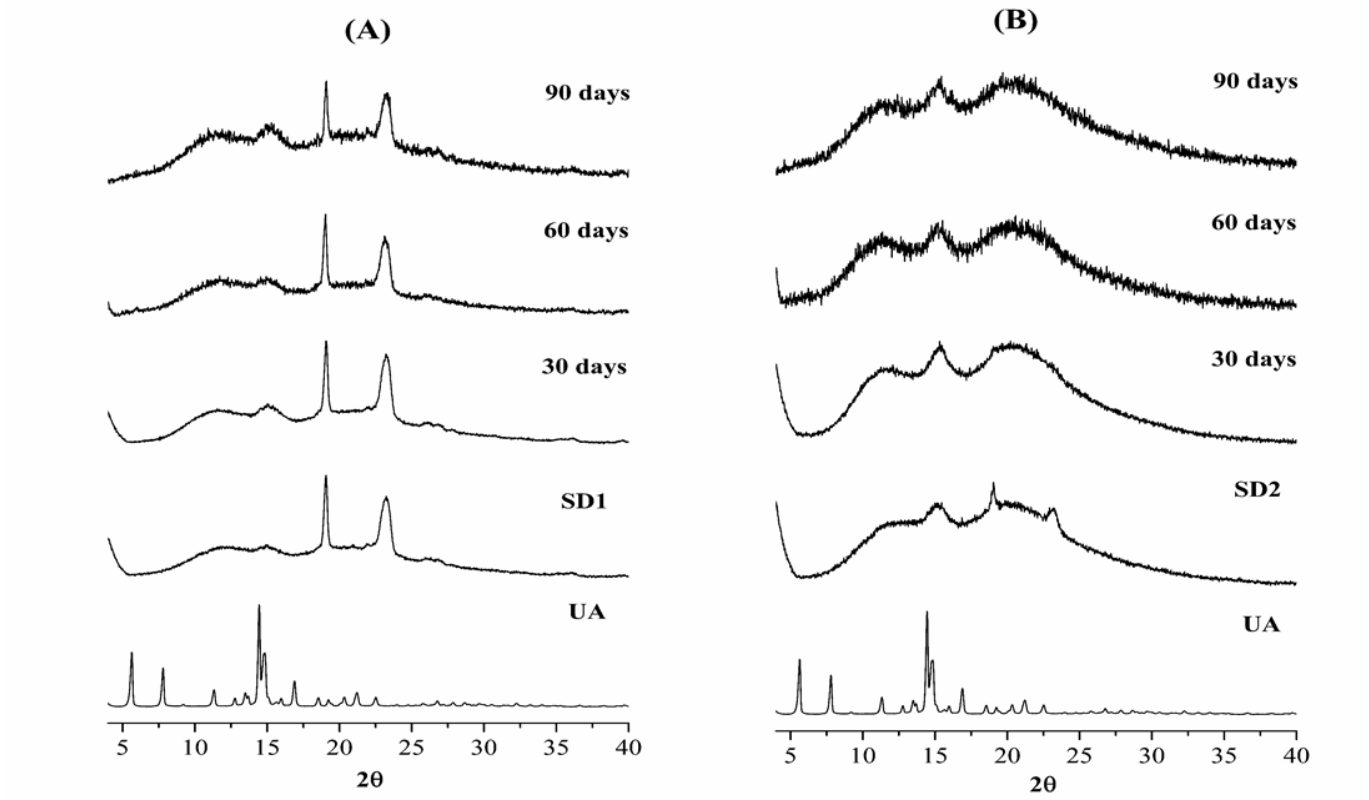

FIGURE 9 - X-ray diffractograms corresponding to the short-term physicochemical stability of the SD1 (A) and SD2 (B) after 0, 30, 60 and 90 days under 60 ± 5% RH and 30± 2°C.

Regarding the evaluation of the drug content during the stability study, it was observed that the UA content remained practically constant in SD1 with a 6.9% reduction in the drug content after 90 days of experiment. However, SD2 showed a more pronounced drug reduction, with a loss of 24.51% and after 90 days. Thus, SD1 was more stable than SD2 in the storage conditions investigated during the stability study, as shown in Table V. Therefore, addition of poloxamer 407 in the formulations provided greater physical and chemical stability of the drug in the SD1, possibly due to the amphiphilic chemical structure of these compounds that allows to enhance the miscibility of drugs in the carriers by the reduction of the interfacial tension, thus preventing the precipitation and drug recrystallization during storage (Vo *et al*., 2013).

TABLE V - Drug content in SD1 and SD2 during stability study under temperature of 30 ± 2°C and RH of 60 ± 5% at 0, 30, 60 and 90 days

Time (days)	Drug content in solid dispersions (%)	
	SD1	SD2
0	103.15 ± 2.51	98.92 ± 1.07
30	100.23 ± 2.37	91.53 ± 0.77
60	100.53 ± 1.04	78.55 ± 2.38
90	96.25 ± 0.61	74.41 ± 3.15

Mean ± SD (n=3)

Before conducting *in vitro* release studies of UA from solid dispersions (SD1 and SD2), drug solubility was determined in media receptor to ensure that release experiments were performed under sink conditions (drug total percentage ≤ 10% solubility value in the receptor medium). If the total amount of drug in the SDs had been released during the test, as an amount of 300 µl of each

formulation was added to the donor medium, the maximum drug concentration reached would be 0.128 mg/ml in the receptor medium (volume 7 ml). Therefore, sink conditions were maintained during the in vitro release study, as UA solubility in medium receptor was 2.28 mg/mL.

Comparing the release of free drug and solid dispersions (Figure 10), the SD1 and SD2 showed highest release rate, since no amount of free UA was released during 24 h, possibly due to the hydrophobic properties of this molecule, while after 120 minutes, SD1 (UA:PVP:P407) and SD2 (UA:PVP:TPGS) showed 10% of UA released. The enhancement in drug release rate was attributed to amorphous nature of the drug molecules in the SD and amphiphilic properties of the vehicles that are able to reduce the interfacial tension between UA and the receptor solution.

Although, the cumulative amounts of drug released from SD1 and SD2 are not statistically different ($p < 0.05$). The flux ($\mu g/cm^2/h$) of UA from the SD1 through synthetic membrane, as well as the its diffusion coefficient (Kd), were about two times higher than the drug flux and Kd obtained for SD2 (Table VI). This can be explained by the greater affinity of the UA (highly lipophilic) by the SD2, which have more lipophilic properties due to the presence of TPGS. In contrast, as SD1 has more hydrophilic properties because it consists of PVP k30 and P407, it is able to provide a better diffusion of the drug from the formulation and consequently a greater flux of the drug through the membrane. In addition, the low cumulative amount of UA released from SD1 and SD2 after 24 h of testing may be due to the presence of a membrane between the donor and receptor compartments, as well as low wettability and solubility of UA. The PES membrane was employed to simulate the skin because it is hydrophobic and possesses rate-limiting properties like skin.

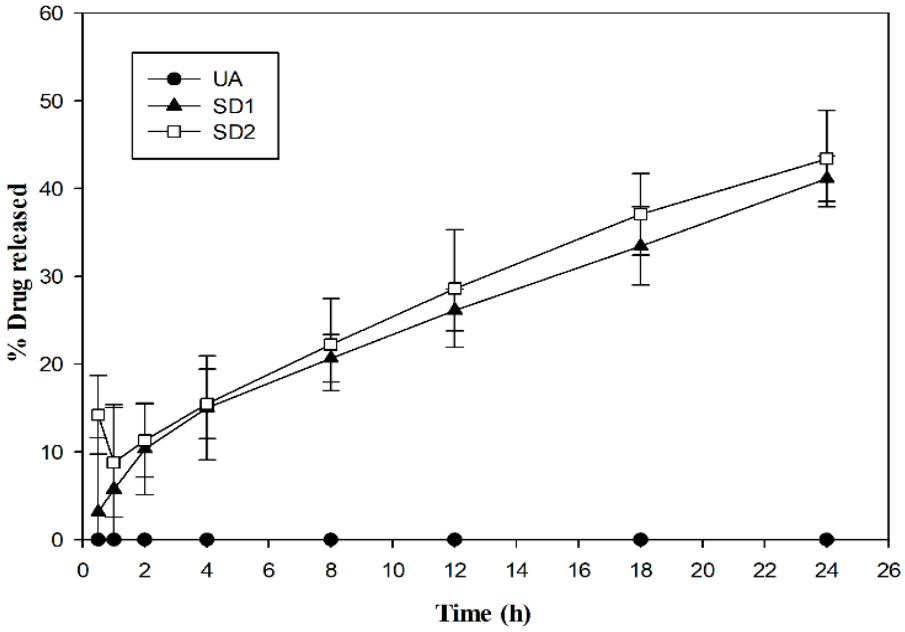

FIGURE 10 - In vitro release of ursolic acid from solid dispersions in sodium phosphate buffer containing 1.25% w/v of sodium lauryl sulfate (pH 7.4) at 32.5°C. SD1 composed by UA:PVP:P407 and SD2 of UA:PVP:TPGS, both in the ratios of 1: 6: 3 (w/w/w).

TABLE VI - Diffusion parameters of drug calculated by linear regression of data obtained for SD1 and SD2

Samples	J (µg/cm ²/h)	Kd (cm²/s)
SD1	32.72 ± 6.73	$3.03 \times 10^{-6} \pm 6.22 \times 10^{-7}$
SD2	14.66 ± 2.70	$1.38 \times 10^{-6} \pm 8.23 \times 10^{-8}$

Abbreviations: J, flux through membrane and Kd, diffusion coefficient**.**

The correlation coefficient (R^2) values in the release study data of UA solid dispersions in accordance with first order, Weibull model, Korsmeyer -peppas model, Baker & Lonsdale model; Hixon & Crowell model and Higuchi model are given in Table VII. According to the values of the correlation coefficient (R^2) the best fit model for solid dispersions was the of Weilbull. This kinetic model can be expressed by the equation:

$$m_{(t)}/m_{\infty} = 1 - e^{-a.t^b}$$ (Colombo *et al*., 1995)

Where $m_{(t)}$ is the amount of drug released in time t, m_{∞} is the maximum release amount of drug, α corresponds to the value of time scale of the release process and b represents the shape of the release curve (Costa, Sousa Lobo, 2001). According to Papadopoulou *et al* (Papadopoulou *et al*., 2006), b is an indicative parameter of transport mechanism of the drug through the carrier matrix, where $b \leq 0.75$ corresponds to simple molecular diffusion (Fick's law), while a combined mechanism (diffusion and swelling-controlled) is related with b values in the range $0.75 < b < 1$. For values of $b > 1$, drug transport follows a complex release mechanism. Thus, b values for SD1 and SD2 were 0,7689 to 0,9324 showing that the drug release occurs by a combination of diffusion and swelling-controlled transport.

TABLE VII - Release kinetic and mechanism of UA release from SD1 and SD2

Samples	First order	Weibull		Korsmeyer peppas		Baker Lonsdale	Hixon Crowell	Higuchi
	r^2	r^2	b	r^2	N	r^2	r^2	r^2
SD1	0.9198	0.9954	0.7689	0.9963	0.5928	0.9717	0.9026	0.9826
SD2	0.7357	0.9867	0.9324	0.9312	0.4822	0.9256	0.7073	0.9305

CONCLUSION

In conclusion, UA solubility was increased in presence of hydrophilic carriers like PVP k-30 in combination with P407 or TPGS in solid dispersions. Particularly, solid dispersions with P407 revealed a greater increase in UA water solubility when compared to other formulations, the same was observed for SD with a lower proportion of drug and a higher proportion of carrier. The increase in UA solubility from SDs can be mainly due to the conversion of drug to amorphous state. SD1 and SD2 were selected because of their ability to carry a greater amount of drug in their matrices. From the results of the stability study, it was found that SD1 proved to be more stable than SD2, indicating that the use of P407 contributed to ensure better stability of UA. Furthermore, SD1 and SD2 equally increased UA release, following Weibull mathematical model, indicating that drug release mechanism occurs by a combined of diffusion and swelling-controlled transport. Based on solubility studies, physicochemical analyzes, stability assay and in vitro drug release studies, SD1 with P407 were the best choice and likely favors the therapeutic application of UA.

ACKNOWLEDGEMENTS

This work was supported by FAPESP, process number: 2016/22544-0 (São Paulo, Brazil), CNPq (Brasília, Brazil), CAPES (Brasília, Brazil) and PACD-FCFAr-UNESP (Araraquara, Brazil). We thankthe Thermal Analysis Laboratory of the Analytical Chemistry Department of the Chemistry Institute - UNESP, Araraquara - SP and also to the Polycrystalline Crystallography and X-Ray Diffraction Laboratory of the Physical Chemistry Department - UNESP, Araraquara - SP.

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Received for publication on 24th March 2021
Accepted for publication on 07th December 2021