

SEPARATION OF IBUPROFEN ENANTIOMERS BY DIASTEREOMIC SALT FORMATION AND ANTISOLVENTE PRECIPITATION IN SUPERCRITICAL CARBON DIOXIDE

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The aim of the investigation is to validate the potential of SAS crystallization process to resolve racemic ibuprofen. The resolution is performed via diastereomic salt formation and subsequent salt precipitation in a supercritical CO₂ environment. Firstly, the operating conditions, using (S)- α -methylbenzylamine as resolving agent, were optimized. Secondly, the measurement of the binary phase diagram of (S,R)-Ibuprofen and L-Arginine was initiated as a previous step in the assessment of L-Arginine as resolving agent of ibuprofen.

INTRODUCTION

Ibuprofen ((R,S)-2-(isobutylphenyl)- propanoic acid) is a Non-Steroidal Anti-Inflammatory Drug (NSAID) widely used for the treatment of a wide range of complaints, including pain, inflammation, arthritis, fever and dysmenorrhea. Like other 2-arylpropionate derivatives (ketoprofen, flurbiprofen, naproxen, etc), ibuprofen contains a chiral carbon in the α -position of the propionate moiety, and therefore two enantiomeric forms of ibuprofen exist. Indeed, it is well recognised that the anti-inflammatory activity resides almost exclusively in the (S)-(+)-ibuprofen (dexibuprofen). Interestingly, when the drug is orally administered as a racemate, the inactive R (-) enantiomer undergoes a unidirectional metabolic inversion to active dexibuprofen, resulting in accumulation of ibuprofen residues in fatty tissue. The long-term effects of the accumulation of ibuprofen residues in fatty tissue are unknown, but the risk of toxic effects can be avoided by administering the pure (S)-enantiomer [1]. A large investment has been made in order to research and develop specific techniques for enantiomerically purify ibuprofen [2].

Another concern related with the conventional oral administration of ibuprofen as free acid, is the relatively slow absorption observed, due to its low solubility in aqueous media (< 20 ppm) [3]. This limitation can be easily overcome by the use of salts derived from the ibuprofen free acid form, since these forms can be freely soluble in water. Nowadays, there are four salt forms available in the market (ibuprofen sodium, lysine, argine and hydrobromide); however none of them represents more than the 5% of all the total formulations that have ibuprofen as active ingredient. This is approximately the same proportion of formulations containing acid dexibuprofen [4].

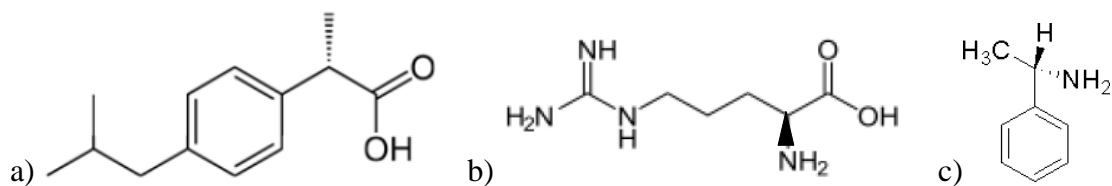


Figure 1. Structure of *S*-Ibuprofen (a), *L*-Arginine (b) and *S*-(α)methylbenzyl-amine (c)

Among the different techniques for separation of enantiomers, diastereoisomeric crystallization with different basis (*R*-(+)-phenylethylamine, *N*-methyl-*D*-glucamine.....) has been the dominant one in the pharmaceutical industry for the resolution of ibuprofen [5,6]. Moreover, the formation of an optically active salt of a naturally aminoacid (*S*-lysine [6], *S*-arginine (Figure 1.b), *S*-histidine) is also desirable since, as it was previously stated, salt is more soluble in water than the corresponding free acid of ibuprofen, and the hydrolysis of the diastomeric salt into free dexibuprofen will be avoided. Pharmacological studies have proven that salt of ibuprofen-arginine salt is better adsorbed in the blood stream than that of ibuprofen-lysine [7]. Thus, an effective, inexpensive, and pharmacologically valuable product can be produced.

Numerous patents can be found involving an IBU-ARG compound in medical applications such as oral liquid [8], tablets [9] or effervescent preparations [10], cream or gel [11], and injection [12]. However, only very few of these are relevant to the synthesis of the Ibuprofen-arginine compound [13, 14, 15], and, up to the actual knowledge of the authors, none of them is related to the formation of the diastereomeric salt with the dual purpose of resolution and increasing bioavailability. Nevertheless, arginine is capable of performing chiral discrimination of ibuprofen, as evidenced by successful trials in resolving ibuprofen by using arginine-impregnated thin layer chromatography [16].

As in any crystallization-based separation process, the operating condition for the resolution can be optimized (e.g. concentration, temperature) in terms of yield and resolution efficiency by construction of the ternary (solubility) phase diagram in the corresponding organic solvent. However, this requires a large amount of experimental data [17]. Consequently, binary (melting) phase diagrams are used as good approximation, especially for selection and qualification of a resolving agent, as the existence of a eutectic mixture, a primary condition for a resolution, can be determined [18, 19, 20]. These diagrams can be experimentally determined by differential scanning calorimetry (DSC) [19, 20] or predicted by the Schröder-van Laar equation (1):

$$\ln x = \frac{\Delta H_a}{R} \left(\frac{1}{T_a} - \frac{1}{T} \right) \quad (1)$$

where x is the mole fraction of the enantiomer in excess; ΔH_a and T_a are the melting enthalpy (J/ mol) and melting temperature (K) of the pure enantiomer; T is the temperature of the mixture; and R is the universal gas constant (J / (mol ·K)). This equation is a simplified expression since it does not account for the heat capacity term found to be negligible in the case of enantiomers [20].

Being $SC\text{-}CO_2$ an excellent media for separation and precipitation techniques, avoiding or reducing the use of organic solvents and giving the possibility to work at mild temperature. $ScCO_2$ chromatography has been successfully applied to the resolution of ibuprofen [21, 22]. It

has resolved via partial diastereomeric salt formation with different ethylamines and subsequent supercritical fluid extraction of the unreacted enantiomer [23,24].

In this general context, the aim of the investigation is to study the potential application of Supercritical AntiSolvent (SAS) crystallization process to resolve racemic ibuprofen via diastereomeric salt formation of ibuprofen-arginine.

Since the melting phase diagram of the L-Arginine with ibuprofen (S and R) is not reported in the bibliography, it would be necessary to determine it experimentally. Even so, the viability of the process for the resolution of racemic ibuprofen would be previously tested using (S)- α -methylbenzylamine ((S)-MBA, Figure 1.c)) as resolving agent, because it was reported in literature for resolution of ibuprofen [25, 26] and data of its binary mixture with IBU are available [19]. Besides, its counter enantiomer was successfully used to resolve mandelic acid by diastereomeric salt formation and crystallization by SAS with good resolution efficiency, the 92% of the R-mandelic acid was recovered [27]. In a previous work [28], the ibuprofen was resolved with (S)-MBA by a modified SAS process operated in batch mode. SC-CO₂ was saturated with racemic ibuprofen before being injected in the crystallizer, where a solution of (S)-MBA in ethylacetate was previously loaded. The precipitation yield was quite low, due to the small amount of ibuprofen that is soluble in SC-CO₂, and the enrichment in (S)-Ibuprofen of almost 20% in one step, from 50% to 69%.

MATERIALS AND METHODS

Materials

Racemic ibuprofen, (S)-Ibuprofen, L-Arginine and (S)-Methylbenzylamine were purchased by Sigma -Aldrich with purity higher than 98%. Carbon dioxide, 99.95% pure, was supplied by Carbueros Metalicos S.A.. Organic solvents ethyl acetate, ethanol and dimethylsulfoxide (DMSO) were given by Panreac Quimica with 99% purity. Ethanol with 96% purity was purchased at Panreac Quimica, as well.

Equipment and procedure

The SAS equipment is schematized in Figure 1. Carbon dioxide is supplied from a gas cylinder (1), cooled in a cryostat (2), delivered by a membrane pump (3, Dosapro MD 140 S(G)6) into a thermostated bath (4) where it is heated to the desired temperature before passing into the crystallizer (5). Carbon dioxide mass flow is monitored by a Coriolis flow meter (FI). Two solutions can be pumped simultaneously (6a, 6b) at the desired flow-rate by two piston chromatographic pumps (7a, 7b, Jasco PU-2080 Plus) through a 1/16" pipe and are mixed with CO₂ in a tee of 1/4". The solutions get into the crystallizer through a 1/16" tube. The crystallizer is an isolated and jacketed stainless steel AISI 316 vessel of 1.5 l internal volume, equipped with temperature and pressure indicators (TI, PI). To avoid product losses, there is a 1 μ m metallic frit at the bottom of the crystallizer vessel and a basket filter (8) in CO₂ outlet to trap solid particles. Pressure is regulated downstream with a needle valve (9, Autoclave Engineers) electrically heated to prevent freezing or plugging. After pressure release, there is a flash vessel (10) to achieve the separation of solvent and CO₂.

The general experimental procedure to resolve ibuprofen by diastereomeric salt formation with MBA, consisted on, first, pumping compressed and preheated CO₂ in the precipitator from the top through the diffuser. The pressure and flow rate were controlled with the outlet valve and pump stroke size. Once the steady state at the desired conditions of pressure, temperature, and CO₂ flow rate (0.6 kg/h) was achieved, the solution of MBA was pressurized and pumped (1 mL/min) into the vessel. Immediately afterwards, the solution of racemic IBU was pumped, as well. After the solutions were completely pumped (1 mL/min) into the vessel, the inlet valve and solution pump were shut off and CO₂ mass flow was increased (3 kg/h) while maintaining crystallizer pressure to ensure that all the remaining traces of solvent which might be present in the vessel were removed. The minimum amount of CO₂ flushed was 1.5 kg. Finally, the system is depressurized and the particles are collected both from the filter and the walls of the precipitator, and weighted in order to determine the yield of the precipitation.

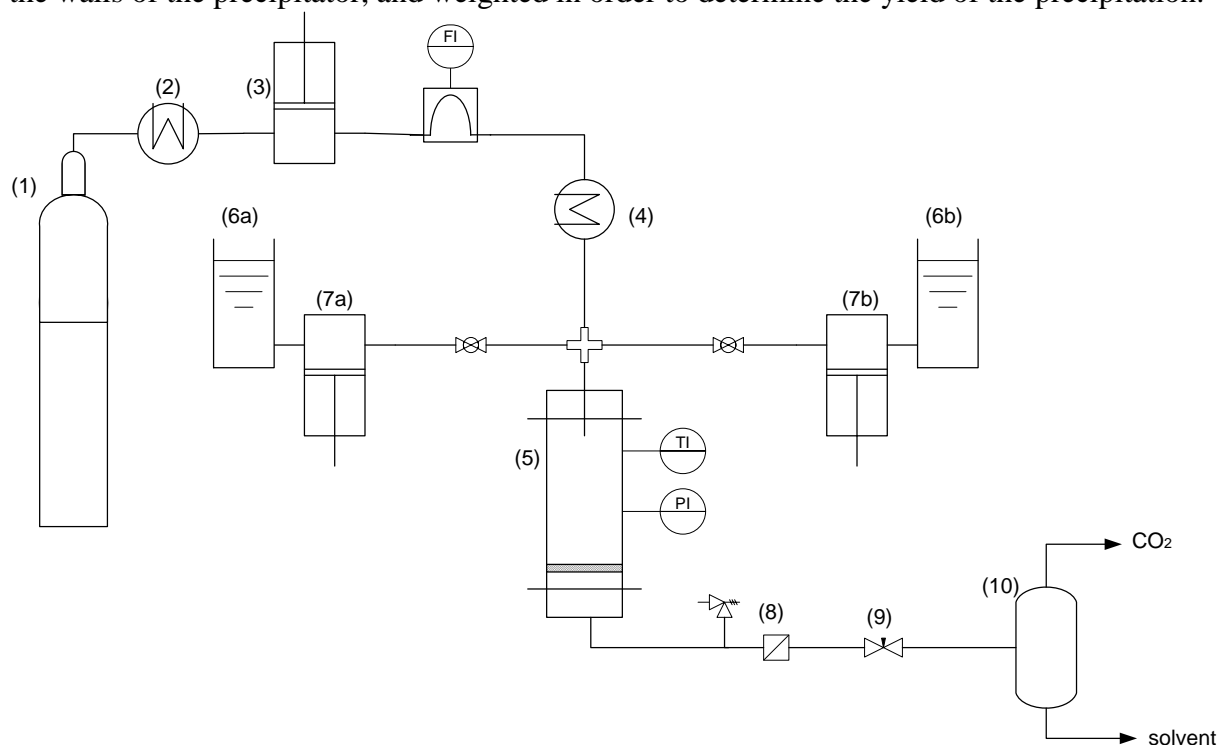


Figure 1. Schematic diagram of the SAS equipment

The ratio between flow rates of CO₂ and solution was established according to the experience of the research group on SAS process [29, 30]. The reaction to form the diastereomeric salt was performed inside the precipitator vessel because better results in terms of yield and enantiomeric excess were reported in the resolution of mandelic acid by SAS process [27]. Similarly, the molar ratio of resolving agent to racemic compound was set at 0.5 as it is widely stated in literature to result in higher resolution efficiency. The selected solvent was ethanol, since it was the less harmful from health point of view of those reported in literature for the resolution of ibuprofen with (S)-MBA[18, 26]. Nevertheless, experiments were programmed to verify whether these parameters were the most adequate.

Analysis

Differential Scanning Calorimetry (DSC) and Capillary Electrophoresis (CE) were employed to determine the efficiency of the enantiomeric separation. These techniques are characterized for being faster and cheaper than other common methods used to quantify each enantiomer individually, such as chiral column chromatography and NMR spectroscopy.

Since the existence of a eutectic mixture is a primary condition for a resolution by diastereomeric salt formation, the melting point DSC does not permit the quantification of the (S,S) salt in the whole range of molar fraction. Nevertheless, DSC analysis allows to identify/quantified compounds different to the diastereomeric salt of interest, (S,S)-salt, such as non-reacted S-ibuprofen, which would be identified by a pick between the melting temperature of the racemic mixture ($T_m = 78^\circ\text{C}$), and that of each individual enantiomer, R or S ($T_m = 52^\circ\text{C}$), or other reaction products. In this sense, this method is more powerful compare against polarimetry, used in the first work [28], because it permits to distinguish between the different compounds in the precipitate which would be not possible global optical rotation measurements. In the case of IBU-MBA salts, according to binary phase diagram of the system experimentally determined by Ebbers and co-workers [19], the composition of the product in terms of (S,S)-salt can be quantified for mixtures with molar ratio higher than 0.5, that means, with melting points above 158°C . Samples of the powder collected from the precipitator were analyzed by DSC (DSC822e FRS5 Mettler Toledo, dt = 1.00, 0.0 – 250.0°C at $10.00^\circ\text{C}/\text{min}$, N_2 60.00 mL/min) without any further pre-treatment.

Electrophoresis analyses were carried out in a Beckman P/ACE MDQ capillary electrophoresis system coupled with a diode array detector (DAD) (Palo Alto, CA, USA). A $50\ \mu\text{m}$, 50 cm (40 cm to detector) fused silica capillary was used and maintained at 25°C . The running buffer consisted of 1% sulfated- β -cyclodextrin in 80 mM phosphate buffer with diethanolamine to achieve pH=2.6. The capillary was rinsed before each run with NaOH 1M, water and running buffer, each for 1 min at 20 psi. Separation occurred at 15kV with reverse polarity for 16 min. All samples were diluted in water at approximately 300 mg/l and injection was by pressure at 0.2 psi for 4 seconds. Detection of ibuprofen enantiomers occurred at 214 nm.

The results of the separation are presented in terms of the enantiomeric excess and the yield of the precipitation. The enantiomeric excess is defined as

$$\text{e.e}(\%) = \left| \frac{R - S}{R + S} \right| \quad (2)$$

The enantiomeric excess is determined from the DSC analysis of the particles for the IBU-MBA salt.

The yield of the precipitation is defined as:

$$\eta(\%) = \frac{\text{S-ibuprofen in particles}}{\text{S-ibuprofen in feed}} \cdot 100 \quad (3)$$

The yield is determined from the weight of the particles collected and the composition analysis of the particles. The amount of S-ibuprofen none reacted or forming compounds other than IBU-MBA is not considered when analysis DSC data. Nevertheless, the global S-Ibuprofen present in the samples is analysed by electrophoresis. This value, when used, is adequately indicated.

RESULTS AND DISCUSSION

Methylbenzylamine as resolution agent

Following the general procedure established previously several series of experiments were carried out in order to determine the more adequate ones.

In the first series, the operating pressure was fixed at 10 MPa and the temperature was varied between 35 and 60°C. The concentration of racemic IBU in ethanol was 80 mg/mL and that of MBA was 11.3 mg/mL, to keep the molar ratio in 0.5 using the same volume of solvent.

The experimental results are shown in Table 1. As an example, the DSC curve for sample c is shown in Figure 3. It shown two peaks; the first and sharp one is that of a salt of IBU-MBA of certain concentration of (S)IBU-MBA according to the binary phase diagram [19]. The second one is an impurity or by-product of the reaction. From DSC analysis, this impurity accounts for the 20% in average and it is present in all the samples. A DSC analysis of racemic ibuprofen ($T_m = 78,7^\circ\text{C}$, in good agreement with those reported in literature [31]) and S-ibuprofen showed the presence of a contaminant in temperature between 154 and 243°C, which is very likely to be the origin of the impurity.

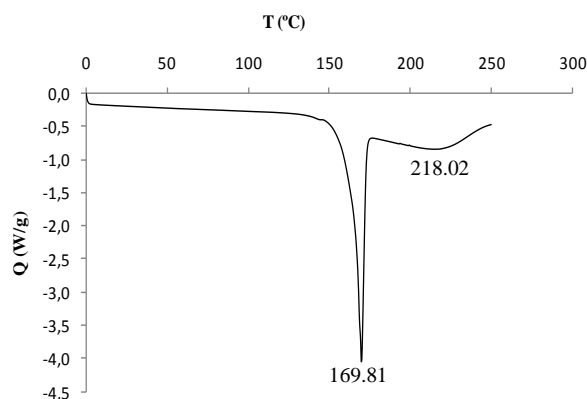


Figure 3. DSC Analysis of Sample c

The temperature of 50°C was selected for the following experiments to optimize the operating pressure in the range of 8 to 12 MPa, as at that temperature the global yield of the process was maximized.

Sample	T (°C)	% e.e. ⁽ⁱ⁾	% η
a	35	57	11
b	40	57	9
c	50	44	24
d	60	42	18

Table 1. Influence of temperature at 10 MPa

(i)The %e.e. is calculated from X(R,S) extrapolated from binary phase diagram [19]at the experimental T_m (IBU-MBA). The mean incertitude is ca. 2%, which has an impact on % η of the same magnitude.

Sample	P (MPa)	% e.e. ⁽ⁱ⁾	% η
e	8	11	< 1
c	10	44	24
f	12	40	12

Table 2. Influence of pressure at 50°C

Although low temperatures improved resolution efficiency, the precipitation yield is considerably decreased. Consequently a temperature of 50°C outcomes good resolution yields. In a similar way, working pressures above 10 MPa results in a smaller precipitation yield as shown in Table 2. The same trend was observed at 40°C (experimental data not included).

Other studied variables were the concentration of the ibuprofen solution (80 – 160 mg/ mL), resolving agent to ibuprofen molar ratio (M.R.=0.25 -1). In the range of operating conditions explored, the better results (e.e = 44% and $\eta = 24\%$) are attained when performing the diastereomic reaction inside the crystallizer at a temperature of 50°C and a pressure equal to 10 MPa. The ibuprofen concentration in solution was 80 mg/mL and the concentration of the resolution agent was 0.5 of the stochiometric amount. The influence of the solvent was tested, as well. It proved that the used of absolute ethanol do not offer any advantage over ethanol of 96% purity; on the contrary, slightly lower yield was achieved.

Although the MBA-IBU salt has to be hydrolyzed in IBU as a previous step to prepare any pharmaceutical formulation, the quality of the product was analyzed in terms of particle morphology and stability. SEM photographs of the particles of MBA-IBU are shown in Figure 2. They were all needle like. The length and width varied slightly with operating conditions, from 0.6 to 1mm and from 1 to 20 μm , respectively.

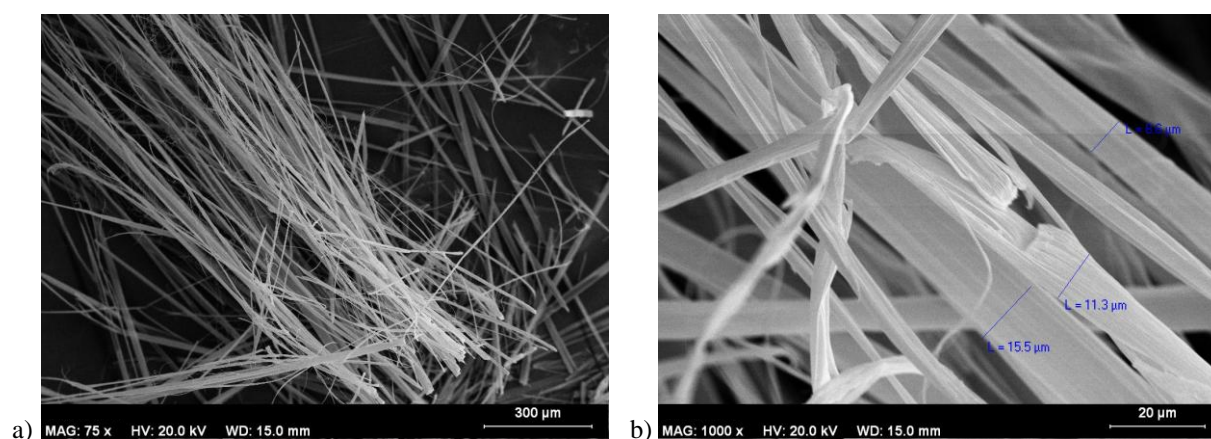


Figure 2. SEM images of particles from experiment f. a) General view. b) detail

The stability of MBA-IBU salt at ambient conditions was analyzed by comparison of the DSC data of the fresh product and that obtained after a-six-month storage. As it is shown on Figure 5, there is a degradation of the IBU-MBA (the peak widens) and, as a result, a peak corresponding to a mixture of (S)-IBU and (R)-IBU, at concentration different to the racemic ($T_m = 78^\circ\text{C}$), appears.

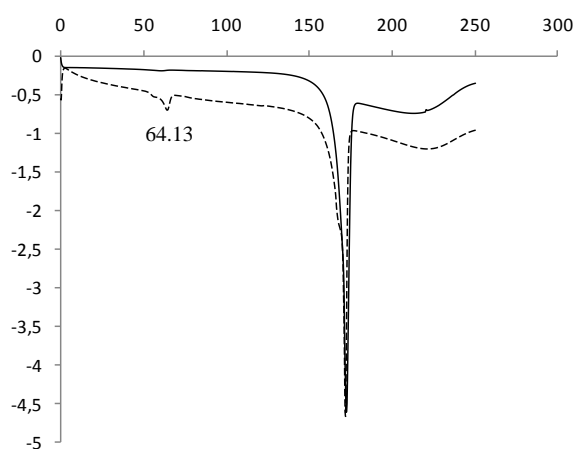


Figure 5. Stability of IBU-MBA salt after storage at room temperature. — $t=0$, ---- $t=6$ months.

Arginine as resolution agent

As it was mentioned previously, the binary diagram for the IBU-ARG salt is not reported in literature, so its determination by DSC was the first step of this part of the work. Firstly, the pure components were analysed. The analysis of raw Ibuprofen, racemic and pure S-enantiomer, was already studied in Figure 4. Regarding the raw arginine, On Figure 6, four peaks can be clearly identified: evaporation of water as a wide peak below 100 °C, L-arginine itself around 240 °C and two contaminants around 100 °C and 220 °C. In order to investigate the nature of these impurities a sample of L-ARG was recrystallized from distilled water and the other was placed in an oven at $T = 70^{\circ}\text{C}$ for 1 day to remove humidity.

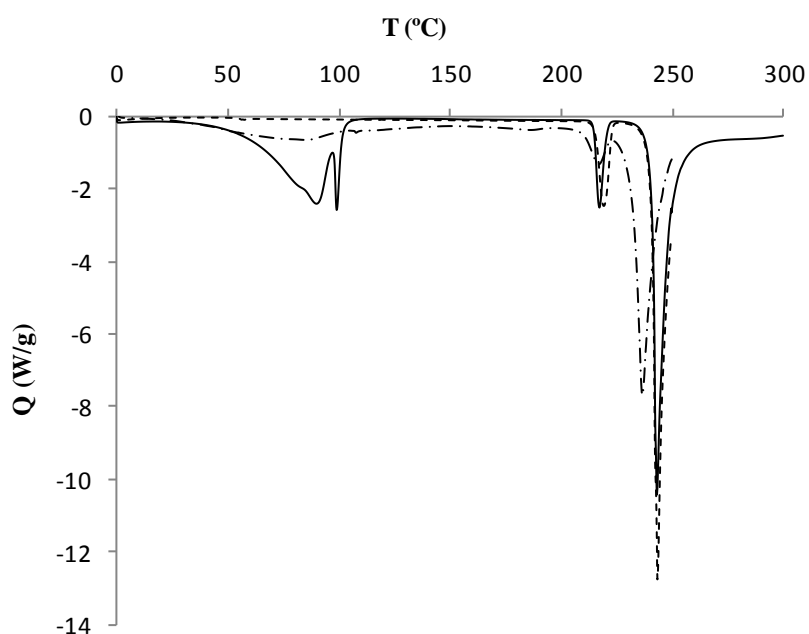


Figure 6. DSC analysis of L-Arginine: — raw material, ---- Heat treatment, - · - recrystallized from water

From these experiments it can be concluded that the contaminant at 100°C appears to be unrelated to humidity but it is eliminated by heat treatment. The contaminant at 218 °C it is likely water-soluble because it exhibits the same peak widening and decrease in peak temperature as arginine after recrystallization.

To ensure good reference samples for DSC analysis, the patented methods of producing the IBU-ARG compound were followed to obtain the salt. Results are sum-up in Table 3. In all the experiments the molar ratio of resolving agent (ARG) to IBU was 0.5.

The process proposed by Liu [13] consisted on dissolving the ibuprofen in 95% ethanol, adding arginine while stirring continuously and maintaining the solution at 40-80 °C for 15-60 minutes. Afterwards, the mixture is cooled to ambient temperature and crystallized. However, a viscous gel was obtained.

The second patent [14] involved dissolving Ibuprofen in absolute ethanol, adding arginine gradually while stirring, diluting the mixture with additional absolute ethanol and continuously stirring until the solution becomes transparent. Stirring is continued until turbidity appears, then for a further 4 hours. The product is filtered, washed with cold absolute ethanol and dried in vacuum. The DSC analysis of the particles obtained according to the last procedure, are presented in Figure 7.

Sample	Patent	Solvent	IBU	Result
A	[14]	Ethanol 96%	(S)	Viscous gel
B	[13]	Ethanol 100%	(S)	Successful
C	[13]	Ethanol 100%	Racemic	Successful

Table 3. Synthesis of IBU-ARG salt according to patented procedures.

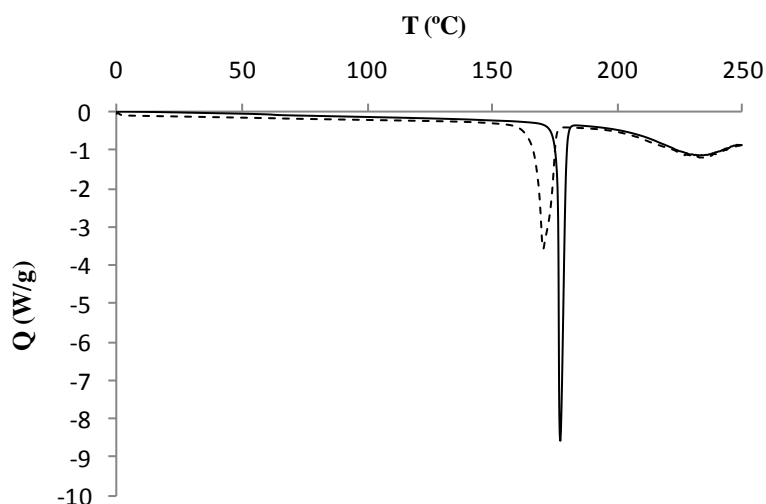


Figure 7. DSC analysis of IBU-ARG compound synthesized according to [13]: — S-IBU, ---- Racemic IBU

Both DSC curves present two peaks: a sharp peak, appearing at 170 and 178 °C, followed by an elongated peak terminating around 250 °C. The first one is bounded to correspond to the IBU-ARG complex. The second peak is unlikely related to the impurity observed in the arginine samples at 218 °C as this was determined to be likely water soluble, and this samples were prepared and filtered from absolute ethanol. It is most likely caused by the impurity observed in the DSC result of Ibuprofen (Figure 4). Moreover, an impurity about 220 °C was also found in the particles obtained when resolving ibuprofen with arginine (Figures 3 and 5). It is noticeable that there is no unreacted ibuprofen, even though, it was in excess (R.M. =0.5). This can be due to the de-localized positive charge of L-ARG, enabling the formation of multiple H-bonds. As it happens with (+)-tartaric acid (although negatively charged) [32], the compound formed by the interaction with a base (e.g. 1,2 cyclohexanediol) was a complex or co-crystal rather than a salt, as it was expected. This complex was stabilized by hydrogen bonds.

Another concern regarding the synthesis and analysis of arginine compound is its tendency to form polymorphs. Raju and co-workers [33], found that arginine salts of a ragaliglitazar, a phenylpropanoic acid as the ibuprofen, gives different polymorphs forms depending on the solvent (ethanol, acetone, isopropanol, methanol, ...) and procedure of the crystallization. They reported up to 11 polymorphs among which only one has suitable solid-state properties for an API (Active Pharmaceutical Ingredient), although all of them proved to be identical in solution. In this sense, precipitation by supercritical antisolvent techniques (SAS) can be also useful, as the control of polymorphism can be achieved with an adequate selection of process conditions (pressure, temperature, concentration, flow ratios and mixer design) as it was shown for ibuprofen sodium [34].

CONCLUSIONS

(S,R)-Ibuprofen has been resolved by SAS crystallization of the diastereomic salt formed with (S)- α -methylbenzylamine. The better resolution efficiency (e.e = 44%) was attained when performing the diastereomic reaction inside the crystallizer at a temperature of 50°C and a pressure equal to 10 MPa. The ibuprofen concentration in solution was 80 mg/mL and the concentration of the resolution agent was 0.5 of the stoichiometric amount. Although the yield of the process was quite low, $\eta = 24\%$, operating conditions can be further optimized decreasing the concentration of ibuprofen in the feed solution and/ or performing the diastereomic reaction outside of the crystallizer.

The experimental determination of the binary phase diagram of L-Arginine is in progress in order to evaluate its potential as resolving agent of ibuprofen.

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