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TERNARY PHASE DIAGRAM OF KETAMINE ((*R*,*S*)-2-(2-CHLOROPHENYL)-2-METHYLAMINOCYCLOHEXANONE) IN ETHANOL AND PRELIMINARY STUDIES AIMING AT ENANTIOSELECTIVE CRYSTALLIZATION OF *S*-KETAMINE

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Abstract - Crystallization is an important industrial-scale process for the purification of enantiomers that depends on a phase diagram. In this work, the ternary phase diagram of R- and S-ketamine in ethanol was determined. The eutectic point indicated that crystallization of pure enantiomers from solutions containing more than 75% of the desired enantiomer is feasible. Solubility studies showed the feasibility of using temperature control to conduct the process. Batch crystallization of ketamine (S/R:80/20) solutions at 25°C provided the isolation of S-ketamine (purity of 100%) with a yield from 65 to 70% and a productivity of 6.5 g/(l h).
Keywords: Ketamine; Enantioseparation; Phase diagrams; Crystallization.

INTRODUCTION

(*R*,*S*)-2-(2-chlorophenyl)-2-ethylaminocyclohexanone (Figure 1), known by its generic name ketamine, is a drug with sedative, analgesic and anesthetic properties, whose molecular structure has a chiral center thus having two enantiomers: *S*- and *R*-ketamine. Since its introduction on the market, several studies have recommended its use for post-operatory anesthesia and analgesia, thereby minimizing the use of opiates, which induce very often hyperalgesia (Luft and Mendes, 2005). The problems associated to this drug when used in the same doses as those used in common anesthesia are the adverse effects, such as undesirable psycho reactions and illusions, caused by the *R*-enantiomer (Nishizawa et al., 2000). A feasible alternative to avoid these problems is the use of an optically pure drug containing only the *S*-enantiomer instead of the commonly used racemic form. Commercialization of pure enantiomers, however, depends on the availability of an industrially efficient process for enantioseparation.

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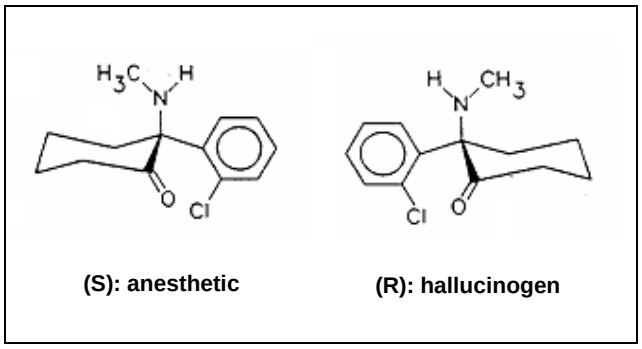


Figure 1: Molecular structure of ketamine with a representation of its stereoisomerism.

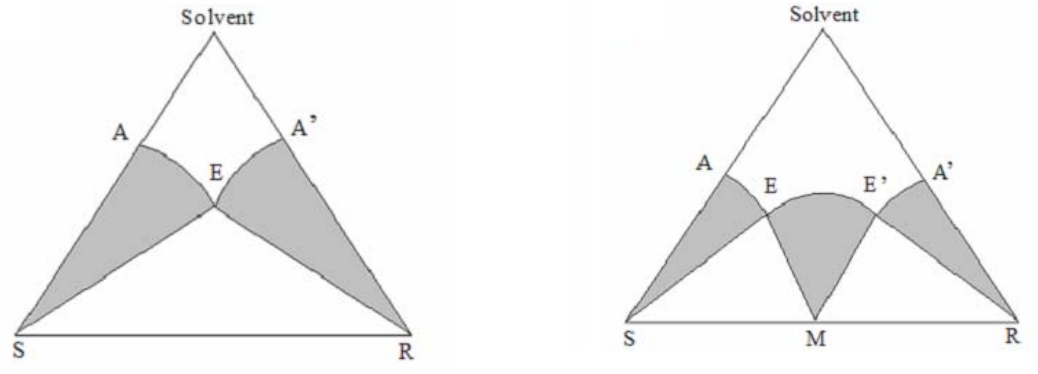
Studies in the literature regarding the enantioseparation of ketamine have been mostly related to chiral chromatography (Silva et al., 2005a; Silva et al., 2005b; Santos et al., 2004) and diastereomeric resolution (Steiner et al., 2000; Russo and Russo, 2003). In the chromatographic processes microcrystalline cellulose triacetate (MCTA) has been used as the chiral stationary phase, achieving purifications of virtually 100%. These processes have been carried out in HPLC (Silva et al., 2005a; Silva et al., 2005b) and in simulated moving bed (SMB) units (Santos et al., 2004). A limitation of these processes has been their low productivity (0.15 to 0.5 gram per day), even when using the SMB technology in a laboratory-scale unit. A decay in productivity as the level of purification increases is usually observed in chromatographic processes.

In the diastereomeric resolution processes, separation of *S*-ketamine is based on the application of *L*-(+)-tartaric acid as the resolution agent, allowing the selective crystallization of *S*-ketamine tartrate salt (Steiner et al., 2000; Russo e Russo, 2003). Diastereomeric resolution is one of the most traditional techniques in the enantioseparation field. However, compared to direct crystallization, it has the disadvantage of requiring additional steps for salt conversion, thereby increasing the overall processing time (Wang et al., 2004). To our knowledge, there is no study in the literature related to the direct crystallization or even the solubility of *rac*-ketamine or its enantiomers.

In this study, we determined the ternary phase diagram of *R*- and *S*-ketamine in ethanol and the primary conditions for the crystallization of *S*-ketamine as well as the feasibility of applying it as the predominant or as an auxiliary process.

ENANTIOSEPARATION VIA CHROMATOGRAPHY AND CRYSTALLIZATION

In the past two decades, remarkable progress has been achieved in the field of enantioseparation, with a significant contribution of chiral chromatography, carried out in preparative HPLC or in simulated moving bed (SMB) units for large-scale production. The SMB technology was a breakthrough in increasing the productivity of chromatographic separations; however, a significative decrease in productivity can still be observed as the level of purification increases. For this reason, some authors have been pursuing the use of hybrid processes, combining SMB chromatography and crystallization in order to obtain an increase in productivity (Lim et al., 1995; Lorenz et al, 2001; Bléhaut et al., 2001; Gedicke, 2005; Lorenz et al., 2006). The general concept of the hybrid process is based on using chromatography as a prepurification stage, followed by a crystallization step to achieve the final purification. The advantage is that it overcomes the problem of low productivity found in the former process, while meeting the usual requirement of prepurified solution in the second process. Crystallization is a less labor and cost demanding process than chromatography; however, in only a few cases (5 to 10%) can it be carried out directly in a racemic solution. In most cases it requires an enriched solution with a molar excess of the desired enantiomer, which makes coupling it with chromatography a very attractive alternative. The design and optimization of the hybrid chromatography-crystallization process is discussed in Fung and Ng (2005), Ströhlein et al. (2003), and Amanullah and Mazzoti (2006).



(a) (b)

Figure 2: Illustration of typical ternary phase diagrams involving enantiomeric systems: a–phase diagram of a conglomerate forming system; b–phase diagram of a racemic compound-forming system.

The feasibility of crystallization as the predominant or the auxiliary process depends on the shape of the ternary phase diagram of the enantiomers in a suitable solvent. When the crystal-forming system characterizes a conglomerate (physical mixture of crystals of the pure enantiomers), the diagram (Figure 2a) has one eutectic point (E) which is exactly at the 50:50 composition and crystals of pure S and R enantiomers are obtained respectively in the SAE and REA' regions of the diagram. On the other hand, when the crystal-forming system characterizes a racemic compound (crystals with equal amounts of the two enantiomers in the crystalline unit), the diagram (Figure 2b) has two eutectic points (E and E') and crystals of pure enantiomers are obtained in the regions SAE and RE'A'. Provided that a certain *rac*-mixture is of the conglomerate type, a straightforward strategy to isolate only one of the enantiomers is to add small crystals of the desired enantiomer to the supersaturated racemic solution, which causes the preferential crystallization of the specie added. On the other hand, provided that the mixture is of the compound type, a previous enrichment that produces a purification level above the eutectic composition is required in order to make crystallization a suitable process. About 90 to 95% of chiral organic molecules are racemic compounds and only 5 to 10% are conglomerates (Jacques et al., 1981; Profir et al., 2002). Therefore, the direct crystallization of enantiomers without a prepurification step is strongly limited and its coupling with an other process, such as chromatography, is required in most cases.

EXPERIMENTAL SECTION

Materials

Ketamine racemate and enantiomers with purities above 98% were kindly supplied by Cristália Produtos Químicos Farmacêuticos (Itapira, SP, Brazil) in their base and hydrochloride forms. A conversion procedure was implemented in order to convert the hydrochlorides into their base forms. Solvents used for the solubility studies and chromatographic analysis were of analytical and HPLC grades, respectively.

Ketamine Hydrochloride Conversion

1.0 M sodium bicarbonate solution was slowly added to the aqueous ketamine hydrochloride solution under stirring until the solution pH was close to 11. Stirring was continued for 16 h, and then the base form of ketamine was extracted with dichloromethane. The solvent was eliminated by evaporation in a rotatory evaporator and an HPLC analysis was performed in order to confirm the purity of the converted product.

X-Ray Powder Diffraction (XRPD)

XRPD measurements were carried out using X PERT PRO MRD Philips equipment (USA) with Cu-Kα radiation. The Bragg-Brentano geometry with a diffracted beam pirolytic graphite monochromator was used and the scans were taken with 40 kV - 40 mA in the 10 - 50 deg (2θ) range with a step size of 0.02 º and a counting time of 3 s.

Infrared Spectroscopy

The solid ATR-FTIR spectra of ketamine racemate and enantiomers were collected in a Bomem-Hartmann & Braun IR spectrophotometer (USA). KBr pellets were properly prepared to contain approximately 1 wt% of each compound and sampled from the average of 150 scans using a resolution of 8 cm^{-1}.

Solubility Studies

Solubility was determined in a temperature range of 5 to 40ºC in small vials containing about 1.0 ml of the ketamine/ethanol suspensions. The amount of total solute in the suspensions varied from 50 to 230 mg/ml. Temperature control and stirring were provided by a thermostatic bath and a bench shaking table. After the equilibrium time (previously determined through kinetic experiments), the suspensions were centrifuged at 2000g for 2 min in a thermostatically controlled centrifuge (Nova Técnica, Brazil). The liquid phase was then filtered with 0.45 μm Millex membranes (Millipore, USA) and analyzed for ketamine concentration, based on the absorbance at 245 nm in a UV-VIS spectrophotometer (Beckman, USA). Equilibrium time was determined at 5 and 25ºC for each of the enantiomers and for the racemate by dissolution assays with periodical measurement of the liquid phase concentration until no change was observed.

In order to determine the ternary phase diagram (at 25ºC), solubility measurements were carried out (in duplicate) for suspensions with different ratios of *R*- and *S*-ketamine, all of which had a total of 300 mg/ml of solute. After equilibrium was reached, concentration of the liquid phases was measured with a UV-VIS spectrometer and both liquid and solid phases were analyzed with regard to the enantiomeric composition in HPLC equipment (Waters, USA).

Batch Crystallization

Batch crystallization was carried out in volumes of 20 to 30 ml of *R/S*-ketamine/ethanol suspensions containing an enantiomeric excess of 80% of the *S*-enantiomer. The solute/solvent ratio varied from 150 to 250 mg/ml and the processing time from 4 to 20 h. Suspensions were initially heated (45ºC for 1 h) for a complete dissolution of the solids and then cooled to a temperature of 25ºC at a cooling rate of 0.67ºC/min. The clear solutions were then stirred at a constant temperature (25ºC) during the established processing times. At the end of the process, the liquid phases were filtered through 0.45 μm membranes and analyzed with HPLC. The solid phases were left in an oven for 30 min with air circulation at 35ºC, and the resulting crystals were redissolved and evaluated with HPLC with regard to *S*-ketamine purity.

RESULTUS AND DISCUSSION

Characterization Studies

Characterization of ketamine samples was done by infrared spectroscopy and XRPD in order to identify the racemate crystal-forming system as belonging to one of the two main classes: conglomerate or racemic compound. In both of these methods, the opposite enantiomers always provide identical results, enantiomers and respective conglomerate provide identical results and enantiomer and respective compound provide different results (Jacques et al., 1981). Therefore, differences in the infrared spectra or in the XRPD patterns of the racemate and the enantiomers are an indication that the product is a racemic compound.

The infrared spectra of S-ketamine and *rac*-ketamine (Figure 3) showed similar peaks for the vibrations of the main chemical bonds, including the aliphatic C-H vibration in the 3000-2850 cm^{-1} range, the aromatic hydrogen vibrations around 3000 cm^{-1}, the methyl group at 2800 cm^{-1} and the carbonyl groups at 1700 cm^{-1}. However, different vibrations are observed mainly around 2900 cm^{-1} within the aliphatic group's range, around 3400 cm^{-1} for N-H bonds, and within the 700-800 cm^{-1} range for the C-Cl bonds. The different vibrations observed in the two spectra suggest that ketamine racemate is certainly a racemic compound.

The PXRD patterns (Figure 4) confirm the hypothesis that the product is a racemic compound. The diffraction patterns of the enantiomer and the racemate are remarkably different from each other.

Solubility Studies and the Ternary Phase Diagram

Solubilization assays conducted at 5 and 25ºC indicated that a time interval of 10 h was enough for the ketamine/ethanol suspensions to reach equilibrium (data not shown). For the racemic mixture at 5ºC, dissolution up to 100 mg/ml was observed within the first hour of solubilization, before effectively reaching the equilibrium concentration (70 mg/ml) in a few

hours. A possible explanation of this is the occurrence of a metaestable conglomerate in the initial stage, which assumed the compound form at equilibrium. A similar fact was observed in Profir et al. (2002) where the authors reported a possible metaestable conglomerate in the initial hours of the crystallization of mandelic acid in water, known to appear as a racemic compound. Polymorphism is a critical issue in the crystallization process, and therefore, further investigation should be performed to elucidate the fact.

The solid-liquid equilibrium curves for the ketamine isomers and racemate were then determined in a temperature range of 5 to 40ºC (Figure 5). The solubility of the enantiomers as well as of the racemate increased with increasing temperature. The solubility of the racemate was higher than those of the pure isomers, which as expected, had identical solubilities. The solubility curves indicated a reasonable influence of temperature, implying that the crystallization of ketamine could be carried out by temperature control.

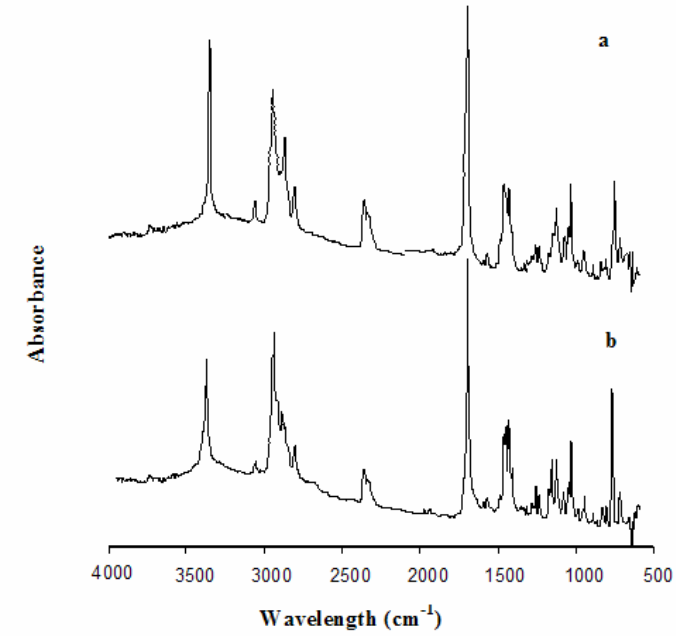


Figure 3: Infrared spectra of racemic ketamine (a) and *S*-ketamine (b) obtained with the KBr method.

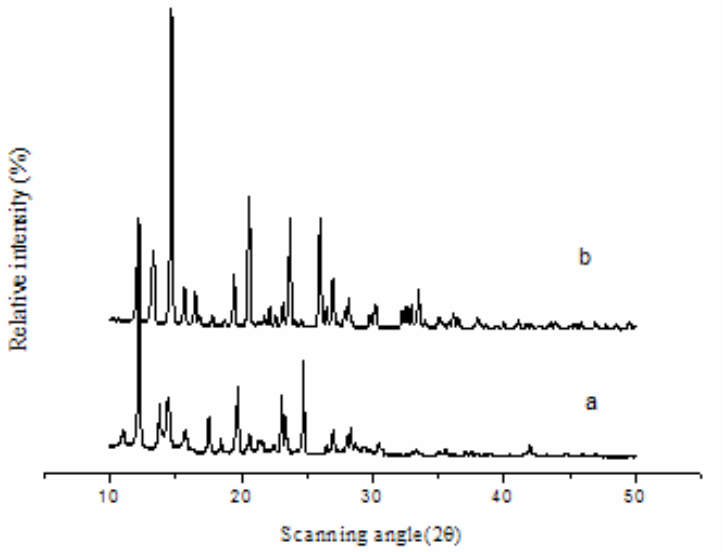


Figure 4: X-ray diffraction patterns of rac-ketamine (a) and *S*-ketamine (b), using Cu-Kα radiation at a scanning rate of 0.020º/s with scans at 40 kV, 40 mA, 2θ between 10 and 50 deg and intervals of 0.02 deg.

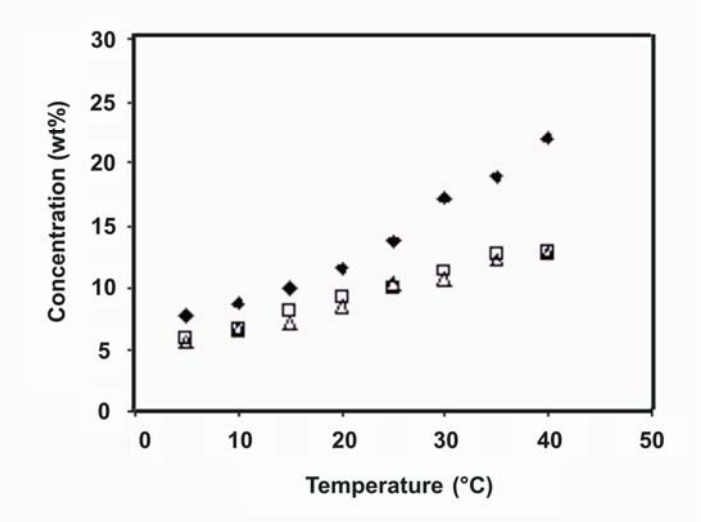


Figure 5: Solubility of racemic ketamine (◆), *S*-ketamine (□) and *R*-ketamine (△) in ethanol. Average of four repetitions with a standard deviation of 0.007 wt%.

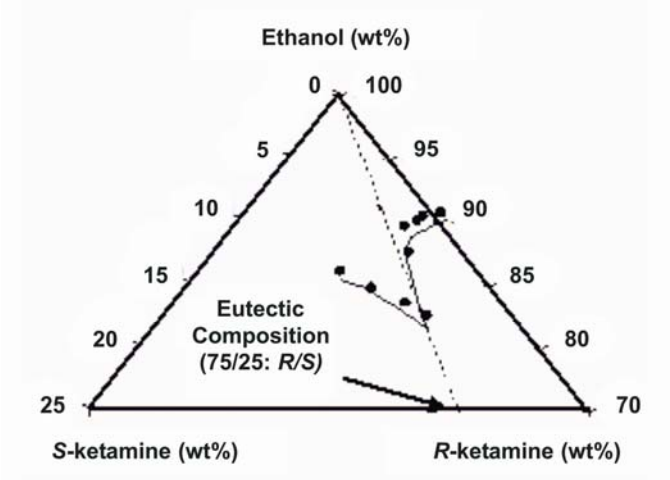


Figure 6: Ternary phase diagram of S/R ketamine in ethanol at a temperature of 25ºC.

The ternary phase diagram was determined (Figure 6), based on the measurement of the solubility of solutions containing different molar fractions of each enantiomer. Only one side of the diagram was determined, given that it is symmetrical when related to stereoisomers. Determination of only one side of the diagram is very common, avoiding unnecessary work and reducing the number of assays. The fact that the diagram was determined on *R*-ketamine and not on the *S*- form, which is the desired isomer, was due to the higher availability of the *R*-enantiomer.

The ternary phase diagram had a eutectic point at the enantiomeric composition of 75/25: *R/S,* indicating that the crystallization of ketamine in the presence of ethanol provides a racemic compound. According to these results, the enantioselective crystallization of ketamine from solutions containing enrichments above 75% of the desired enantiomer is feasible.

Batch Crystallization

Batch crystallizations were conducted at different supersaturations (150 and 250 mg/ml) and processing times (4 and 20 h). The highest productivity under the specified conditions was 6.5 g/(l h), obtained from a suspension of 150 mg/ml stirred for 4h. A purity of 100% and yields of 65 to 70% were observed independent of the supersaturation and the processing times. A prognosis based on these results was that a

combination of the highest supersaturation (250 mg/ml) and the shortest processing time (4h) could possibly provide a productivity of 24 g/(l h). Since these assays consisted in preliminary trials, only possible yields and productivities were estimated and an optimum condition was not determined. The conditions applied were chosen arbitrarily and certainly an optimization of the enantioselective crystallization of *S*-ketamine as well as the feasibility of its coupling with chromatography depends on more detailed studies. Nonetheless, these preliminary results demonstrated the potential of coupling crystallization with chromatography as a mean for increasing the productivity. A parallel analysis with the productivity of 0.5 g/(l h) reported by Santos et al. (2004) for the chromatographic separation of *S*-ketamine indicates that the coupling of these two processes is a very attractive alternative for the resolution of *S*-ketamine.

In a possible coupling of crystallization with chromatography, the productivity of the overall process must take into account other aspects, such as the time required for the concentration step. Usually the solution obtained by chromatography is diluted, and a crystallizer requires supersaturated solutions. In the particular case of ketamine, the final concentration of *S*-enantiomer obtained in a typical SMB chromatographic unit (Santos et al., 2004) was 5 mg/ml, which is 30 to 50 times lower than the concentration used in the crystallization assays in the present work.

CONCLUSIONS

The present study has demonstrated the potential of applying crystallization for the enantioselective separation of ketamine. According to the ternary phase diagram using an enriched solution with an enantiomeric excess above 75% was required to crystallize the pure stereoisomer. This made the process more suitable as an auxiliary process, designated to complement a previous chromatographic separation step. Chromatographic purification only up to 75% would reduce the number of cycles significantly more than full purification, thus increasing productivity. A purity of virtually 100% and yields from 65 to 70% were found for preliminary batch crystallizations regardless of processing time and supersaturation. A combination of the best conditions studied allowed the projection of a possible productivity of 24 g/(l h).

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