

# SIMULATED MOVING-BED ADSORPTION FOR SEPARATION OF RACEMIC MIXTURES

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**Abstract** - The two enantiomers that constitute a racemate have different activities when employed as pharmaceuticals. Due to this fact, fully recognized today, the pharmaceutical industry has been forced to market pure enantiomers instead of the racemic mixture whenever a chiral compound is involved. The simulated moving bed (SMB) is a chromatographic process that, unlike traditional HPLC systems, operates continuously without losing the enantiomeric purity of the outlet streams. The present work describes the enantioseparation of the anesthetic ketamine in a semipreparative-scale SMB unit. The chiral stationary phase employed was the microcrystalline cellulose triacetate. The outlet streams were analyzed by an on-line system, composed by an UV/VIS meter and a polarimeter, and also by HPLC. The analysis indicated purity values up to 100% for the stream of interest and up to 97.7% for the other stream.

**Keywords:** SMB chromatography, enantiomer separation, preparative chromatography, ketamine.

## INTRODUCTION

A general theme in the field of separation processes is the improvement of the selectivity of solutes. The power of modern synthetic chemistry in conjunction with separation science and technology can be used to develop new separating agents and equipment with an enhanced selective separation function. There is a growing demand in the pharmaceutical industry for efficient and cost effective methods to purify optical isomers (McCoy, 2000). The use of continuous chromatographic processes has been pointed to as an important tool to meet the objectives of the chemical manufacture of several chiral compounds (Nicoud, 1999). The simulated moving bed (SMB) is a large-scale version of traditional high-performance liquid chromatography (HPLC), but unlike normal HPLC,

it operates continuously, without loss of the enantiomeric purity of the outlet streams. The process consists in simulating the countercurrent movement of the bed of adsorbent by switching the positions of the inlet and outlet streams, producing two outlet streams, one rich in the more adsorbable component (extract stream) and the other rich in the less adsorbable one (raffinate stream), which is adequate for binary separations, as in the case of racemates. SMB has been used by several authors to separate components from racemic mixtures (Juza et al., 2000), obtaining the two enantiomers of a chiral molecule with a high enough purity and in sufficient quantities to carry out clinical tests or even production stages. The variety of chiral selectors used as the stationary phase and the vast number of racemic mixtures produced by the pharmaceutical industry make this technique a powerful tool, and

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create a stimulating and challenging area of interest for both laboratory-scale studies and production plant design. The aim of the present work was to design and build a laboratory-scale unit to separate racemic mixtures of pharmaceutical compounds originating from an organic synthetic route. The anesthetic ketamine was utilized as a model of a molecule to be separated in the system. The stationary phase utilized in this study was cellulose-triacetate in a microcrystalline form, which has been considered one of the most convenient stationary phases for the resolution of the racemic mixtures. This unit is, to the best of our knowledge, the only SMB chromatographic research-oriented and laboratory-scale unit operating in Brazil.

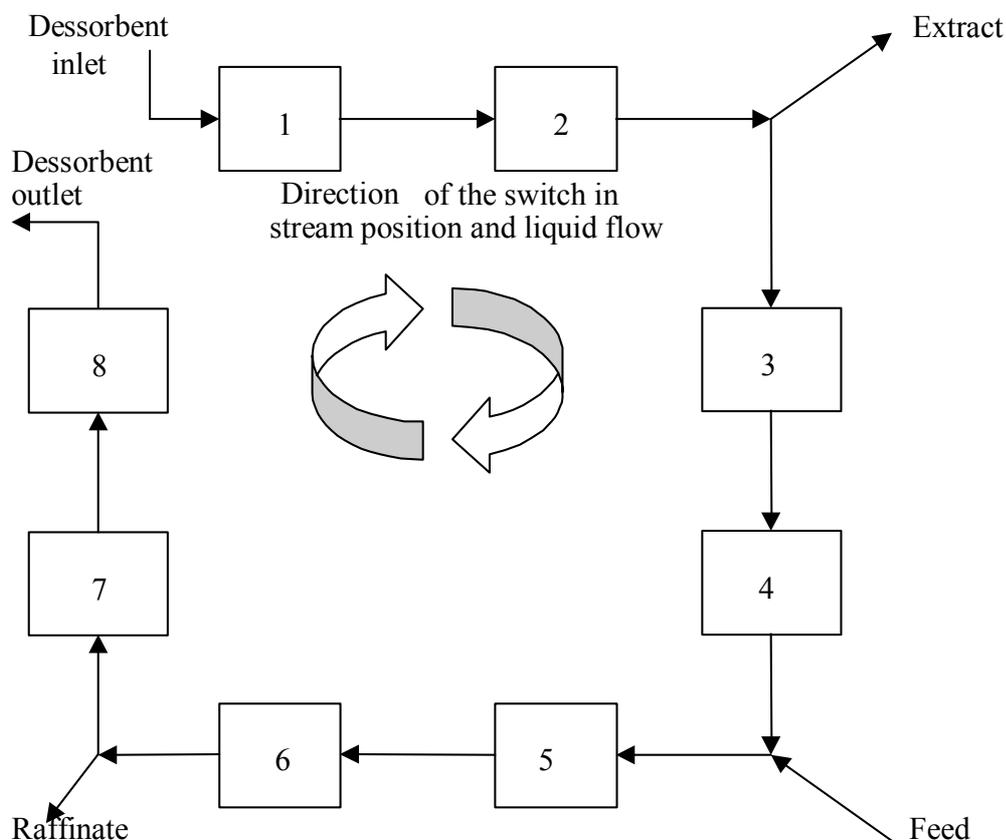
## MATERIALS AND METHODS

### Description of the Simulated Moving Bed (SMB) Chromatographic Unit

The laboratory-scale SMB unit built has eight stainless steel columns 0.77 cm in diameter and 20

cm in height. The columns are distributed between four different regions containing two columns each (Figure 1). The desorbent is recycled outside of the series of columns by using a multiposition valve instead of a solvent reflux pump. Another four multiposition valves are responsible for the position change of feed, desorbent inlet, raffinate and extract at preset switch times. These valves are connected to four semipreparative liquid chromatographic pumps (Shimadzu LC-6AD). In Figure 2 some details of the complete setup are shown. Multiposition valves (Valco Instruments Co.) are electrically commanded and linked to a computer by a data-acquisition board. Each valve automatically operates the unit at the selected flowrates by a program developed with the Labview software.

The unit also contains a sampling valve connected to one of the columns of the series, which allows collection of internal samples. Analysis of these samples enables determination of the internal profile of concentrations of R and S enantiomers, which illustrates the dynamics of separation inside the series of columns.



**Figure 1:** Basic scheme of the SMB laboratory-scale unit

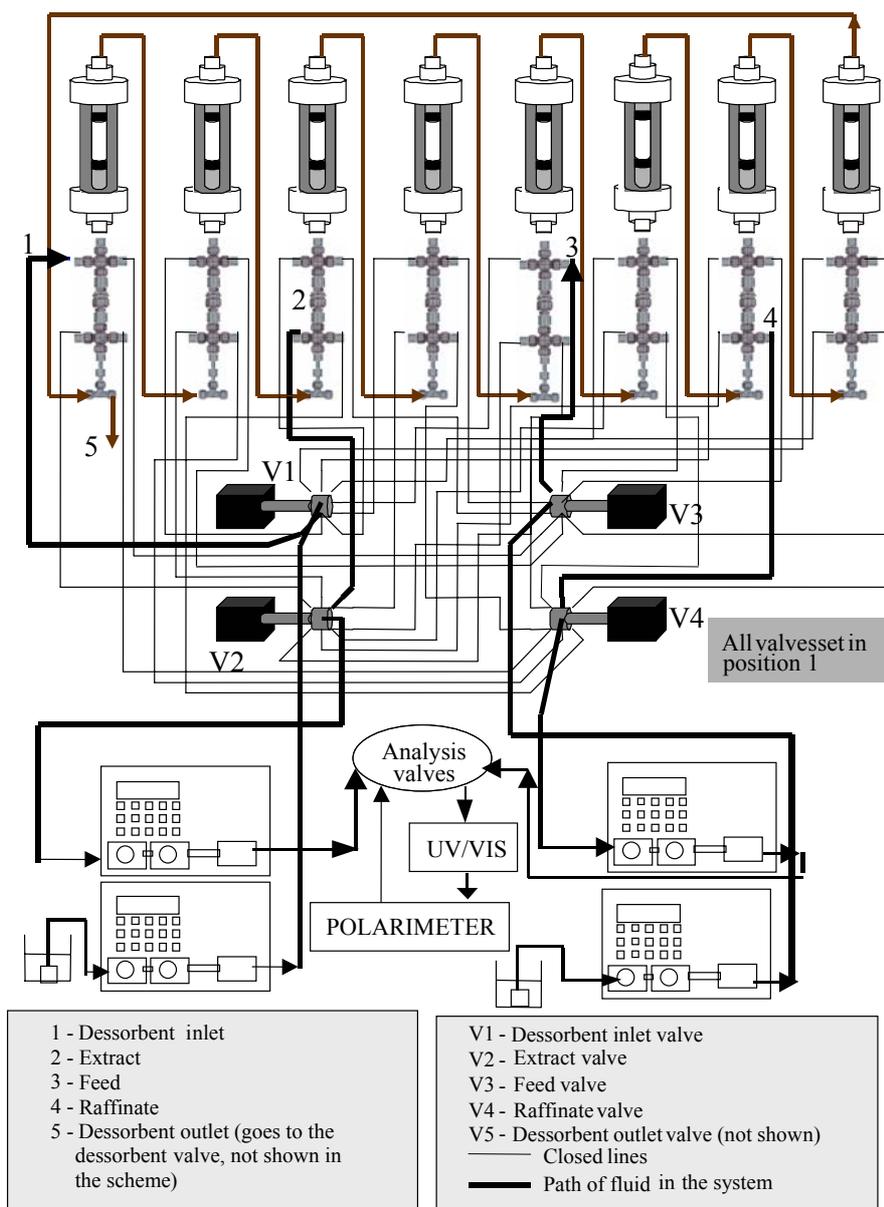


Figure 2: General setup of the SMB unit

### Analytical System of the SMB Unit

The purity of the raffinate and extract streams is continuously monitored in order to determine separation efficiency. The raffinate must have the highest possible purity for the less adsorbed component (in the case of ketamine, the S enantiomer) and the extract must have the highest purity of the more adsorbed component (the R ketamine enantiomer). Extract purity is referred to as

$P_{Ex}$  and raffinate purity as  $P_{Raf}$ . The equations relating these purities to the component concentrations  $C_S$  and  $C_R$  are respectively

$$P_{Ex} = \left( \frac{C_R^{Ex}}{C_R^{Ex} + C_S^{Ex}} \right) \cdot 100 \quad (1)$$

$$P_{Raf} = \left( \frac{C_S^{Raf}}{C_S^{Raf} + C_R^{Raf}} \right) \cdot 100 \quad (2)$$

The total amount of solute in raffinate and extract is determined with an UV/VIS detector equipped with a flow cell. The difference between these concentrations can be determined with a polarimetric detector also equipped with a flow cell. A similar procedure for the quantitative detection of chiral molecules was successfully utilized by Lodevico et al. (1997).

The UV/Visible detector is a Shimadzu model SPD-10AV detector, while the polarimetric detector is a Jasco P-1010. A Shimadzu DGU-14A membrane degasser is used to avoid air bubbles in the system.

### Racemic Mixture Used for Separation

A racemic mixture of anesthetic ketamine in the form of free base as well as samples of the standard pure enantiomers used for calibration were kindly provided by Cristália Pharmaceutical Industries, Itapira, São Paulo, Brazil. The structure of the ketamine molecule is depicted in Figure 3.

The ketamine molecule has chirality and was separated in a column of cellulose-triacetate in a microcrystalline form (MCTA) by Blaschke (1986), using ethanol as the mobile phase. The S enantiomer is the one of interest, due to its anesthetic properties, and must be separated from the R enantiomer before marketing.

### Column Packing and Mobile Phase

MCTA, the stationary phase, has been considered one of the most convenient stationary phases for the resolution of racemic mixtures (Francotte et al., 1985). It was purchased from Merck. The mobile phase used was HPLC-grade ethanol, in which

ketamine is easily dissolved. The columns were slurry-packed following the protocol described by Nicoud (1993).

### Determination of Purity by HPLC

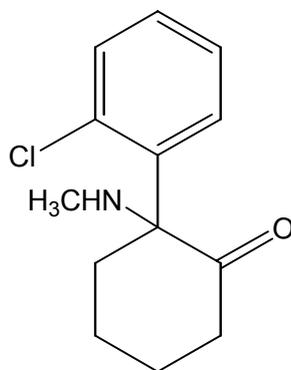
In order to ensure the purity of the streams, samples were collected throughout the experimental run, for subsequent analysis in a HPLC system, which furnishes values of purity averaged over the time of collection. The column used for the HPLC tests was packed with the same stationary phase as that employed in the SMB columns, MCTA, and is 200 mm high with an ID of 4.6 mm.

### Porosity Measurements

Porosity of the bed of MCTA packed in the columns was measured following the protocol described by Pedferri et al. (1999). Each of the eight columns necessary for operation of the SMB unit, containing the MCTA packed bed, was individually coupled to the HPLC system, and 1,3,5 - Tri-tert-butylbenzene was injected into each one of them. This compound does not interact with the adsorbent and hence residence time throughout the bed is proportional to total bed porosity. The relation between porosity and residence time is

$$\varepsilon = \frac{t_0 \cdot Q}{V} \quad (3)$$

where  $\varepsilon$  is the total bed porosity including the particle pores,  $t_0$  is the residence time of Tri-tert-butylbenzene flowing throughout the bed,  $Q$  is the liquid flow rate and  $V$  is the total volume of the bed.



**Figure 3:** Chemical structure of ketamine

## Linear Isotherms Parameters from Chromatographic Experiments

Before running the SMB unit, it was necessary to know the interactions between the enantiomers of the racemic mixture and the chiral stationary phase. This information can be obtained by injecting racemic ketamine into each of the eight columns of the SMB unit, coupled individually to a HPLC system, as in the porosity tests. Then the retention time of each enantiomer is measured, which allows determination of the linear isotherms, valid for dilute systems. The isotherms are represented by the Henry constants, given by (Pedferri et al., 1999)

$$H_i = \frac{(t_i^R - t_0)}{t_0} \cdot \frac{\varepsilon}{1 - \varepsilon} \quad (4)$$

where  $t_i^R$  is the retention time of the  $i$  enantiomer.

It is worth observing that in typical publications in the area of chromatography, the Henry constants defined above are very similar to the capacity factors  $K_i$ , which are defined by Cass et al. (1997)

$$K_i = \frac{(t_i^R - t_0)}{t_0} \quad (5)$$

## RESULTS AND DISCUSSION

### Bed Porosity and Henry Constants

Total porosity  $\varepsilon$  was determined from the pulse experiments for the eight columns. The average values and standard deviations (sd) are  $\varepsilon = 0.634$ ,  $sd = 0.02$ ;  $H_R = 4.74$ ,  $sd = 0.34$ ; and  $H_S = 2.33$ ,  $sd = 0.31$ .

### Continuous Runs in the SMB Unit

After determination of the Henry constants, the procedure described by Mazzotti et al. (1997) allowed choice of the operational conditions for SMB unit operation. It was assumed that the system operates under conditions dilute enough to be correctly represented by the linear isotherms.

The conditions chosen for the experimental runs are summarized in Table 1.

The experimental run under set of conditions number 4 was chosen as an example, and its results will be presented in order to illustrate how SMB

operation is assessed, based on the response of the system of analysis.

The UV/VIS and polarimetric detectors register signals as the experimental runs are carried out, providing a good way to instantaneously assess the separation. From preliminary polarimetric measurements with the R and S standard ketamine enantiomers, a positive rotation was observed for the former and a negative rotation for the latter. Therefore, for a successful separation it is expected that the raffinate stream, which must contain the S enantiomer (less adsorbed), must show a negative signal in the polarimeter, while the signal of the extract stream, which must contain the R enantiomer (more adsorbed), must be positive.

The signals of the UV/VIS and polarimetric detector are given in Figures 4 and 5 respectively for the extract and raffinate streams in the experimental run under set of conditions 4. It is necessary to conduct two identical runs, one for each of the streams, in order for both extract and raffinate to be analyzed by the on-line system.

Through the calibration curves of the meters, the data presented in Figures 4 and 5 can be mathematically treated to furnish the concentrations of the R and S enantiomers as a function of time. Figure 6 shows the results obtained with this treatment for the extract and raffinate streams.

During the experimental runs, samples from the extract and raffinate streams were collected for analysis in the HPLC system. The components of the streams are identified and quantified by comparison of the retention times obtained with those obtained with the injection of a solution of racemic ketamine. Figure 7 shows a comparison between the chromatogram obtained with the injection of a solution of racemic ketamine (a) and the chromatogram obtained with the injection of an extract sample (b), both at the same mobile phase flow rate (1 mL/min). The predominance of the R enantiomer in the extract is clearly observable for a retention time of about 7 min.

Figure 8 shows a comparison between the chromatogram obtained with the injection of a solution of racemic ketamine (a) and the chromatogram obtained with the injection of a raffinate sample (b), both at the same mobile-phase flow rate (0.25mL/min). The predominance of the S enantiomer in the raffinate is clearly observable for a retention time of about 18 min.

Figure 9 shows the chromatogram resulting from injection of a solution containing the standard ketamine S enantiomer. Comparing Figures 9 and

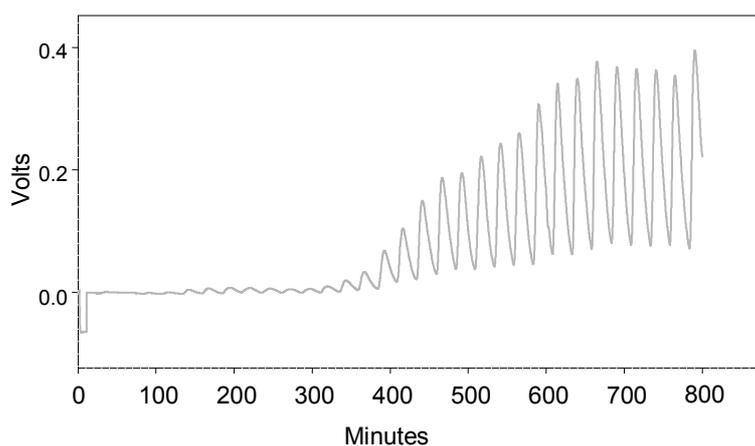
8(b), it may be seen that the raffinate stream has an enantiomeric purity comparable to that of the standard S enantiomer.

The sampling valve installed on top of the first column allows collection of samples from the nodes

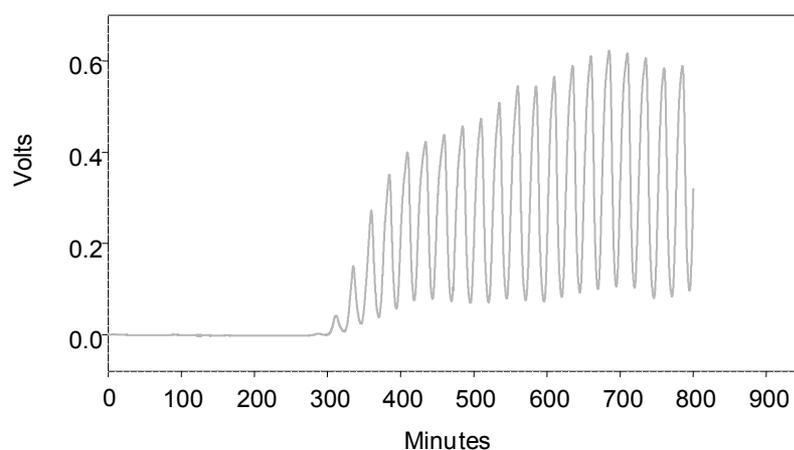
between the columns. Analysis of these samples in the HPLC system allows determination of an internal concentration profile. Figure 10 shows this profile for one of the experimental runs, when the steady state had already been established.

**Table 1: Conditions designed for the experimental runs**

Experimental condition	Stream flow rates (mL/min)					Flow rates in the zones (mL/min)				Switch time (min)	C <sub>F</sub> (g/L)
	Ex	Raf	F	D <sub>In</sub>	D <sub>Out</sub>	Q <sub>1</sub>	Q <sub>2</sub>	Q <sub>3</sub>	Q <sub>4</sub>		
1	0.32	0.31	0.15	0.85	0.37	0.85	0.53	0.68	0.37	30	5
2	0.47	0.43	0.18	1.1	0.38	1.1	0.63	0.81	0.38	25	2.5
3	0.47	0.43	0.18	1.1	0.38	1.1	0.63	0.81	0.38	25	1.5
4	0.44	0.39	0.1	1.1	0.37	1.1	0.66	0.76	0.37	25	5

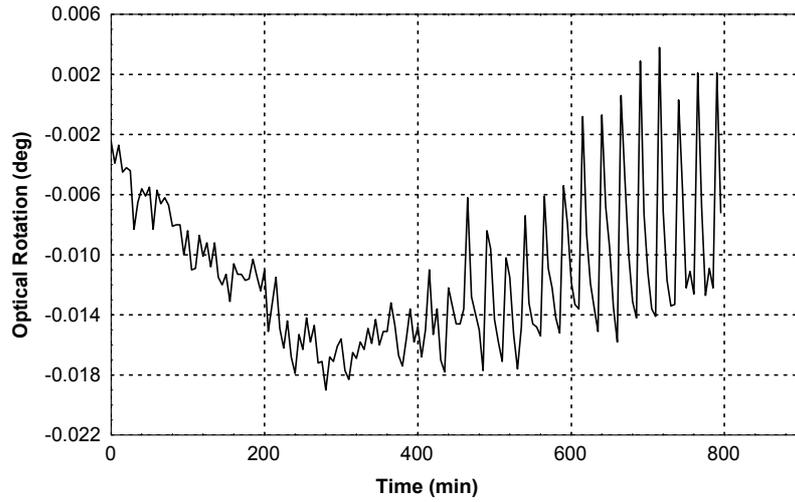


(a)

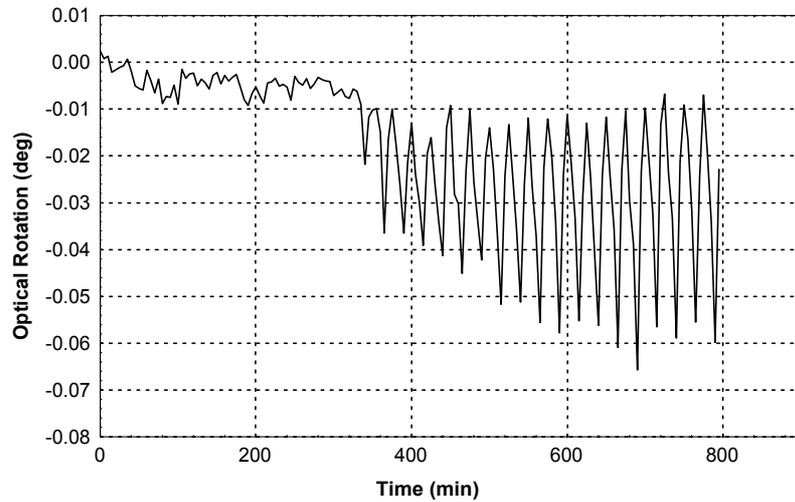


(b)

**Figure 4:** Response of the UV/VIS to flow of the extract (a) and raffinate (b) streams in the experimental run under set of conditions 4.

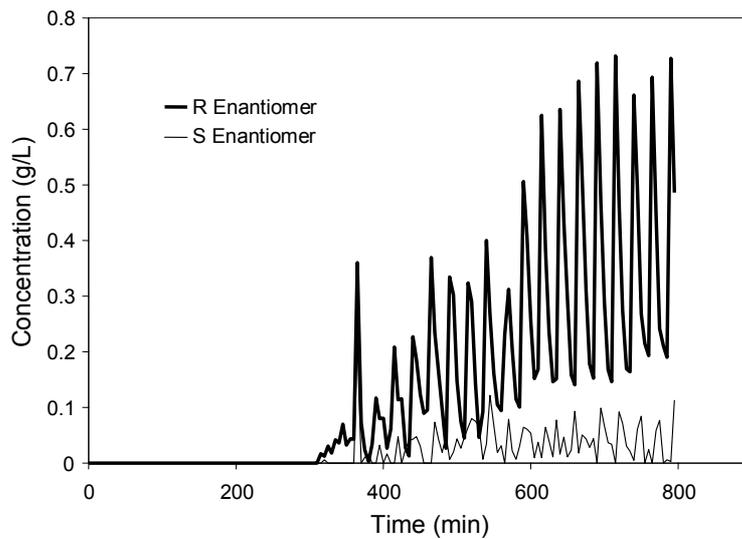


(a)

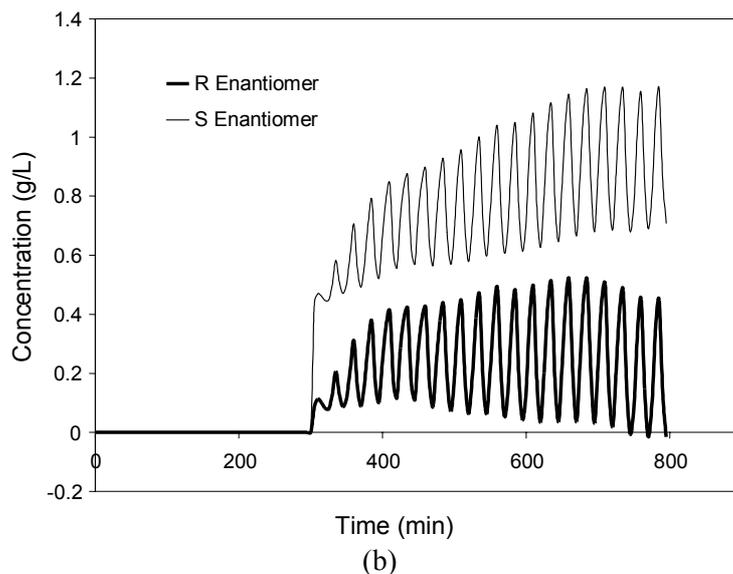


(b)

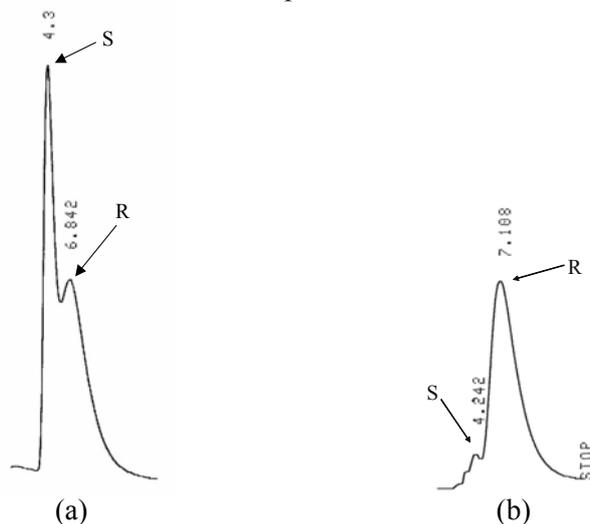
**Figure 5:** Response of the polarimetric detector to flow of the extract (a) and raffinate (b) streams in the experimental run under set of conditions 4.



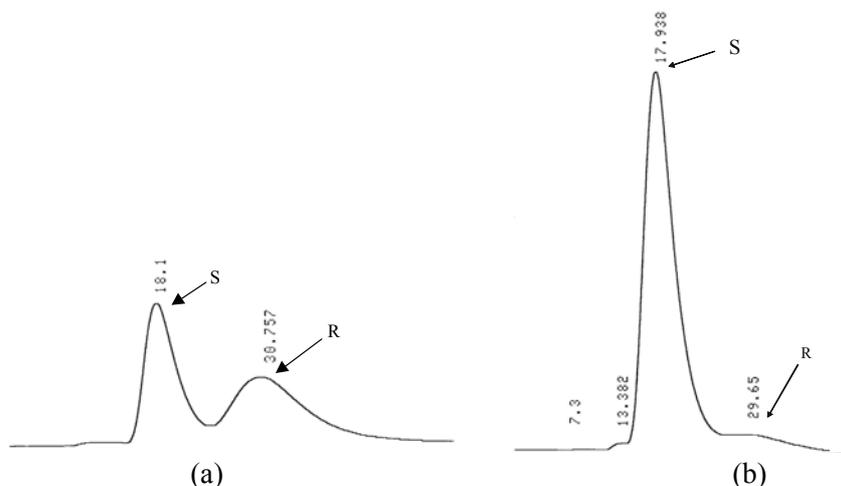
(a)



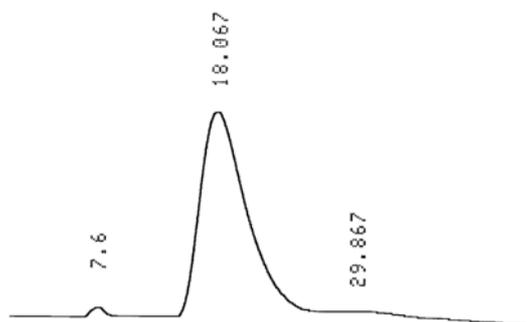
**Figure 6:** Evolution of the concentrations of R and S enantiomers in the extract (a) and raffinate (b) streams in the experimental run under set of conditions 4.



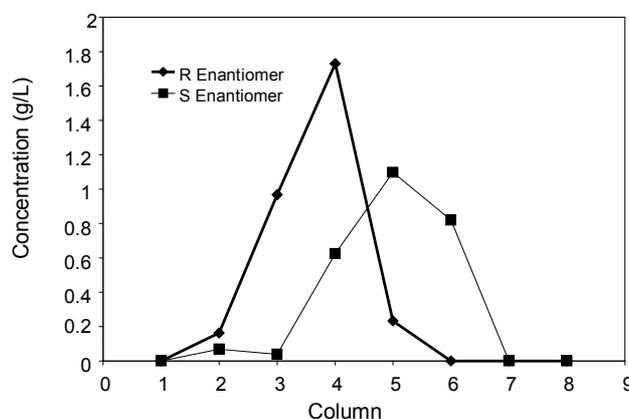
**Figure 7:** Chromatograms resulting from injections in the HPLC system. (a) solution of racemic ketamine, 1.5 g/L. (b) extract sample. Both were injected at the same mobile-phase flow rate (1mL/min).



**Figure 8:** Chromatograms resulting from injections in the HPLC system. (a) solution of racemic ketamine, 1.5 g/L. (b) raffinate sample. Both were injected at the same mobile-phase flow rate (0.25 mL/min).



**Figure 9:** Chromatogram resulting from the injection of a solution of the standard ketamine S enantiomer, 1.5 g/L, at a mobile-phase flow rate of 0.25 mL/min in the HPLC system.



**Figure 10:** Concentration profiles in the set of eight columns of the SMB unit at the steady state for the experimental runs under the set of conditions 4

## CONCLUSIONS

Results on stream purity from the experimental runs under the conditions shown in Table 1, are summarized in Table 2.

The enantiomers of racemic ketamine were successfully separated by the SMB unit under the conditions designed and employed. The method adopted for the design of the experimental conditions (Mazzotti et al., 1997), as well as the assumption of linearity of the isotherms which describe the system were adequate for the range of concentrations tested, not higher than 5 g/L in the feed. The levels of enantiomeric purity were high, mainly for the stream

of interest, i. e., the raffinate, whose purity was comparable to that of the standard ketamine S enantiomer. The purity of the extract, although not as high as that of the raffinate, was high enough to avoid large losses of the S enantiomer, which should be completely recovered in the raffinate stream. The processing of racemic ketamine was in the range of 0.32 to 1.08 g/day, and the production of pure S enantiomer, 0.15 to 0.5 g/day.

Studies to determine competitive isotherms are currently being carried out in order to develop equations that can represent systems with higher concentrations and thereby improve the productivity of the SMB unit in the future.

**Table 2: Results of the SMB experimental runs**

Experimental condition	Stream flow rates (mL/min)					$C_F$ (g/L)	Switch time (min)	Purity	
	F	$D_{In}$	$D_{Out}$	Ex	Raf			Ex	Raf
1	0.15	0.85	0.37	0.32	0.31	5	30	95.1	98.8
2	0.18	1.1	0.38	0.47	0.43	2.5	25	97.7	100.0
3	0.18	1.1	0.38	0.47	0.43	1.5	25	100.0	100.0
4	0.1	1.1	0.37	0.44	0.39	5	25	95.7	99.0

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### NOMENCLATURE

$C_R$	concentration of R enantiomer (g/L)
$C_S$	concentration of S enantiomer (g/L)
$H_i$	Henry constant (dimensionless)
$K_i$	capacity factor of the column for component i (dimensionless)
$P_{Ex}$	extract purity (equation 1) (dimensionless)
$P_{Raf}$	raffinate purity (equation 2) (dimensionless)
$Q$	flow rate through the bed (mL/min)
$t_0$	retention time for an inert compound in the packed column (min)
$t_i^R$	retention time for component i in the packed column (min)
$V$	total volume of the bed (mL)
$\varepsilon$	total bed porosity (dimensionless)

### Subscripts and Superscripts

Raf, Ex refer to raffinate and extract, respectively.

### REFERENCES

- Blaschke, G., Chromatographic Resolution of Chiral Drugs on Polyamides and Cellulose Triacetate, *Journal of Liquid Chromatography*, 9 (2), 341-368 (1986).
- Cass, Q.B., Degani, A.L.G., Tiritan, E., Matlin, S.E., Curran, D.P. and Balog, A., Enantiomeric Resolution by HPLC of Axial Chiral Amides Using Amylose Tris((S)-1-Phenylethylcarbamate), *Chirality*, 9, 109-112 (1997).
- Francotte, E.R., Wolf, R.M., Lohmann, D. and Muller, R., Chromatographic Resolution of Racemates on Chiral Stationary Phases. I. Influence of the Supramolecular Structure of Cellulose Triacetate, *Journal of Chromatography*, 345, 25-37 (1985).
- Juza, M., Mazzotti, M. and Morbidelli, M., Simulated Moving Bed and Its Application to Chiraltechnology, *Trends in Biotechnology*, 18, 108-118 (2000).
- Lodevico, R.G., Bobbitt, D.R. and Edkins, T.J., Determination of Enantiomeric Excess Using a Chiral Selective Separation Mode and Polarimetric Detection, *Talanta*, 44, 1353-1363 (1997).
- Mazzotti, M., Storti, G. and Morbidelli, M., Optimal Operation of Simulated Moving Beds for Nonlinear Chromatographic Separations, *Journal of Chromatography A*, 769, 3-24 (1997).
- McCoy, M., SMB Emerges as a Chiral Technique, *Chemical and Engineering News*, vol. 78, No. 25, 1-3 (2000).
- Nicoud, R. M., A Packing Procedure for High Flow Rate and High Stability Columns Using Cellulose Triacetate, *LC - GC Int.*, 6, 636-637 (1993).
- Nicoud, R.M., The Separation of Optical Isomers by Simulated Moving Bed Chromatography (Part II), *Pharmaceutical Technology Europe* 11, 28-34 (1999).
- Pedefferri, M.P., Zenoni, G., Mazzotti, M. and Morbidelli, M., Experimental Analysis of a Chiral Separation Through Simulated Moving Bed Chromatography. *Chemical Engineering Science*, 54, 3735-3748 (1999).