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PhD THESIS
SUMMARY

STUDY OF SOME BIOLOGICAL COMPOUNDS BY MACROCYCLIC RECEPTORS

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CONTENTS

INTRODUCTION

The present paper aims to demonstrate the importance of the involvement of the macrocyclic receptors for physico-chemical processes underlying analytical techniques, taking into account the fact that such chemical species are the ones that realize supramolecular architectures that are part of the supramolecular chemistry.

The present thesis presents aspects of applications of macrocyclic receptors in the study of compounds with biological relevance. So, it studies the macrocyclic calixarenic receptors, α- and β-cyclodextrins and cucurbit[*n*]uril, n = 6, 7 in the complex and separation processes of amino acids, dipeptides and nucleobases. Furthermore, it is studied the possibility of enantioseparation of native and methylester aromatic amino acids through bulk liquid membranes by using β-cyclodextrin functionalized as chiral carrier. The main objectives of the thesis are the following:

a) The characterization and comparative study of the complexes formed between the important biological compounds amino acids, dipeptides, nucleobases and: *p*-sulfonatocalix [n]arenes, n = 4 ,6, α-cyclodextrin and β-cyclodextrin functionalized (heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin, cucurbit[6]uril (CB[6]) and cucurbit[7]uril (CB[7]) by UV-Vis spectrophotometric methods, calorimetric titration, nuclear magnetic resonance and circular dichroism spectroscopy.

b) Studies concerning the possibilities of biological compounds (aromatic amino acid in native and methylesters form) separation by using macrocyclic receptors. Thus, it has been studied aspects of liquid-liquid extraction regarding separation of aromatic amino acids with heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin: distribution equilibrium involved in the biphasic and triphasic system, the effect of the parameters that influence the process of extraction (pH, temperature, shaking time), the determination of extraction yields of amino acids, the determination of extraction constants of amino acids.

c) Studies regarding the transport of aromatic acids in native and methylesters form by liquid chloroform membrane with heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin aiming separation. In this case, the following aspects are studied: the stages of the underlying transport through liquid membrane, the factors that influence the transport through liquid membrane (pH, shaking time, solvent phase membrane, ion pair, temperature), the transport mechanism of aromatic amino acids native and methylesters through liquid chloroform membrane by the cyclodextrinic functionalized receptor, the possibilities of enantioseparation of aromatic amino acids through bulk liquid membrane by using the chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin.

The thesis has eight main chapters, the first three chapters (macrocyclic receptors used in analytical chemistry, thermodynamic aspects of host-guest complexes form with biological compounds, analytical applications of macrocyclic receptors in the separation processes) refers to the theoretical part and the following five chapters to the experimental part (original part).

In the theoretical part are presented synthetically general aspects regarding the importance of the involvement of macrocyclic receptors in the recognition of chemical and biological compounds as well as aspects of their applications in the separation of compounds of interest. There are highlighted the studies concerning the analytical applications of the macrocyclic receptors towards chemical, biochemical and environmental compounds.

The original part is divided into five chapters, of which the first three chapters present the results obtained from studies on the processes of complexation of cucurbituril, calixarenes, and cyclodextrines with amino acids, nucleobases and dipeptides by means of calorimetric techniques, UV-Vis spectrometry, and nuclear magnetic resonance (NMR). In

Chapter 7 there are presented the applications of chiral β-cyclodextrin functionalized (heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin) in the processes of extraction and transport of native and methylesters amino acids through liquid membranes for their separation. In Chapter 8, to complete the information on the possibilities of enantioseparation of aromatic amino acids, the circular dichroism spectroscopy is used.

The general conclusions chapter includes a synthesis of the results obtained during this thesis. This thesis ends with bibliographical references and the published contributions resulted from the experiments carried out.

EXPERIMENTAL PART

I Comparative study of the complexes formed between p-sulphonatocalix[n]arenes, cyclodextrins and cucurbit[n]uril with biological compounds

Chapter 4. Thermodynamic aspects of the amino acids and dipeptides complexation with p-sulphonatocalix[n]arenes, n = 4.6

4.1. General considerations

It is well known the fact that in supramolecular chemistry the thermodynamic parameters characteristic to the complexes formation represent essential data for the molecular recognition. In order to study them, various techniques (like it was mentioned in chapter I.5) are used: UV~Vis spectrometry, NMR, calorimetric, potentiometric and conductometric titrations, chromatographic methods and mass spectrometry.

The amino acids, as fundamental constituents of a large and various category of biological macromolecules, represent attractive targets in the supramolecular chemistry. It is well known that the properties of the amino acids in aqueous solution is of great importance both in developing the synthesis processes, their purification and separation, as well as in elucidating the principles of transport through biological membranes. Moreover, the reactivity and biological activity of proteins in the aqueous phase depend on the hydration of their structural fragments as well as on that of the amino acids residues [9].

On the other hand, investigating the nature of the interactions involved in the formation of the ligand-peptide complexes is important for the understanding of the biomolecular interactions specific to the regularization of the cellular processes. The study of the factors that contribute to the formation of the complex is also important in the chemistry of peptides [25]. The research group of A. W. Hamilton studied the proteins molecular recognition establishing the principles that govern their recognition by the functionalized macrocycles [30].

The water soluble calix[n]arenes can form complexes with numerous biological species, and the inclusion properties of the guests investigated are correlated with their structural properties [64, 133, 151, 181, 182]. Arena and his collaborators proved through NMR and computational studies the possible formation of complexes between α-amino acids (L-alanine, L-valine, L-leucine, L-phenylalanine, L-tyrosine, L-tryptophan) and *p*-sulphonatocalix(4)arens through the insertion of the aromatic or aliphatic group inside the hydrophobic cavity of the calixarene [183].

In this chapter we have studied, using the calorimetric method, the possibility of the formation of the *p*-sulphonatocalix[n]arenes, n = 4, 6 complexes with a series of amino acids, dipeptides and nucleobases in aqueous solution.

4.2. Experimental conditions

4.2.1. Apparatus and Reagents

The thermodynamic studies were done by calorimetric titrations with a calorimeter Tronac 450 (Tronac Company). In order to record the thermic signal versus time a microvoltmeter is used, model *197* (*Keithley Instruments GmbH* Company, Germering) and a plotter –x,y, model *SE 130* (*ABB Goertz* Company).

Reagents used

Amino acids: L-proline (L-Pro), L-arginine (L-Arg), L-lysine (L-Lys), L-histidine (L-His), L-aspartic acid (L-Asp), L-serine (L-Ser), L-isoleucine (L-Ile) and L-tyrosine (L-Tyr) of purity > 99% were obtained from Sigma-Aldrich (Figure 4.2) and the dipeptides were obtained from Fluka. It was used distilled water throughout the experiments (Millipore). *p*-sulphonatocalix[4]arene (Figure 4.3) and *p*-sulphonatocalix[6]arene (Figure 4.4) of analytical purity (>98 %) were obtained from Acros Organics.

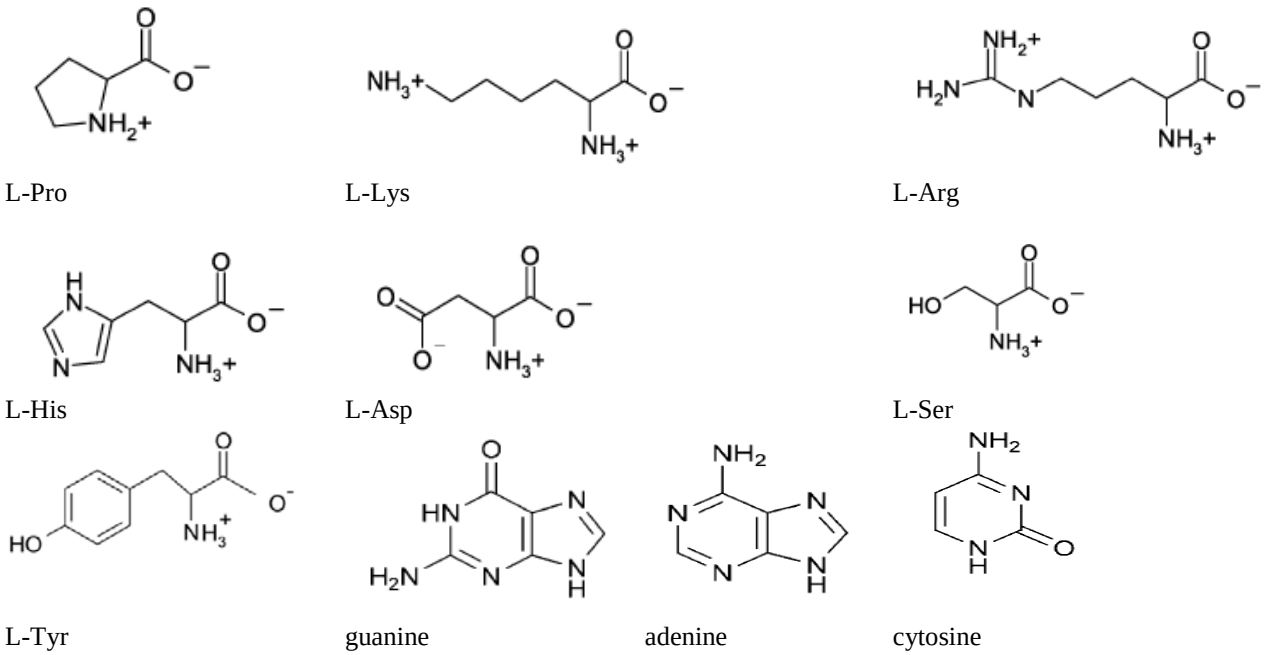

Fig. 4.2 *The structure of the compounds used in experiment*

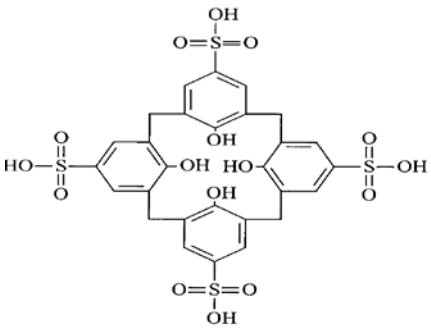

Fig. 4.3 *The chemical structure of p-sulphonatocalix[4]arene*

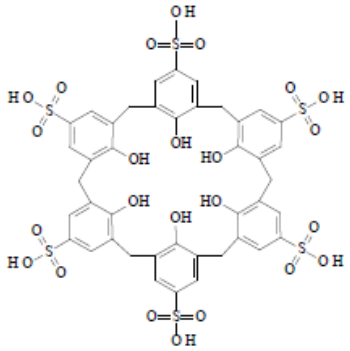

Fig.4.4 *The chemical structure of p-sulphonatocalix[6]arene*

4.2.2. Procedure

During the calorimetric titrations a corresponding ligand solution (0,01 – 0,02 mol/L) was continuously added to the amino acid or peptide solution (0,001-0,002 mol/L). The heat

measured Q is connected to the enthalpy reaction ΔH and the moles number of the complexes formed Δn through the following equation:

$$Q = \Delta H \, \Delta n \qquad (4.1)$$

Δn depends on the stability of the complex. The calculation of the stability constant from the titration curves is done with the help of a specialized program.

4.2.3 Results and discussion

4.2.3.1 Determination of the stability constant and thermodynamic parameters of amino acids complexes with p-sulphonatocalix [4]arene in aqueous solution

The values of the stability constants and of the thermodynamic parameters studied for the complexes formed between *p*-sulphonatocalix[4]arene and a series of amino acids in aqueous solution are presented in Table 4.1. The study was done in aqueous solution without a buffer solution in order to avoid any influence on the formation of the complex. It is known the fact that the amino acids exist in a neutral medium under an amphionic form (zwitterionic). The results obtained are compared to those obtained in the literature by mean of NMR studies. As it can be noticed, they differ in case of some complexes. From the results presented in Table 4.1 one can observe that *p*-sulphonatocalix[4]arene forms complexes with the amino acids studied and the complexation is favoured by enthalpic contributions and disadvantaged by entropic contributions.

Table 4.1. *The stability constants log K (K in M^{-1}) and the values of the thermodynamic parameters ΔH and $T\Delta S$ for the complexes of some amino acids with p – sulphonatocalix[4]arenes in aqueous solution at 25^0 C.*

Amino acids	***Log K***	***-ΔH (kJ/mole)***	***TΔS (kJ/mole)***
L-His	1,8 ± 0,02 1,3[183]	20,1 ± 0,3 15[183]	- 28,2 ± 2,4 - 26[183]
L-Pro	3,5 ± 0,1 3,1[184]	36,2 ± 0,2 30[184]	- 28,5 ± 1,3 - 25[184]
L-Tyr	2,9 ± 0,05 2,3 [185]	31 ± 2,1 27[185]	- 26,5 ± 1,4 - 25[185]
L-Ser	1,9 ± 0,1 2,08[184]	22,5 ± 1,3 25,4[184]	- 28,7 ± 1,5 - 24[184]
L-Asp	3,3 ± 0,07 2,7[184]	30,5 ± 1,4 30,1[184]	- 25,7 ± 1,2 24[184]
L-Ile	3,07 ± 0,04	46,3 ± 1,2	- 32,5 ± 1,6
L-Lys	3,2 ± 0,02 3,08[185]	33,7 ± 1,2 30,9[185]	- 28,4 ± 2,1 - 35,6[185]
L-Arg	3,01 ± 0,02 3,19[184]	31,6 ± 2,1 30[184]	- 29,6 ± 2,1 - 28[184]

$C_{L\text{-}His} = 1{,}1 \times 10^{-3}$M ; $C_{L\text{-}Pro} = 1{,}2 \times 10^{-3}$M ; $C_{L\text{-}Tyr} = 1{,}5 \times 10^{-3}$M ; $C_{L\text{-}Ser} = 1{,}1 \times 10^{-3}$M ; $C_{L\text{-}Ser} = 1{,}8 \times 10^{-3}$M ; $C_{L\text{-}Asp} = 1{,}3 \times 10^{-3}$M ; $C_{L\text{-}Ile} = 2{,}0 \times 10^{-3}$M ; $C_{L\text{-}Lys} = 1{,}6 \times 10^{-3}$M ; $C_{L\text{-}Arg} = 1{,}4 \times 10^{-3}$M ; $C_{L\text{-}His} = 1{,}1 \times 10^{-3}$M ; $C_{calix4} = 1{,}5 \times 10^{-2}$M.

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4.2.3.2 Determination of the stability constant and thermodynamic parameters of the dipeptides complexes with p-sulphonatocalix [4]arene in aqueous solution

The study was done in aqueous solution without buffer solution. From the results that were obtained one can notice that it is the case of a complexation favoured by enthalpic

contributions. The results obtained are compared with those obtained in the literature by other techniques like the NMR technique. Also no significant differences can be noticed between the values of the stability constants for the complexes studied.

4.2.3.3 Determination of the stability constant and thermodynamic parameters of the dipeptides complexes with p-sulphonatocalix[6]arene in aqueous solution

In the case of *p*-sulphonatocalix[6]arene, due to its conformational possibilities, there is the possibility of forming both inclusion and exclusion complexes. The values of the reaction enthalpy are big and they suggest the formation of a complex but more studies are required using other techniques in order to establish exactly the type of interactions present in the formation of the complex. In conclusion, p–sulphonatocalix[6]arene forms complexes with dipeptides whose stability constant values are between log K = 3.00 (L-Leu-L-Ala) and log K = 3.36 (Gly-L-Ala), but the receptor does not present selectivity for the dipeptides studied.

4.2.3.4 Determination of the stability constant and thermodynamic parameters of some nucleobase complexes with p-sulphonatocalix[4]arene in aqueous solution

By using calorimetric titration was studied the possibility of complex formation between *p*-sulphonatocalix[4]arene and a few nucleobases : guanine, cytosine and adenine (Figure 4.2). Thus, an aqueous solution of *p*-sulphonatocalix[4]arene (2.0×10^{-2}M) was added continuously to the nucleobasis solution (1.5×10^{-3} M). The procedure was the one described at 4.2.2.

Table 4.2. *The stability constants log K (K in M^{-1}) and the values of the thermodynamic parameters ΔH and $T\Delta S$ for the complexes of some nucleobases with p – sulphonatocalix[4]arenes in aqueous solution at 25^0 C.*

Nucleobases	***Log K***	***-ΔH (kJ/mole)***	***TΔS (kJ/mole)***
Adenine	2.2 ± 0.02	22.1 ± 0.1	- 15.2 ± 2.4
Cytosine	1.5 ± 0.1	18.2 ± 0.2	- 11.5 ± 1.3
Guanine	2.1 ± 0.03	20.1 ± 0.2	- 10.2 ± 1.6

$C_{calix4} = 2{,}0 \times 10^{-2}$M ; $C_{nucleobases} = 1{,}5 \times 10^{-3}$M.

4.3 Conclusions

From the results presented one can notice that *p*-sulphonatocalix[4]arene forms complexes with the amino acids studied and the complexation is favoured by enthalpic contributions and disadvantaged by entropic contributions. The values of the stability constants and of the thermodynamic parameters studied for the complexes formed between *p*-sulphonatocalix[4]arene and dipeptides have large values suggesting the formation of the complex favoured by enthalpic contributions. The results obtained are compared to those obtained in the literature through other techniques, in this case with the NMR technique. Also, no significant differences are noticed between the values of the stability constants for the complexes studied. From the studies in solid state for the host-guest complex formed by *p*-sulphonatocalix[4]arene with histidine it was noticed that the responsibility of complex formation is given by the hydrogen bonds (N-H…O) formed between the amine group of the amino acid and the SO_3^- groups of the calixarene [186].

In conclusion, p–sulphonatocalix[6]arene forms complexes with the dipeptides whose stability constant values range between log K = 3.00 (L-Leu-L-Ala) and log K = 3.36 (Gly-L-Ala), but the receptor does not present selectivity for the dipeptides studied. So, there is the possibility to form both inclusion and exclusion complexes. The values of the stability constants obtained in the case of the nucleobases study have small values ranging between log K = 1.1 and log K = 2.2 for cytosine and adenine respectively.

Chapter 5. Study of complexation of some amino acids and dipeptides with α-Cyclodextrin

5.1 General considerations

5.2 Study of the amino acids and dipeptides complexes with α-cyclodextrin by calorimetric method

5.2.1. Apparatus and Reagents

5.2.2. Procedure

5.2.3. Results and discussion

5.2.3.1. Determination of the stability constant and thermodynamic parameters of the complexes of some amino acids and dipeptides with α-cyclodextrin

All the amino acids and the peptides studied form the complexes with α-cyclodextrin**.** The complexation is characterized by small values of the enthalpy reaction and the contribution of the entropy reaction. Dipeptides complexation with α-cyclodextrin is favoured by the entropy reaction**.** As in the case of amino acids, removing of the two water molecules from the cavity of α-cyclodextrin influence the entropy reaction [189]**.** The values of the entropy reaction are near zero.

5.3 Conclusions

As a conclusion, complexation reaction of cyclodextrins in aqueous solution are characterised by small values of the enthalpy reaction, and for certain components nearly equal to the values of the entropy reaction. One can mention that the complexation reaction of some amino acids and dipeptides with α-cyclodextrin is favourised by the enthalpic contributions as well as entropic contributions. In the formation of the complexes are involved hydrophobic interactions.

Chapter 6. Termodynamic aspects of cucurbit[n]uril, n = 6,7 complexation with some dipeptides and nucleobases by colorimetric method, UV-Vis and NMR

6.1 General considerations

In recent years the family of synthetic receptors named cucurbit[n]uril with n = 5-11 is becoming more involved in chemical and biological processes because of their affinity to form complexes with organic compounds, inorganic, and biological species. As it was mentioned in the literature part, these receptors have hydrophobic cavities of different size that allows binding various guest compounds. They have different solubilities in water and organic solvents.

In this chapter will be presented some aspects concerning the complexation possibilities of cucurbit[6]uril and cucurbit[7]uril with dipeptides and nucleobases using calorimetric method and nuclear magnetic resonance.

6.2 Study of cucurbit[6]uril (CB[6]) complexes with dipeptides and nucleobases by calorimetric method

6.2.1. Apparatus and Reagents

Reagents used

Dipeptides and nucleobases used in experiments: glycyl-leucine, glycyl-valine, glycyl-tryptophan, glycyl-alanine, glycyl-histidine, adenine, cytosine, guanine and uracil were obtained from Fluka and were of analytical grade. It was used distilled water (Millipore). The cucurbit[6]uril receptor (Figure 6.2) has been synthesized and purified in lab. Duisburg-Essen University. Complexation of dipeptides with CB[6] were studied in aqueous acidic solution (formic acid 50%) at 25^0C because of CB[6] low solubility in water.

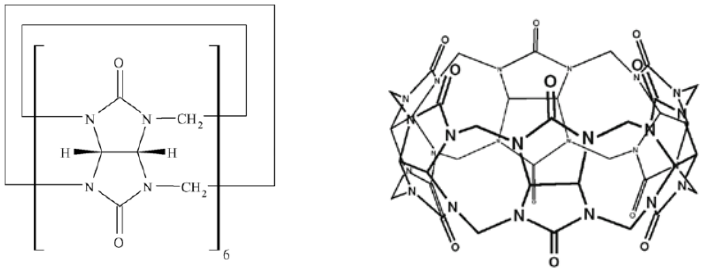


Fig. 6.2 *Cucurbit[6]uril* (*CB[6]).*

6.2.2 Procedure

6.2.3 Results and discussion

6.2.3.1 Determination of the stability constant and thermodynamic parameters of cucurbit[6]uril with dipeptide complexes in aqueous acidic solution

Note that the stability constant values are almost identical between them and they are ranging from log K = 2,64 (Gly-His) to log K = 2,90 (Gly-Trp). It is assumed that the interaction involved in the formed complexes of cucurbit[6]uril, with the studied dipeptides, are dipole ion interactions between the amino protonate group of the dipeptide and the carbonyl group of cucurbit[6]uril. Also it can be observed that the structure of different peptides examined do not have influence on the value of the stability constant. Even the reaction entropy has close values for all the complexes studied. These results can be explained by forming exclusion complexes.

6.2.3.2 Determination of the stability constant and thermodynamic parameters of cucurbit[6]uril complexes with nucleobases in aqueous acidic solution

In Tabel 6.2. are presented the constant stability values and thermodynamic parameters, enthalpy and entropy reaction which characterize the complexes formed between CB[6] and

some nucleobases in acidic aqueous solution. The results demonstrate that CB[6] forms complexes with the studied nucleobases.

Tabel 6.2. *The stability constants log K (K în M^{-1}) and thermodynamic parameters ΔH and TΔS for some nucleobase complexes with CB[6] in aqueous acidic solution (formic acid 50%) at 25^0C.*

Nucleobases	***Log K (M^{-1})***	***-ΔH (kJ/mol)***	***TΔS (kJ/mol)***
Adenine	4.30 ± 0,1	2.88 ± 0,2	21.8 ± 0,9
Guanine	3.30 ± 0.05	2.32 ± 0.6	20.4 ± 1.2
Cytosine	2.65 ± 0.06	3.2 ± 0.4	11.9 ± 1.0
Uracil	2.64 ± 0.01	2.57 ± 0.1	12.5 ± 0.8

$C_{CB[6]} = 5.0 \times 10^{-2}M$; $C_{adenine} = 3.0 \times 10^{-3}M$; $C_{guanine} = 2.7 \times 10^{-3}M$; $C_{uracil} = 2.8 \times 10^{-3}M$.

The stability constants are between log K = 2,64 for uracil and log K = 4,30 for adenine. In case of the studied nucleobases one can be observed small values of the reaction enthalpy and the complexation is characterized by enthalpic contributions and entropic contributions.

6.3 Study of cucurbit[7]uril (CB[7]) complexation with some nucleobases by NMR

The experiments have continued with the study of possibilities of cucurbit[7]uril and nucleobases complexation. The cucurbit[7]uril compared to cucurbit[6]uril is water soluble, which confers to it interesting applications in the study of the biological relevant compounds. Cucurbit[7]uril shows a moderate solubility in water, of 2×10^{-2} mol/L, which is similar with β-cyclodextrin, 1.6×10^{-2} mol/L [41]. So, the results obtained by NMR studies concerning the possibility of CB[7] to form complexes with adenine and cytosine are presented. Because of its solubility, guanine has not been yet studied.

6.3.1 Apparatus

To obtain ^{1}H- RMN spectra it has been used the spectrometer Varian Inova 400 (Institute of Organic Chemistry "C.D. Nenițescu", Romanian Academy). The samples have been studied in NMR tubes of 5 mm (Norell 507).

6.3.2 Procedure

1 mg of sample (adenine or cytosine) was dissolved in 650µL D_2O. The samples were sonicated for 5 minutes, for degassing and mixing.

6.3.3 Results and Discussion

In Figure 6.4 is presented ^{1}H- RMN spectrum of cucurbit[7]uril in D_2O (6 mg CB[7] in 650 µL D_2O) and in Figure 6.5, ^{1}H- RMN spectrum of adenine in the presence of CB[7] with different concentrations. From ^{1}H- RMN spectrum of adenine presented in Fig. 6.5 in the absence and in the presence of increasing concentrations of CB[7] it signals the presence of two protons H_x and H_y of adenine. They have a chemical shift with increasing the amount of CB [7] from base to top of the spectrum. This suggests the possibility of forming an inclusion complex that means the adenine can be included in the cavity of CB[7] receptor. This obvious aspect must be correlated with other techniques to demonstrate the complex formation. The

experiments for studying the possible formation of a complex between CB [7] and cytosine have continued. ^{1}H-NMR spectrum of cytosine in the absence and in presence of increasing amounts of CB[7] is shown in Figure 6.6. Also in the cytosine spectrum case, the absence and the presence of increasing amounts of CB [7] indicates the presence of two protons Hx and Hy of cytosine. They have a chemical shift in the same time with the increasing amount of CB[7] from base to top of the spectrum. This suggests the possibility of forming an inclusion complex which means that cytosine could be included in the cavity of CB[7] receptor.

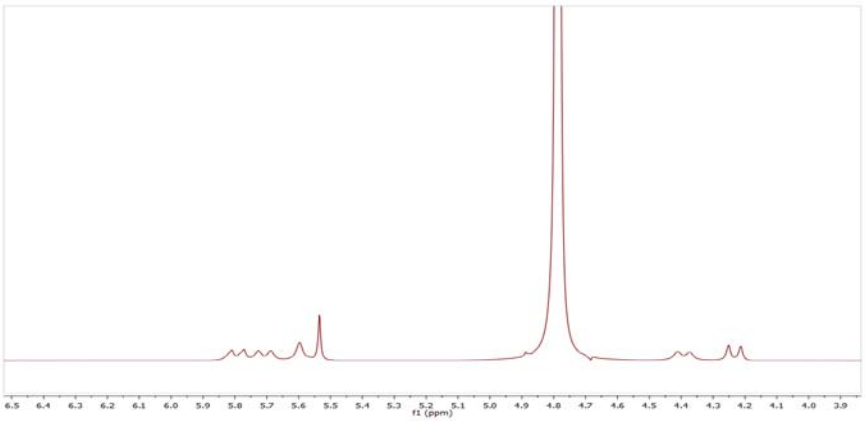
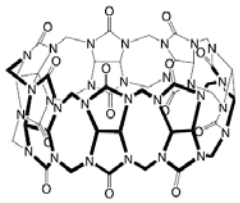


Fig.6.4 *^{1}H-NMR spectrum of cucurbit[7]uril in D_2O*



adenine CB[7]

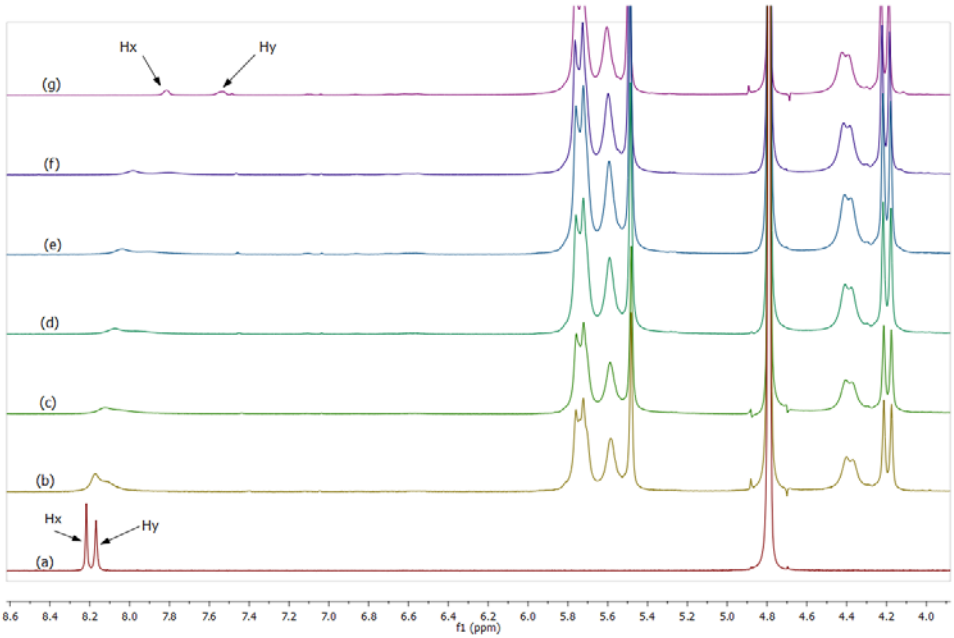
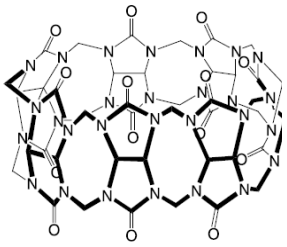


Fig.6.5 *^{1}H-NMR spectrum of adenine in the absence (a) and in the presence of 0.2 echiv. (b), 0.4 echiv (c), 0.6 echiv (d), 0.8 echiv (e), 1.0 echiv (f) and 2.0 echiv (g) CB[7] in D_2O.*



cytosine CB[7]

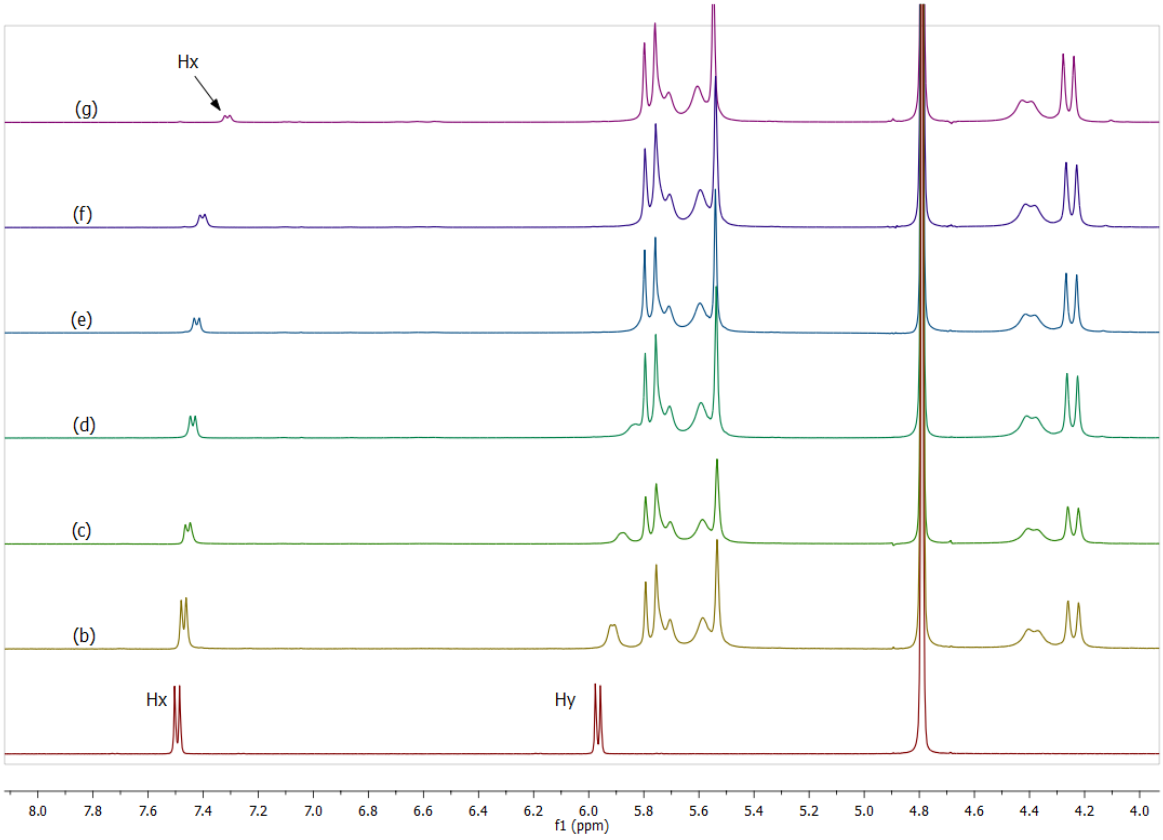


Fig.6.6 *^{1}H-NMR spectrum of cytosine in the absence (a) and in the presence of 0.2 echiv. (b), 0.4 echiv (c), 0.6 echiv (d), 0.8 echiv (e), 1.0 echiv (f) and 2.0 echiv (g) CB[7] in D_2O.*

6.4 The UV-Vis study of nucleobase complexes with CB[7]

Based on the results obtained by ^{1}H-NMR studies regarding the possible formation of inclusion or exclusion complexes between CB[7] and adenine or cytosine has been studied the spectrophotometrical behaviour of nucleobases in the presence of cucurbit [7]uril.

6.4.1 Apparatus

The absorbances measurement carried out by UV-Vis spectrophotometer Jasco V-530 and for measuring pH of solutions has been used a pH meter Pracitronic MV-870 with glass electrode and calomel electrode.

6.4.2 Procedure

Necessary quantities of adenine, cytosine and CB[7] were weighed in order to prepare the solutions. In this matter were prepared aqueous solutions of CB[7] with increasing concentrations ranging from 2.0 x 10^{-4} M - 7.0 x 10^{-4} M, solution of adenine 1.0 x 10^{-4} M, cytosine aqueous concentration 1.0 x 10^{-3} M. Adenine absorbs in the ultraviolet domain at λ = 256 nm and cytosine at λ = 265 nm. CB [7] does not absorb in the ultraviolet domain.

6.4.3 Results and discussion

In Figure 6.7 is presented UV-Vis spectrum of adenine in the absence and presence of various increasing concentrations of CB[7]. From UV-Vis spectra it can be observed a decreasing of adenine absorption related to increasing amount of CB[7] (2.0 x 10^{-4} - 7.0 x 10^{-4} M). From the graphical representation of the absorbance versus concentration of cucurbit[7]uril is obtained a straight line. Correlating the results obtained by UV-Vis spectrometry with those obtained by NMR measurements could be suggested that it is possible the formation an inclusion complex of CB [7] with adenine.

The same experiment was performed for cytosine too. Thus in Figure 6.8 is presented the cytosine UV-Vis spectrum in the absence and presence of various increasing concentrations of CB[7] (2.0 x 10^{-4} – 9.0 x 10^{-4} M). As for adenine from UV-Vis spectra it can be observed a decreasing of cytosine absorption based on the increasing amount of CB [7]. However there are some differences. So, as it is increased the concentration of CB [7] over 6.0 x 10^{-4}M one can observed the displacement of cytosine maximum absorption from the originally value of 265 nm to 271.5 nm. Thus, in the case of cytosine the decreasing of absorption in the presence of CB [7] is linear in the concentration range 2.0 x 10^{-4}- 5.0 x 10^{-4}M and over this value of receptor concentration, probably is formed other complexes.

Correlating the results obtained by UV-Vis spectrometry with those obtained by NMR measurements one can be suggested that it is possible to form an inclusion complex of CB[7] with cytosine only in according to the adequate receptor concentration.

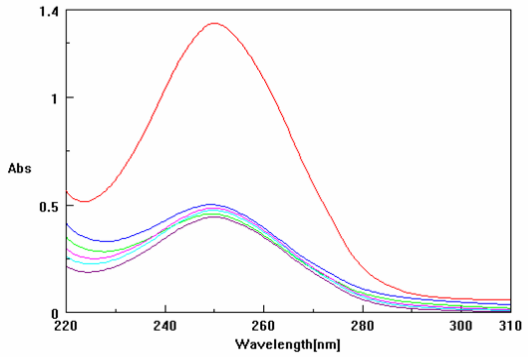


Fig. 6.7 *UV-Vis spectrum of adenine in aqueous solution in the presence of increasing amounts of CB [7], λ = 256 nm and graphical absorbance depending on the concentration.*

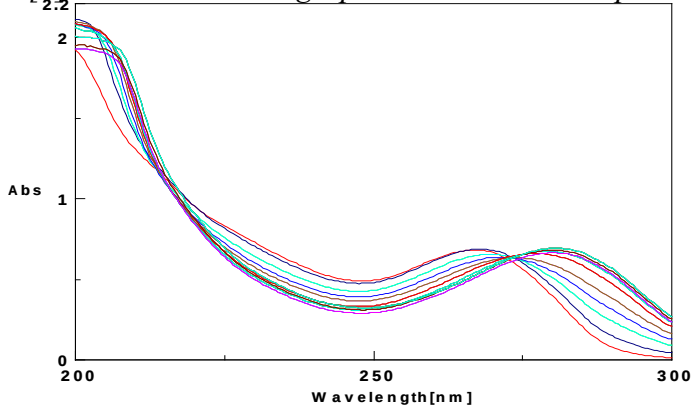


Fig. 6.8 *UV-Vis spectrum of cytosine in aqueous solution in the presence of increasing amounts of CB [7], λ = 265 nm.*

6.5 Conclusions

The values of stability constants for complexation of CB[6] with dipeptides are almost identical to each other and are between log K = 2.64 (Gly-His) and log K = 2.90 (Gly-Trp). The interactions involved in the formation of cucurbit[6]uril with dipeptide complexes suggest that are ion dipole interactions between amino protonated group of dipeptides and carbonyl group of cucurbit[6]uril. The experimental results prove that the structure of different dipeptides do not influence the values of the stability constant. Even the reaction entropy has similar values for all studied complexes. These results can be explained by the formation of exclusion complexes. In case of the nucleobases complexation with CB[6] one can observed low values of enthalpy reaction and the complexation is characterized by enthalpy contributions as well as entropic contributions.

From ^{1}H-NMR spectrum of adenine, in the absence and presence of increasing amounts of CB [7], was reported chemical shift of the two protons Hx and Hy of adenine along with the increasing amount of CB [7] from base to top spectrum. This suggests the possibility of forming an inclusion complex meaning that adenine could be included in the cavity of receptor CB [7].

Correlating NMR results with those obtained by UV-Vis spectrometry it can be observed that it is possible to form an inclusion complex of CB[7] with adenine. Like adenine, the same results were observed by using cytosine concerning the decreasing absorption of cytosine by increasing the amount of CB [7]. There are some differences suggesting the possibility of forming other types of complexes too. Thus the results obtained by UV-Vis spectrometry with those obtained by NMR investigations one can be suggested that it is possible to form an inclusion complex of CB [7] with cytosine only in according to the adequate receptor concentration.

II. Aspects concerning the separation possibilities of amino acids in native and derivatives form by using functionalized β-cyclodextrin

Chaptor 7. Extraction and transport of native and methylesters amino acids by using functionalized β-cyclodextrin

In this chapter have been studied the possibilities of using functionalized β-cyclodextrin, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin (Figure 7.1) in extraction procedure of amino acids from the aqueous phase into the organic phase and as a carrier through liquid membrane aiming the separation of some native and methylesters aromatic amino acids [190, 191].

7.1. General considerations

7.2 Extraction of native and methylesters amino acids by using functionalized β-cyclodextrin

7.2.1 Distribution equilibrium in liquid-liquid extraction

It is well known that depending on the pH, amino acids can be transferred in aqueous solution in protonated form according to the equilibrium:

$$R-NH_2 + H_3O^+ \Leftrightarrow R-NH_3^+ + HOH \qquad (7.1)$$

characterized by the acidity constant K_a,

$$K_a = \frac{[R-NH_2][H_3O^+]}{[R-NH_3^+]} \quad (7.2)$$

This cationic form of the amino acid exists in solution at a pH depending on the constant value K_a and it can be complexed by the neutral macrocyclic receptors:

$$R-NH_3^+ + L \Leftrightarrow R-NH_3^+L \quad (7.3)$$

characterized by the stability constant β_1:

$$\beta_1 = \frac{[R-NH_3^+L]}{[R-NH_3^+][L]} \quad (7.4)$$

where:

$[R-NH_3^+L]$ - represents the concentration of the complex after extraction (mol/L);

$[R-NH_3^+]$ - represents the amino acid concentration in the aqueous phase before extraction (mol/L);

$[L]$ - represents ligand concentration in the organic phase (mol/L).

The cationic complex formed can be extracted from water into an organic solvent as an ion pair if the anion A^- will be compatible with the size and structure with the ligand. In this case the formed complex will be $(R-NH_3^+LA^-)$ in according to equilibrium:

$$(R-NH_3^+A^-)_w + (L)_{org} \Leftrightarrow (R-NH_3^+LA^-)_{org} \quad (7.5)$$

with the extraction constant K_{ex}:

$$K_{ex} = \frac{[R-NH_3^+LA^-]_{org}}{[R-NH_3^+A^-]_w[L]_{org}} \quad (7.6)$$

In which:

w – aqueous phase

org – organic phase

$[R-NH_3^+LA^-]$ - represents the complex concentration (mol/L);

$[R-NH_3^+A^-]$ - represents the amino acid concentration as a ion pair form;

$[L]$ - ligand concentration (mol/L);

The anion A^- can be conjugated base of an organic acid HA and is formed in solution on the base of equilibrium:

$$HA + H_2O \Leftrightarrow H_3O^+ + A^- \quad (7.7)$$

characterized by the acidity constant $K_a^{'}$:

$$K_a^{'} = \frac{[H_3O^+][A^-]}{[HA]} \quad (7.8)$$

The acidity constant $K_a^{'}$ determines the pH domain in which anion A^- predominate in solution.

In the baze of equilibrium, macrocyclic ligand L will be distribute between the two liquid phases of the system, characterized by the repartition constant K_L:

$$(L)_w \Leftrightarrow (L)_{org} \quad (7.9)$$

Repartition constant K_L:

$$K_L = \frac{[L]_{org}}{[L]_w} \quad (7.10)$$

So it can be determined the global equilibrium of the extraction of ion pair based on individual equilibrium presented in the above relations:

$$(R-NH_3^+)_w + (A^-)_w + (L)_{org} \Leftrightarrow (R-NH_3LA)_{org} \quad (7.11)$$

Having the global extraction constant K_{ex}:

$$K_{ex} = \frac{[R-NH_3LA]_w}{[R-NH_3^+]_w[A^-]_w[L]_{org}} \quad (7.12)$$

Because the values of constants involving charged species in organic solvent are very low, extracting significant equilibrium is defined as:

$$(RNH_3^+L)_w + (A^-)_w \Leftrightarrow (RNH_3LA)_{org} \quad (7.13)$$

with the extraction constant K'_{ex}:

$$K'_{ex} = \frac{[RNH_3LA]_{org}}{[RNH_3^+L]_w[A^-]_w} \quad (7.14)$$

Distribution ratio of amino acid in water-solvent system

The distribution ratio (D) of amino acid between the two phases (org) and (w) is defined as the ratio between amino acid concentration at equilibrium between organic phase and aqueous phase:

$$D = \frac{[R-NH_3LA]_{org}}{[R-NH_2]_w + [R-NH_3^+]_w} \quad (7.15)$$

Distribution ratio D defined above is in fact a measure of extractability and stability of the complex formed between ligand and amino acid in the organic phase.

7.2.2 Experimental conditions

7.2.2.1. Reagents used

All amino acids used (Fig. 7.1): L-phenylalanine, L-tryptophan, L-tyrosine, L-tryptophan methylester (L-TrpOMe), L-phenylalanine methylester (L-PheOMe) and L-tyrosine methylester (L-TyrOMe) were obtained from Fluka (purity > 99.5 %). The chiral receptor, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin was obtained from Ciclolab Company (Hungary) and used as received. Distilled water was used throughout the experiments (Millipore). The organic solvent chloroform (Merck, dielectric constant $\varepsilon_r = 4.81$) was saturated with distilled water before usage.

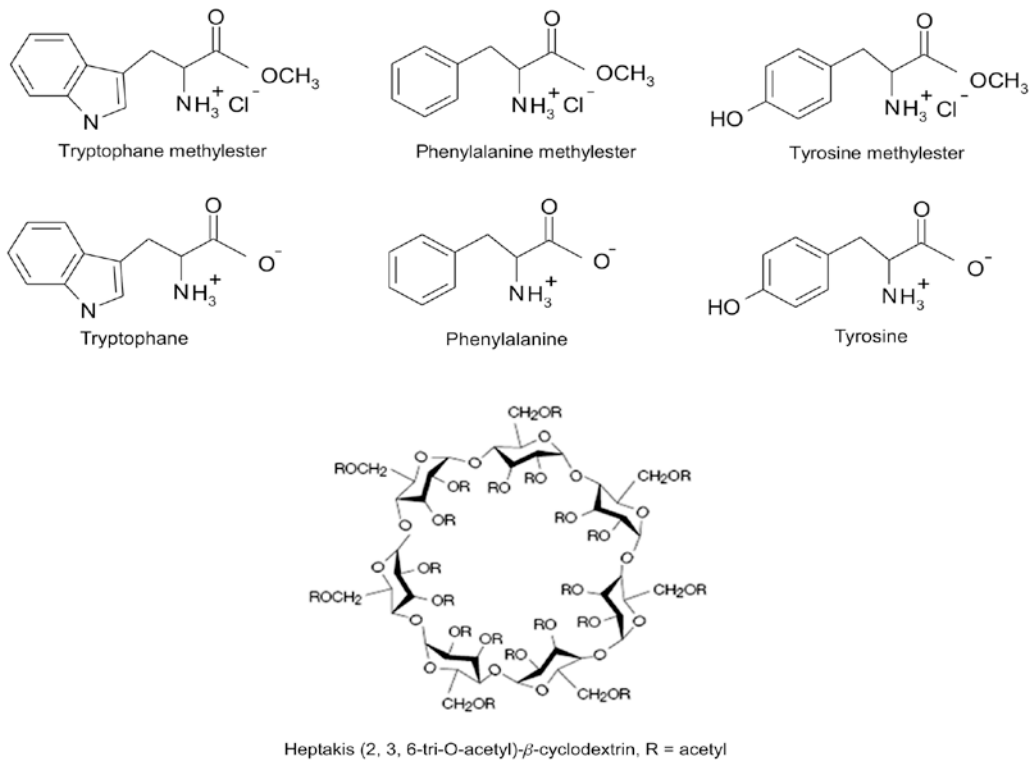


Fig. 7.1 *Amino acids and functionalized β-ciclodextrin used in experiments*

The spectrophotometric measurements were carried out using JASCO V-530 UV-Vis spectrophotometer. The pH was measured by digital MV-870 Pracitronic pH-meter with glass electrode and saturated calomel electrode.

7.2.2.2 Procedure

Liquid-liquid extraction

For liquid-liquid extraction of native and derivates amino acids were prepared stock solutions of amino acids (in saturated water with chloroform) of concentrations between 2.5 x 10^{-4} and 5.0 x 10^{-4}M and heptakis (2,3,6 -tri-O-acetyl)-β-cyclodextrin in chloroform water-saturated of concentration 1.0 x
10^{-3}M using flasks of 25 or 50 ml. The extraction of amino acids from the aqueous phase into the organic phase has been achieved in separating funnels.

Equal volumes (10 mL) of aqueous solution of amino acid native or methylester at pH = 5,5 (NaOAc/HAc) and solution of heptakis (2,3,6-tri-O-acetyl) - β-cyclodextrin in chloroform (10 mL) were mixed and shaken for 30 minutes at T = 25^0 C. The hydrochloric acid was used for pH adjusted of the aqueous solutions. Absorbances of amino acids were determined at λ = 278 nm (L-Trp and L-TrpOMe), λ = 258 nm (L-Phe and L-PheOMe) and λ = 274 nm (L-Tyr and L-TyrOMe).

The extraction efficiency was calculated according to Pedersen's procedure [192]:

$$E\% = \frac{A_0 - A}{A_0} \times 100 \qquad (7.20)$$

where:

A_0 - absorbance of the aqueous phase before extraction;

A - absorbance of the aqueous phase after extraction;

or using solutions concentrations, the above expression can be rewritten:

$$E\% = [(C_0 - C)/C_0] \times 100 \quad (7.21)$$

where:

C_0 and C are initial and final concentrations before and after extraction. Each experiment was run 5 times.

7.3 Results and discussion

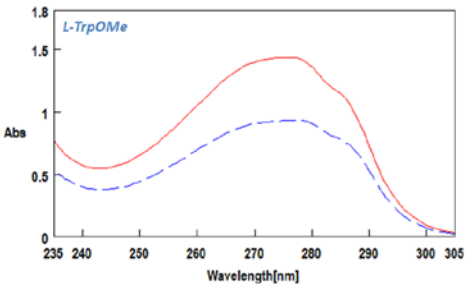
7.3.1 Extraction of native amino acids: L-Trp, L-Phe and L-Tyr

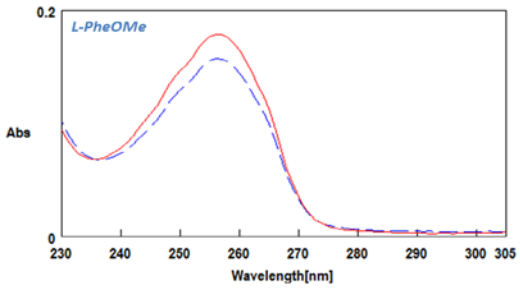
Experimental results showed that the optimum pH of the amino acid solution in extraction is 5.5. It was also performed the optimal stirring time for mixing phases for all amino acids included in the study (30 minutes). L-tryptophan, the essential amino acid in the structure of protein has an indole group that absorbs in the ultraviolet domain, $\lambda \approx 278$ nm, being the highest hidrofobic amino acid. As mentioned above, native and derivatives aromatic amino acids, L-Trp, L-TrpOMe, L-Phe, L-PheOMe, L-Tyr, L-TyrOMe were extracted from the aqueous phase at a pH ≈ 5,5 (NaOAc/HAc) in an organic phase using as extractant chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin with 1.0 x 10^{-3}M concentration in chloroform. The procedure is similar to that described in subchapter 7.2.2.2. Extraction percentage was calculated according to relation (7.20), subchapter 7.2.2.2. Absorbances of the aqueous phase before and after extraction were performed spectrophotometrically. Percentage extraction values obtained for L-Trp, L-Phe and L-Tyr were 17.5%, 15.3% and 8%. In this case, the extractant, chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin, did not show a high affinity for these amino acids.

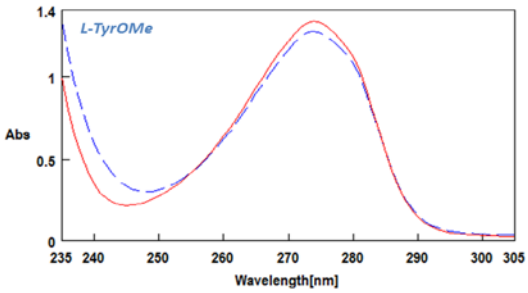
7.3.2 Extraction of methylesters amino acids: L-TrpOMe, L- PheOMe and L-TyrOMe

The extraction of methylesters amino acids: L-TrpOMe, L- PheOMe and L- TyrOMe with chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin was performed in according to subchapter 7.2.2.2. The values of extraction yields calculated according to relation (7.20) for L-TrpOMe, L-PheOMe and L-TyrOMe were the following: 50.2%, 35.1% and 17%. Unlike values of extraction yields obtained for native amino acids, in this case the values are much higher. So, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin exhibits a higher affinity towards methylesters amino acids.

UV-Vis spectra obtained after extraction of methylesters amino acids by functionalized β-cyclodextrin are shown in Figure 7.6.







(_____) UV-Vis spectrum of amino acids before the extraction

(__ __) UV-Vis spectrum of amino acids after the extraction

(L-TrpOMe λ=278 nm; L-PheOMe λ=258 nm; L-TyrOMe λ=274 nm)

Fig. 7.6. *UV-Vis spectra obtained during the extraction of methyl amino functionalized with β-cyclodextrin*

The results obtained for extraction of native aromatic amino acids and methylesters with chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin are shown in Table 7.1. The graphical representation of the extraction of native and methylesters aromatic amino acids from the aqueous phase in chloroform with modified cyclodextrin is shown in Figure 7.7.

Table 7.1 *Extraction yields of native and methylesters aromatic amino acids with chiral heptakis (2,3,6-tri-O-acetyl) - β-cyclodextrin*

Amino acid	***Extraction yields***	***RSD (%)***
L-TrpOMe 2,5 x 10^{-4} M	50,2	2,5
L-Trp 5 x 10^{-4} M	17,5	1,3
L-PheOMe 2,5 x 10^{-4} M	35,1	2,1
L-Phe 5 x 10^{-4} M	15,3	1,2
L-TyrOMe 2,5 x 10^{-4} M	17,0	1,3
L-Tyr 5 x 10^{-4} M	8,0	0,9

The extractability yields as can be seen from Table 7.1 show the following sequence of aromatic amino acid methylesters: L-TrpOMe (50.2 %) > L-PheOMe (35.1 %) > L-TyrOMe (17.0 %). In the case of native aromatic amino acids, the yields of extractability are significantly lower than amino acid methylesters ranging from 8 % (L-Tyr) to 17.5 % (L-Trp) with the following sequence: L-Trp > L-Phe > L-Tyr. The same issue can be seen in the case of native and methylesters aromatic D-amino acid extraction by using as extractant chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin (Fig.7.8).

In Table 7.2 are presented the concentrations of native aromatic amino acids and methyl in L and D form as extracted with the functionalized β-cyclodextrin [191].

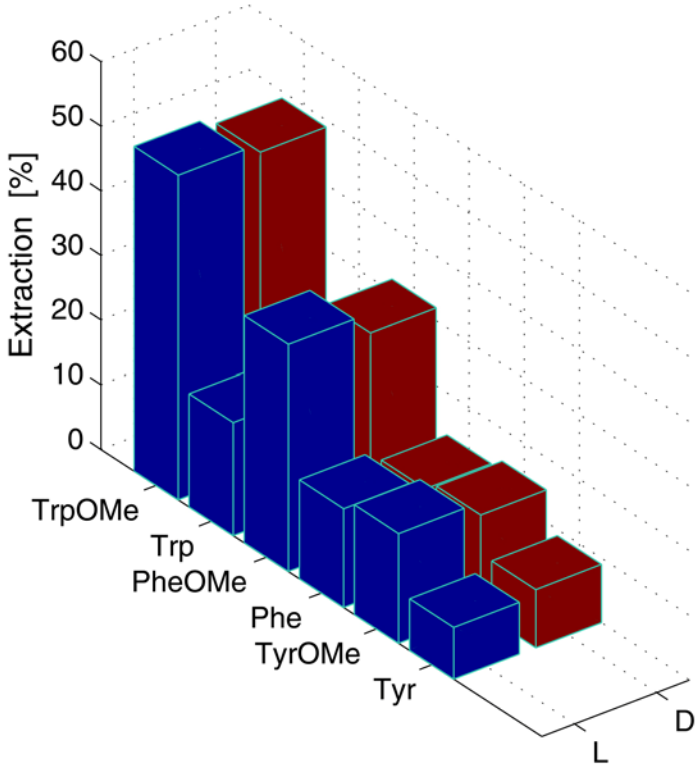


Fig.7.8 *Graphical representation of extraction (%) of L-, and D-amino acid native and methylester from aqueous phase in chloroform with β-cyclodextrin functionalized [191].*

Tabel 7.2 *The values of molar concentration and extraction yields for amino acids studied with β-CD functionalized*

Amino acid	*The initial concentration* $Mx10^{-4}$	*Concentration L-amino acid extracted* $Mx10^{-4}$	η_L *(%)*	*Concentration D-amino acid extracted* $Mx10^{-4}$	η_D *(%)*
TrpOMe	2,5	1,26	50,2	1,22	48,9
Trp	5,0	0,88	17,5	0,48	9,5
PheOMe	2,5	0,88	35,1	0,80	32
Phe	5,0	0,77	15,3	0,68	13,5
TyrOMe	2,5	0,43	17	0,37	15
Tyr	5,0	0,40	8	0,45	9

7.3.3 Relationship between aromatic amino acid extractability and their hydrophobicity

In Figure 7.9 is presented the relationship between the percentage extraction of aromatic amino acids studied and their hydrophobicity. It can be observed that there is a correlation between them, amino acids with higher hidrofobicity are extracted with higher yields. Thus L-TrpOMe (-1.16) > L-PheOMe (-1.45) > L-TyrOMe (- 2.11).

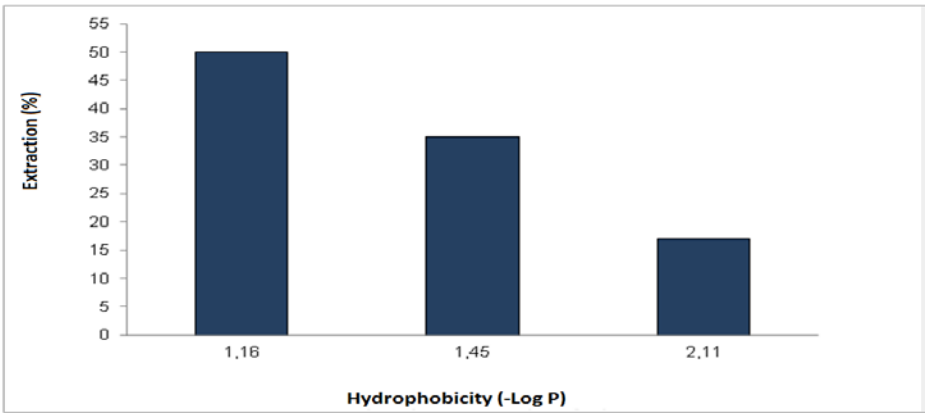


Fig. 7.9 *The relationship between extractability and hydrophobicity of amino acids (-LogP[193]), L-TrpOMe (-1,16); L-PheOMe (-1,45); L-TyrOMe (-2,11).*

7.3.4. Determination of amino acids extraction constants by using functionalized β-cyclodextrin

7.3.4.1. Determination of extinction coefficient ε

7.3.4.2. Determination of extraction constants K_{ex}

To determine the extraction constants, the extractions were performed at pH = 5,5 (NaOAc/HAc) maintaining constant the concentration of amino acid and varying the concentration of functionalized β-cyclodextrin. Measuring absorption and knowing the extinction coefficient, was calculated RNH_3L concentration in the organic phase. In these conditions was possible to calculate the concentrations of species involved at equilibrium , aspect which allowed the calculation of the extraction constants $K_{ex.}$

The complex formation of RNH_3L can be represented by the following relationship:

$$R-NH_3^+ + L \Leftrightarrow R-NH_3^+L \qquad (7.24)$$

where:

$R-NH_3^+$ - aromatic amino acid;

L - heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin;

$R-NH_3^+L$ - the complex formed after the extraction;

The extraction constant is represented by the equation:

$$K_{ex} = \frac{[R-NH_3^+L]_o}{[R-NH_3^+]_w[L]_o} \qquad (7.25)$$

where:

$[R-NH_3^+L]_o$ - the complex concentration after the extraction (mol/L);

$[R-NH_3^+]_w$ - amino acid concentration in the aqueous phase (mol/L);

$[L]_o$ - functionalized β-cyclodextrin concentration in the organic phase (mol/L);

For each amino acid, were performed three extraction constants for different concentrations of functionalized β-cyclodextrin. Extraction constant values obtained are presented in Table 7.3.

Tabelul 7.3. *The extraction constant of native aromatic amino acids and methylester with functionalized β-cyclodextrin*

Amino acid	*ε (L/mol·cm)*	*Log K_{ex}*
L-TrpOMe	5427	3,50 ± 0,06
L-Trp	3274	2,07± 0,10
L-PheOMe	4780	2,94 ± 0,08
L-Phe	2750	1,70 ± 0,09
L-TyrOMe	2573	1,50 ± 0,07
L-Tyr	2428	-

$C_{\text{amino acid}} = 3{,}0 \times 10^{-5} – 5{,}0 \times 10^{-4}$ M; $C_{\beta\text{-CD}} = 1.0 \times 10^{-2} – 1.0 \times 10^{-3}$ M (in chloroform); pH = 5,5 (NaOAc/HAc), T = 25° C.

Values of extraction constants of aromatic amino acid methylester (L-TrpOMe, L-PheOMe, L-TyrOMe) are higher than the native aromatic amino acids (L-Trp, L-Phe, L-Tyr) as shown in Table 7.3. It is also noted that extraction constant values depend on amino acid hydrophobicity. Thus, L-TrpOMe being the most hydrophobic from the amino acids studied, has the highest extraction constant value ($\text{Log } K_{ex} = 3{,}50$). For tyrosine, in methylate form, the extraction constant value ($\text{Log } K_{ex} = 1{,}50$) is small and for tyrosine, in native form, the extraction constant can not be calculated under the conditions studied. So, both extractant structure and amino acid structure influence the determination of extraction constants.

7.4 Study of native and methylesters amino acids transport through liquid membrane by using functionalized β-cyclodextrin

Compared with liquid-liquid extraction, liquid membranes as liquid membrane supported, liquid membrane emulsion, and bulk liquid membrane, offers many economic advantages such as: low loss of carrier, high membrane lifetime, continue operate in one direction (source phase→ receiving phase), compact equipment, applying different driving forces (chemical potential gradient, pressure or temperature between different compartments, electric fields etc.). The study of selectivity and transport of amino acid through liquid membranes is important for the separation and the concentration of amino acids and for the understanding of the transport process of amino acid through cell membrane. Development of supramolecular chemistry, synthesis of new macrocyclic carriers is a milestone in the development of facilitated transport of amino acids.

Chirality is a fundamental feature of all living organisms at both molecular and macroscopic scale. The extraordinary preference of the living organisms for only one of the two enantiomers, named homochirality is a complex and enigmatic phenomenon, yet fully incomprehensible. Macrocyclic receptors such as cyclodextrins, crown ethers and other organic compounds, show applicability in this case, acting as selectors for chiral recognition of important optically active compounds.

Most molecules involved in the structure of living systems are optically active molecules and biochemical processes often prefer an enantiomer or the other. Nature has this remarkable ability to distinguish between enantiomeric compounds. Understanding the molecular principles which stays at the base of the mechanisms which govern the chiral recognition is a top subject in scientific research [1]. It is

well known that natural products such as proteins, peptides, amino acids, hormones, nucleic acids, etc. are optically active compounds. For enantiomers it has been observed high differences in their biological action, especially for pharmaceutical products or food additives. Depending on their chirality, these compounds exhibit an activity or a specific functionality. The separation of enantiomers is an important aspect for the study of optically active compounds, especially in drug industry. By using chiral macrocyclic receptors this is a possible thing.

Obviously, there are many enantiomers separation techniques such as: gas and liquid chromatography, supercritical fluid chromatography or capillary electrophoresis. The advantage of liquid membranes is that it offers an opportunity to achieve the separation of enantiomers in solution, unlike other techniques that use immobilized chiral selectors, something that leads to lower chiral recognition.

In this section are presented the results of research on the transport through liquid membrane of some L-, and D-amino acids using as a chiral carrier, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin for the study of separation possibility of the two enantiomers.

7.4.1 Experimental conditions

7.4.1.1 Reagents used in transport process

All amino acids used (Figure 7.1): L-phenylalanine, L-tryptophan, L-tyrosine, L-tryptophan methylester (L-TrpOMe), L-phenylalanine methylester (L-PheOMe) and L-tyrosine methylester (L-TyrOMe) were obtained from Fluka (purity > 99.5 %). The chiral receptor, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin was obtained from Ciclolab Company (Hungary) and used as received. Distilled water was used throughout the experiments (Millipore). The organic solvent chloroform (Merck, dielectric constant $\varepsilon_r = 4.81$) was saturated with distilled water before usage.

7.4.1.2 Devices used in transport process

Devices used in transport experiments of native and methylester aromatic amino acids from an aqueous sourse phase into a receiving aqueous phase, using as carrier, receptor heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin in chloroform are shown in Figure 7.1. It is used a device consisting of two concentric tubes (Figure 7.10) and a U-shaped tube (Figure 7.11)

7.4.1.3 Procedure

The transport experiments were carried out using a device (Figure 7.10) consisting of two concentric tubes: the inner one contained the source phase, 5 mL of amino acid aqueous solution (5 x 10^{-4}M) which also acted as a stirrer, whereas the aqueous receiving phase, 5 mL (pH=1.5) together with the membrane phase, 35 mL of heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin (1 x 10^{-3} M) in chloroform, were introduced in the outer tube. The phases were stirred at 180 rpm for 24 h. Each experiment was repeated three time. Similar transport experiments were performed for reference in the absence of carrier.

The amino acid concentration from the two aqueous phases (source and receiving) was determined spectrophotometrically by means of UV-Vis Jasco V-530 spectrophotometer. Distilled water and chloroform were saturated with each other before the transport and the pH of the aqueous solution was measured with a pH meter Pracitronic MV-870 with glass electrode and saturated electrode of calomel and was adjusted with HCl solution.

Transport efficiency was calculated by the following equation:

$$T\% = \frac{A_r}{A_0} x100 \qquad (7.26)$$

where:

A_r - the absorbance of the aqueous receiving phase, after transport;

A_0 - the absorbance of the aqueous source phase, before transport.

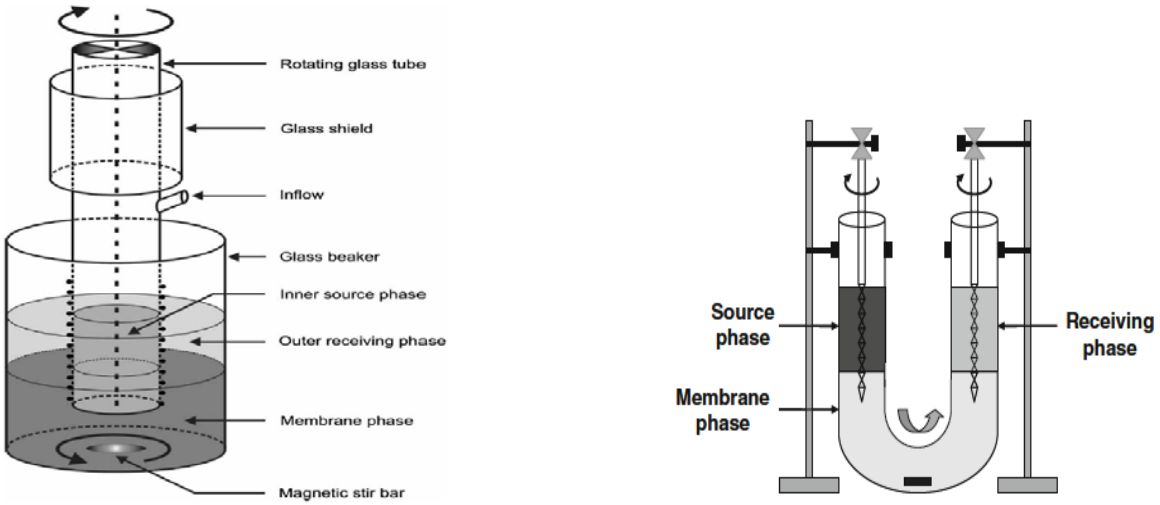


Fig.7.10 *Devices used for transport through membrane*

7.4.2 Results and discussions

7.4.2.1 Transport of aromatic L-amino acids in native and methylesters form using functionalized β-cyclodextrin

7.4.2.1.1 Steps underlying transport through liquid membranes

There are three stages that characterize the transport through liquid membranes. In this case they are: amino acid extraction (complexation) at interface source phase- membrane phase, the transport of amino acid through membrane (diffusion) and decomplexation at the interface membrane phase - receiving phase.

1 Amino acid extraction from the aqueous phase (source) into the organic phase (membrane)

Amino acid in source phase through diffusion reaches at the interface source phase- membrane phase where it meets the receptor from membrane phase forming the complex: amino acid-receptor according to the following equilibrium:

$$(R-NH_3^+)_w + (L)_{org} \Leftrightarrow (R-NH_3L)_{org} \qquad (7.27)$$

with the extraction constant, K_{ex}:

$$K_{ex} = \frac{[R-NH_3L]_{org}}{[R-NH_3^+]_w \cdot [L]_{org}} \qquad (7.28)$$

2. Amino acid transport through liquid membrane

The amino acid existing in the liquid membrane in form of the complex receptor-amino acid, is transported by diffusion to the interface with the aqueous receiving phase according to the following equilibrium:

$$(R-NH_3L)_w \Leftrightarrow (R-NH_3L)_{org} \qquad (7.29)$$

3. Decomplexation of amino acid in the receiving phase.

At the *membrane-receiving phase* interface, the complex is dissolved due to the conditions created in the receiving phase (pH = 1.5) and thus the amino acid moves to the receiving phase and the receptor returns through difusion to the interface with the source phase:

$$(R-NH_3LA)_{org} \Leftrightarrow (R-NH_2)_w + (L)_{org} \qquad (7.30)$$

7.4.2.1.2 Transport of aromatic L-amino acids in native and methylesters form

UV-Vis spectra obtained after transport of amino acids methylesters with modified β-cyclodextrin are shown in Figure 7.12.

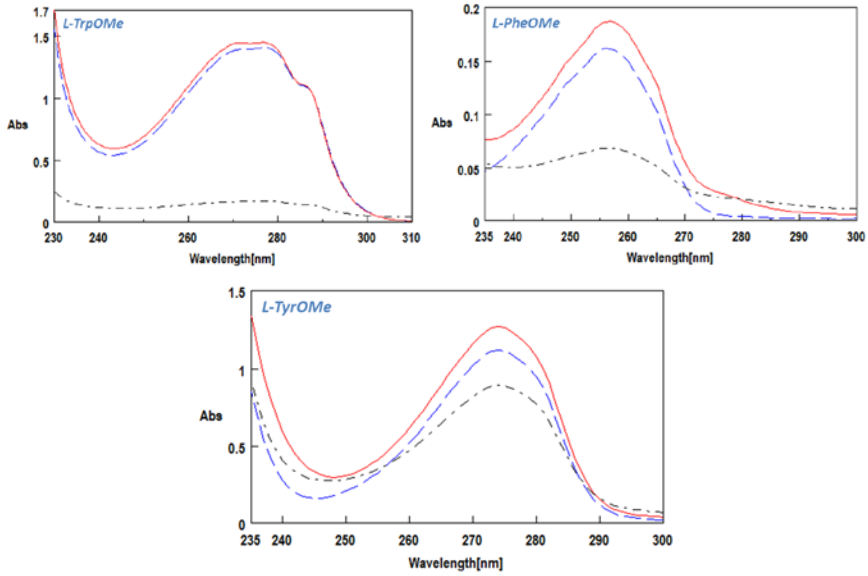


Fig. 7.12. *UV-Vis spectra obtained in the transport process of aromatic L-amino acid methylesters with β-cyclodextrin modified*

(_____) UV-Vis spectra of amino acids before the transport ;

(_ _ _ _ UV-Vis spectra of amino acids in receiving phase after the transport;

(_ _ _) UV-Vis spectra of amino acids in the source phase after the transport.

(L-TrpOMe λ=278 nm; L-PheOMe λ=258 nm; L-TyrOMe λ=274 nm)

The results obtained from transport of aromatic L-amino acid native and methylesters from an aqueous source phase into an aqueous receiving phase using as carrier, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin in chloroform are shown in Figure 7.13. As in amino acid extraction case, heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin as carrier through liquid membrane exhibits a higher affinity for L-TrpOMe. Hence higher transport efficiency for this amino acid. The transport sequence through liquid membrane is as follows: L-TrpOMe > L-PheOMe > L-TyrOMe with yields between 20% (L-TyrOMe) and 96 % (L-TrpOMe).

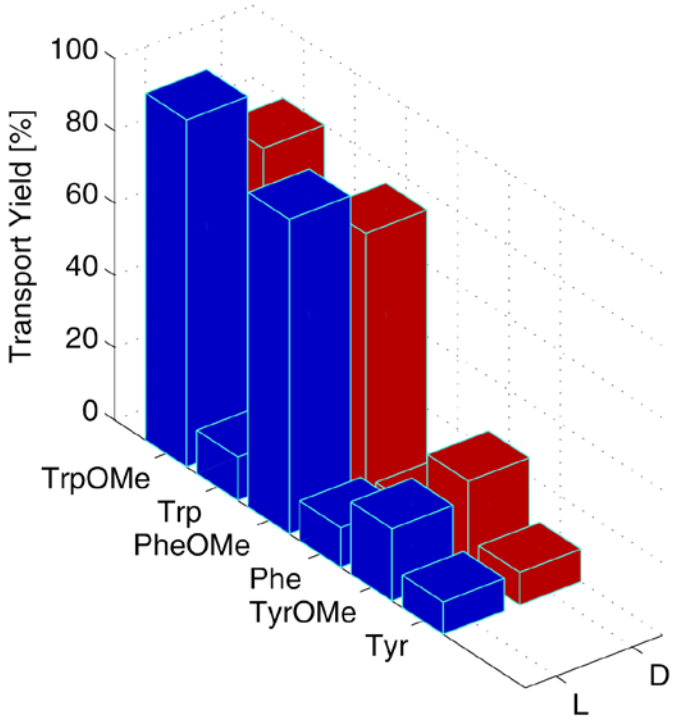


Fig. 7.13 *The transport yields of L- and D-amino acids by using chiral β-cyclodextrin functionalized, T = 25^0 C [191].*
Aqueous source phase (5 mL): $c_{L-,D\text{-amino acid}} = 5 \times 10^{-4}$ M; pH = 5,5 (NaOAc/HAc)
Membrane phase(35 mL): $c_{carrier} = 1 \times 10^{-3}$ M in chloroform;
Receiving aqueous phase (5 mL): distilled water (pH = 1,5; HCl 0,5N)

The quantity of transported amino acid from source phase to receiving phase using β-cyclodextrin functionalized as carrier has been determined by spectrophotometric measurements at the wavelength specific to each amino acid. The amino acid absorbances present in the source phase, membrane phase and receiving phase were determined. It is known that the functionalized β-cyclodextrin does not carry out absorbance in the ultraviolet domain where it absorbs the amino acid.

7.4.2.1.3 Relationship between the aromatic amino acids transport and their hydrophobicity

As in the case of the extraction, in the transport process it is also noted the ratio between the amino acids transport yields and their hydrophobicity (figure 7.14) so, the amino acids with higher hydrophobicity are better extracted compared with those of lower hydrophobicity .

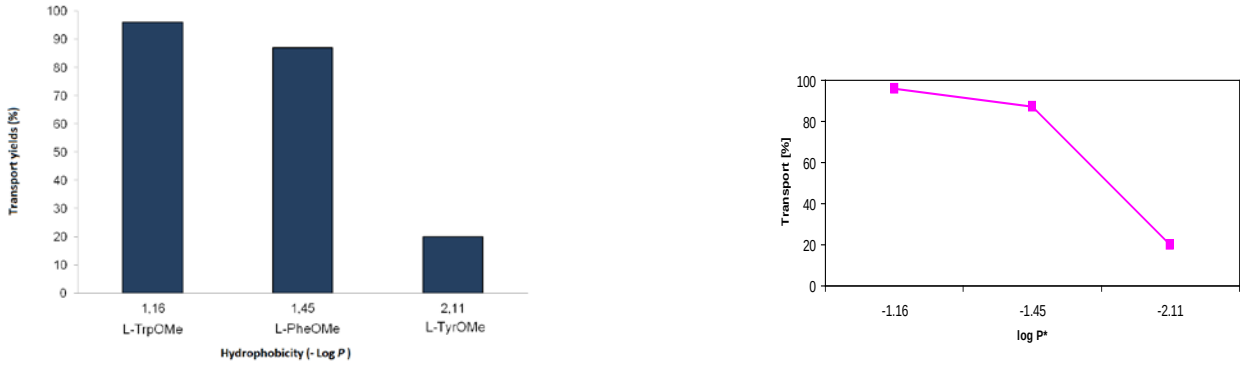


Fig.7.14 *Relationship between the transport yields of amino acids and their hydrophobicity (-Log P[191])*

7.4.2.2 Transport of aromatic D- amino acids in native and methylesters form by using functionalized β-cyclodextrin

Due to the results obtained in the transport experiments of L-aromatic amino acids native and methylesters through chloroform liquid membrane using chiral heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin as carrier, it was continued the experiments with the transport of D-aromatic amino acids through chloroform liquid membrane using chiral heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin to highlight the chiral properties of carrier. The experimental data of the transport displayed in Figure 7.13 and Table 7.4 suggested that chiral heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin acts also as a very good host and carrier for D-amino acids. The sequence of the transport through liquid membrane is the same as in the case of L-amino acids, but with different values for some of the D-amino acids: D-TrpOMe > D-PheOMe > D-TyrOMe with yields between 25% for L-TyrOMe and 80% for L-TrpOMe. For the native D-amino acids the sequence is D-Trp > D-Tyr > D-Phe with the values between 8% D-Phe and 10% for D-Trp.

Based on the results of the transport through liquid membrane with chloroform of the two forms L- and D-amino acids using heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin (Figure 7.13), there are noted differences at L-TrpOMe and D- TrpOMe, L-PheOMe and D- PheOMe and also at L-TyrOMe and D-TyrOMe. The $\alpha = \eta_L/\eta_D$ ratio representing the transport yields of the two enantiomers L- and D-amino acid is shown Table 7.4.

Table 7.4 *The ratio of the transport yields of the L and D forms of the studied amino acids*

Amino acid	η_L ***(%)***[130]	η_D ***(%)***	α_T
TrpOMe	96	80	1.20 (L)
Trp	12	10	1.20 (L)
PheOMe	87	75	1.16 (L)
Phe	11	8	1.38 (L)
TyrOMe	20	25	1.25 (D)
Tyr	9	9	1.00 (L)

As can be seen from Table 7.4 and Figure 7.13 the transport yields of L-TrpOMe is higher than of D-TrpOMe. The same situation is for L-PheOMe compared with D-PheOMe. Comparing the two amino acids, the transport yield of L-TyrOMe is lower than of D-TyrOMe. The enantiomer L form of amino acids methylesters studied had a higher transport yields compared with the transport yields of the enantiomer D, except for D-TyrOMe. The L/D selectivity as can be seen from Table 7.4 is 1.20 for TrpOMe and 1.16 for PheOMe, respectively. In the case of tyrosine methylester, the D/L selectivity has a value of 1.25.

Concerning the transport of aromatic amino acids in native form, even though the values of transport yields are significantly lower compared with those of aromatic amino acids methylesters, the chiral heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin showed enantioselectivity (L/D) for them with values between 1.00 (Tyr) and 1.38 (Phe). The best enantioselectivity was obtained for Phe. Responsible for the differences in the transport yields of aromatic amino acid native and methylesters with chiral functionalized β- cyclodextrin used in the experiments may be attributed to the structural characteristics, especially to the hydrophobicity of the amino acids [191].

The molar concentration values of the studied amino acids are shown at Table 7.5. In addition, the circular dichroism measurements were carried out to prove the chiral recognition of aromatic amino acids by chiral functionalized β-cyclodextrin (Chapter 8).

Table 7.5 *Transport data of aromatic amino acids through liquid membrane by chiral heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin (1.0 x 10^{-3} M in chloroform)[191].*

Amino acid	***Concentration of amino acid in source phase*** $Mx10^{-4}$	***Concentration of L-amino acid in receiving phase*** $Mx10^{-4}$[130]	η_L ***(%)***	***Concentration of D-amino acid in receiving phase*** $Mx10^{-4}$	η_D ***(%)***
TrpOMe	5.0	4.8	96	4.00	80
Trp	5.0	0.60	12	0.05	10
PheOMe	5.0	4.35	87	3.75	75
Phe	5.0	0.55	11	0.40	8
TyrOMe	5.0	1.00	20	1.25	25
Tyr	5.0	0.45	9	0.45	9

7.4.2.3 Transport mechanism through the liquid membrane

The transport of aromatic amino acids native and methylesters studied with heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin as carrier through a liquid membrane with chloroform is an active transport assisted by a pH gradient between the source phase and receiving phase.
The active transport mechanism for the studied experiments are shown in figure 7.15

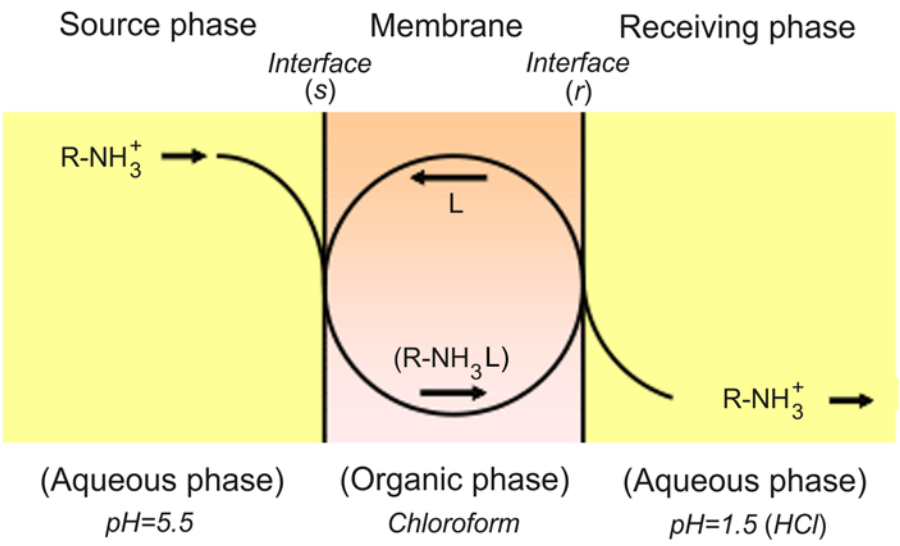


Fig. 7.15 *Active transport assisted by a pH gradient [130].*

At the source phase/membrane phase interface, a complexion process between the present receptor and the compound of interest from the aqueous source phase (pH = 5,5) takes place. This complex is extracted in the membrane phase (an extraction process) and goes to the interface with the receiving phase through a diffusion process. The decomplexion process (re-extraction) takes place at interface membrane-receiving source and due to the conditions existent in receiving phase (pH = 1.5). Thus, the amino acid released from the complex goes into the receiving aqueous phase, and the ligand returns to the interface with the source phase through a diffusion process and thus the process continues.
It is worth noting that in the case of (2,3,6-tri-O-acetil)-β-cyclodextrin compound as carrier through a liquid membrane, no anion is needed to form a pair of ions between the amino acid-receptor-anion for the transport of the amino acid, as frequently used with other receptors.

7.5 Conclusions

The results of the transport through chloroformic liquid membrane of the two forms L- and D-amino acids using chiral heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin noticed that there are differences at L-TrpOMe and D- TrpOMe, L-PheOMe and D- PheOMe and also at L-TyrOMe and D- TyrOMe. The $\alpha = \eta_L/\eta_D$ ratio representing the transport yields of the two enantiomers L- and D-amino acid shows that the L form of the amino acids have a higher transport yield than that of D form, with the exception of D-TyrOMe which has a higher yield than L-TyrOMe. An active transport of the amino acids through the liquid membrane under pH gradient was carried out.

Chapter 8. Study of enantioseparation of some amino acids by circular dichroism spectroscopy

8.1 General considerations

The aim of the determinations consisted in highlighting the property of the newly synthesized cyclodextrin, heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin (CD) to discern between the two enantiomers of some amino acids, using the circular dichroism spectroscopy. Two aromatic amino acids,

phenylalanine and tryptophan in the forms: D-, L- and DL- phenylalanine and L-, DL- tryptophan were used.

8.2 Experimental conditions

The circular dichroism measurements were performed by using a JASCO-815 spectrometer in 240-350 nm range; cuvettes with path length of 1 cm, accumulation, scan speed of 50 nm/s and 8 s response time. The stock solutions were prepared in methanol with the following concentrations: 5.0 x 10^{-3} M (heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin), 2.5 x 10^{-4} M L-Trp and 2.5 x 10^{-4} M DL-Trp. In the case of phenylalanine study the stock solutions of 1.0 x 10^{-2} M heptakis (2, 3, 6-tri-O-acetyl)-β-cyclodextrin and 5.0 x 10^{-3} M L-Phe, 5.0 x 10^{-3} M D-Phe, and 5.0 x 10^{-3} DL-Phe were prepared in methanol [191].

8.3 Results and discussion

The absorption and dichroism spectra for heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin (Figure 8.1) showed that, in the field of interest, these present no signals, therefore no interferences with the spectra of the two amino acids are expected.

The circular dichroism and absorption spectra of the D, L enantiomers and racemic mixture (DL) of phenylalanine are shown in figure 8.2. It can be noticed that the racemic is completely achiral, while the spectra of the two enantiomers have spectra with maximums on the same wavelengths as those in the absorption spectra and are characterised by the object: mirror image relation; the D enantiomer has in the 240-280 nm wavelength range, a positive signal with maximums at 249, 255, 262, 269 nm, the other enantiomer being characterised by a negative dichroic signal. The obtained spectra are the same with those in the literature [201, 202].

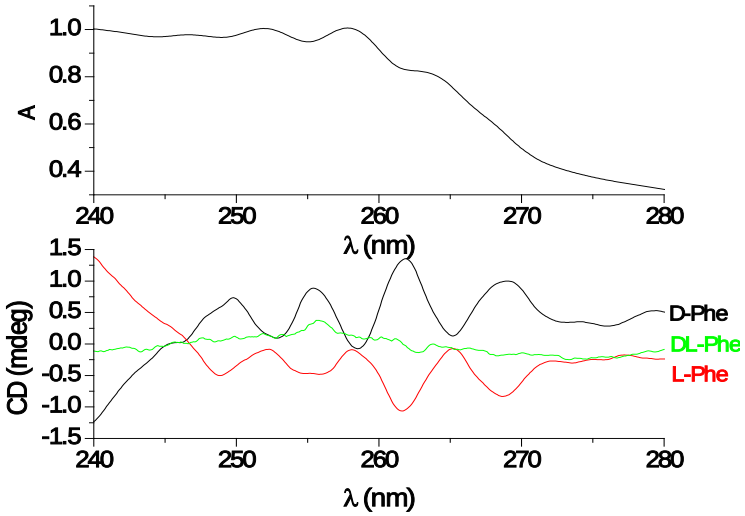


Fig.8.2 *The absorption and circular dichroism spectra for D-Phe, L-Phe and DL-Phe in methanol*

The circular dichroism spectra obtained in the presence of heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin are shown in figure 8.3 by comparison with those of the free enantiomers. Based on the spectra examination, one can note:

- In the case of D enantiomer, a decreasing of dichroic signal is noted in the presence of functionalised cyclodextrin, which is an indication of the interaction with the cyclodextrin and thus of the inclusion of the D-Phe in the cavity. Nevertheless, the noticed differences are small and do not allow for a quantitative evaluation of the binding constant. (Figure 8.3A).
- Taking into account the results obtained for L-Phe, there were insignificant differences of the dichroic signal in the presence of the functionalized cyclodextrin.
- Comparison of the spectra of the two conformers shows a privileged interaction of the D-Phe enantiomer. (Figure 8.3A).
- No clear induced circular dichroism signal is noted in the case of the racemic mixture (Figure 8.3B)

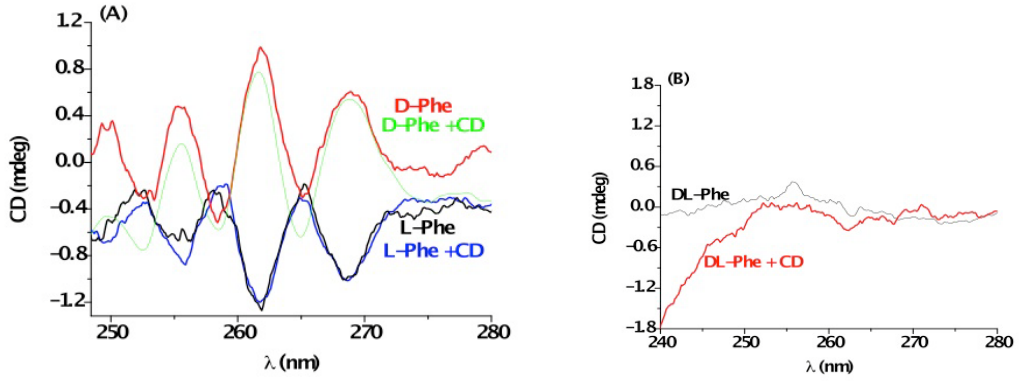


Fig. 8.3 *The circular dichroism spectra in the absence and presence of cyclodextrin for: (**A**) D-Phe, L-Phe şi (**B**) DL-Phe.*

Similar results reffering to the complexation of β-cyclodextrin and β-cyclodextrin functionalized and quaternary ammonium reported in the literature showed that the differences between the association constants of the D- and L-Phe are very small, to the limit of experimental errors [203, 204].

The absorption and circular dichroism spectra for the heptakis-CD –L-Trp systems are shown in fig. 8.4. An increase of the signals (absorbance and dichroic signal) can be noted at the both spectra, indicating the process of amino acid inclusion.

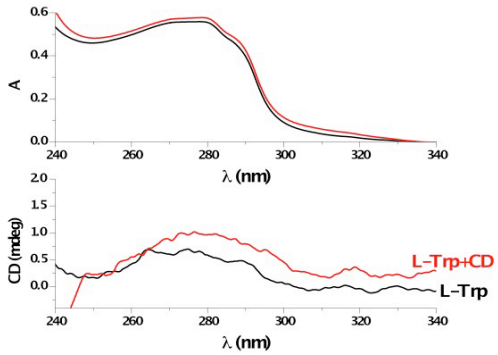


Fig. 8.4 *The absorbance and circular dichroism spectra in the presence and absence of β-cyclodextrin for L-Trp [191].*

As in the case of DL-Phe, for DL-Trp, no circular dichroism signal was obtained. (Figure 8.5)

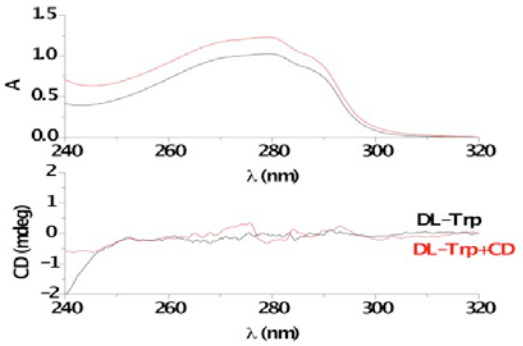


Fig.8.5 *The absorbance and circular dichroism spectra of D L-Trp in the absence and presence of functionalized cyclodextrin.*

8.4 Conclusions

- In this chapter, the possibilities of the functionalized cyclodextrin, heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin to discern between the two enantiomers of some amino acids were studied, using the circular dichroism spectroscopy.
-

General conclusions

The main objective of the thesis *Study of biological compounds with macrocyclic receptors* was the study of applicative possibilities of some macrocyclic receptors of calixarenes, cyclodextrins and cucurbituril in the separation processes (liquid-liquid extraction and bulk liquid membranes) of biological interest compounds: amino acids, dipeptides and nucleobases. In order to accomplish the targeted objective, there is carried out a comparative study of the complexes formed between *p*-sulphonatocalix[n]arene (n = 4, 6), α-, functionalized β-cyclodextrin and cucurbit[*n*]uril (n = 6,7) and the mentioned biological compounds. The separation of the enantiomers of the native aromatic amino acids and methylesters through bulk liquid membranes using the functionalized β-cyclodextrin as chiral carrier is another aspect studied in the herein thesis.
The experimental results allow for the following conclusions to be drawn:

- The calixarenic, *p*-sulphonatocalix[4]arene receptor forms complexes with the studied amino acids (L-proline, L-arginine, L-lysine, L-histidine, Acid L-aspartic, L-serine, L-isoleucine, L-tyrosine) and the complexation process being favoured by enthalpic contributions and disfavoured by entropic contributions. The values of the stability constants and of the thermodynamic parameters under study for the complexes formed between the *p*-sulphonatocalix[4]arene and dipeptides (Lys-Arg, Arg-Arg, Arg-Lys, Lys-Lys) have higher values suggesting the formation of the complex favoured by enthalpic contributions. The results obtained are compared with those obtained in the scientific literature through other techniques, in this particular case through NMR technique. Likewise, no significant differences are noted between the values of the stability constants for the studied complexes.

- The calixarenic *p*– sulphonatocalix[6]arene receptor forms complexes with the dipeptides (Gly-Gly, Gly-L-Val, Gly-L-Leu, L-Leu-Gly, L-Leu-L-Ala, Gly-L-Phe) with values of the stability constants between log K = 3,00 (L-Leu-L-Ala) and log K = 3,36 (Gly-L-Ala), but the receptor do not show selectivity to the studied dipeptides. Therefore, there is the possibility of formation both of the inclusion and exclusion complexes.
- The values of the stability constants obtained in the study of the nucleobases (adenine, cytosine and guanine) have lower values between log K = 1.1 and log K = 2.2 for cytosine and adenine.
- The complexing reactions of α-cyclodextrin in the aqueous phase with a series of amino acids and dipeptides (L-leucine, L-isoleucine, L-valine, L-alanine, glycine, L-phenylalanine, L-tryptophan, L-tyrosine, glycyl-leucine, glycyl-valine, glycyl-tryptophan) are characterised by lower values of the reaction enthalpy, for some compounds almost equal to those of the reaction entropy. It can be stated that the complexation reaction of some amino acids and dipeptides with α-cyclodextrin is favoured by enthalpic contributions and entropic contribution and hydrophobic interactions are involved in the complex formation.
- The values of the stability constants in the case of complex formation of CB[6] with dipeptides (glycyl-leucine, glycyl-valine, glycyl-tryptophan, glycyl-alanine, glycyl-histidine) are almost identical and ranged between log K = 2.4 (Gly-His) and log K = 2.90 (Gly-Trp). The interactions involved in the complexes formed by cucurbit[6]uril with the studied dipeptides suggest dipole ion interactions between the protonated amino group of the dipeptide and carbonyl group of the cucurbit[6]uril. Likewise, the structure of different studied dipeptides does not influence the value of the stability constant. Even the reaction enthropy has closed values for all studied complexes. These results can be explained by formation of the exclusion complexes.
- As for the studied nucleobases (adenine, cytosine, guanine and uracil) with CB[6], there are noted low values of the reaction enthalpy and the complexation is characterised both by enthalpic contribution and entropic contributions.
- The ^{1}H- RMN spectrum of the adenine suggested the possibility of inclusion complex formation between adenine and receptor CB[7].
- By correlation of the results obtained through NMR with those obtained by UV-Vis spectrometry, it can be suggested that there is possible the formation of an inclusion complex of CB[7] with the adenine.
- As in the case of the adenine from the UV-Vis spectra, a decreasing of the cytosine absorbance is noted based on the increase of CB[7] quantity, but with some differences, suggesting the possibility of formation and other types of complexes. Thus, the results obtained by spectrometry in UV-Vis and those obtained by NMR led to conclude that it is possible the formation of an inclusion complex of the CB[7] with the cytosine, respecting the receptor concentration conditions.
- The experimental results obtained in the liquid-liquid extraction of some native aromatic amino acids and methylesters with chiral heptakis (2, 3, 6-tri-O-acetil)-β-cyclodextrin show that the receptor structure and the amino acid hydrophobicity influence the extraction process. The native aromatic L-amino acids and the derivative ones are extracted from the aqueous phase into the organic phase by heptakis (2, 3, 6-tri-O-acetil)-β-cyclodextrin with yields between 17.0 % (L-TyrOMe) – 50,2 % (L-TrpOMe) for the aromatic amino acid methylesters and 8.0% (L-Tyr) – 17.5 % (L-Trp) for the native amino acids. The extractability depends on the receptor structure, pH and nature of the amino acid. For the tryptophan methylester, due to its high hydrophobicity, there are higher extraction yields than at the other amino acids.
- The extraction constant values of the aromatic amino acids methyl esters (L-TrpOMe, L-PheOMe, L-TyrOMe) are higher than of the native aromatic amino acids (L-Trp, L-Phe, L-Tyr).

It was noticed that the extraction constant values depend on the hydrophobicity of the studied amino acid. Thus, L-TrpOMe , the most hydrophobic of all studied amino acids has the highest extraction constant value (Log K_{ex} = 3.50). The extraction constant value (Log K_{ex} = 1.50) is lower for the methylated tyrosine, and for the native tyrosine, no calculation of the extraction constant value is possible in the conditions mentioned in the study. Thus, both the structure of the ligand used for extraction and the amino acid structure influence the determination of the extraction constant.

- The results after the transport of native aromatic L amino acids and methylesters from an aqueous source phase to a receiving phase using as carrier, the heptakis receptor (2,3,6-tri-O-acetil)-β-cyclodextrin in chloroform suggest that the heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin shows a higher affinity to L-TrpOMe. As a result, a higher transport yield for this amino acid. The transport sequence through the liquid membrane is the following: L-TrpOMe > L-PheOMe > L-TyrOMe with yields between 2o% for L-TyrOMe and 96 % for L-TrpOMe.
- The results after the transport of native aromatic D-amino acids and methylesters from a source aqueous phase to a receiving phase using as carrier, the heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin in chloroform showed the same transport sequence as in the case of L-amino acids, but with different values for some of D-amino acids: D-TrpOMe > D-PheOMe > D-TyrOMe with yields between 25 % for L-TyrOMe and 80 % for L-TrpOMe. For the native D-amino acids the sequence is D-Trp > D-Tyr > D-Phe with values between 8% D-Phe and 10% for D-Trp.
- The results of the transport through the chloroform liquid membrane of the two forms, L- and D-amino acid using chiral heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin, differences were noted at L-TrpOMe and D- TrpOMe, L-PheOMe and D- PheOMe, as well as at L-TyrOMe and D-TyrOMe. The $\alpha = \eta_L/\eta_D$ ratio representing the transport yields of the two enantiomers L- and D-amino acid from the carried out transport studies indicate that the L form of the studied amino acids has a higher transport yield than of D form, except for D-TyrOMe which has a higher yield than L-TyrOMe. Thus, heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin can be used as chiral carrier for optically active compounds through optimisation of the transport conditions.
- In the experiments regarding the transport of the native aromatic amino acids and methyl esters with heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin as carrier through a liquid membrane is an active transport assisted by pH gradient between the source phase and receiving phase.
- From the study of the possibilities of the functionalized cyclodextrin, heptakis (2,3,6-tri-O-acetil)-β-cyclodextrin to discern between the two enantiomers of aromatic amino acids: DL-phenylalanine and DL-tryptophan, using the circular dichroism spectroscopy, it were observed the following:

a) A decreasing a dichroic signal for D enantiomer of phenylalanine in the presence of cyclodextrin is observed highlighting the interaction with cyclodextrin and inclusion of D-Phe in the cyclodextrin cavity. But the differences are quite small and the binding constant can not be evaluated. In the case of L-Phe, the differences of spectrum in the presence and absence of cyclodextrin are not significantly. By comparing the spectra of L-Phe and D-Phe one can infer a special interaction of cyclodextrin with the D enantiomer. No clear induced circular dichroism signal is noted in the case of the DL-Phe racemic mixture.
b) From the absorption and circular dichroism spectra registered for heptakis-CD –L-Trp system, an increase of the signals (absorbance and dichroic signal) was noted, indicating the process of amino acid inclusion. As in the case of DL-Phe, for DL-Trp, no circular dichroism signal was obtained.

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List of scientific publications

1. **Ana Delia Stancu**, Mihaela Hillebrand, Cristina Tablet, Lucia Mutihac

β-cyclodextrin derivative as chiral carrier in membrane transport of some aromatic amino acids
Journal of Inclusion Phenomena and Macrocyclic Chemistry/ Doi: 10.1007/s10847-012-0271- 0/ **acceptată**, on line.

2. L. Kim, **A.D**. **Stancu,** E. Diacu, H.-J. Buschmann, L. Mutihac
Extraction and transport behavior of aromatic amino acids by modified cyclodextrin
Supramolecular Chemistry, 21, 131-134, 2009.

3. **A. D. Stancu**, H.-J Buschmann, L. Mutihac
Survey on thermodynamic properties for the complexation behavior of some calixarene and cucurbituril receptors. *Review*
Journal of Inclusion Phenomena and Macrocyclic Chemistry/ DOI :10.1007/s10847-012-0137- 5/**acceptată**/on line.

4. L. Kim, A. Hamdi, **A. D. Stancu**, R. Souane, L. Mutihac, J. Vicens
Selective membrane transport of amino acids by functionalized calix[4]arenes
Journal of Inclusion Phenomena and Macrocyclic Chemistry,66, 55-59, 2010.

5. **A.D**. **Stancu,** M. Ceausescu, L. Kim, E. Diacu, L. Mutihac
Cyclodextrins involved in separation of amino acids by liquid membranes
Trends in membranology, EMS-XXVII Summer School 14-19 June 2010, Eds. Printech, Bucureşti

Conference participations

1. *Some analytical applications of modified cyclodextrins*, L. Kim, C. Baltariu, **A. D. Stancu**, E. Diacu, H.-J. Buschmann, L. Mutihac, **II International Symposium on Macrocyclic and Supramolecular Chemistry,** June 24-29, 2007, Salice Terme, Italy, poster, PSB 19.

2. *Functionalized calixarenes as transporters through liquid membrane* **A. D. Stancu,** L. Kim, P. Z. Iordache, L. Mutihac, **ISMSC** 13-18, July, 2008, Las Vegas, SUA, poster, p. 14.

3. *Extraction aspects of functionalized receptors upon biological compounds*, **A.D. Stancu**, A. Taqi, A. Hamdi, R. Souane, J. Vicens, L. Mutihac, **ISMSC**, 21-25 June, 2009, Maastricht, The Netherlands, poster, p.297

4. *Selective membrane transport of amino acids by functionalized calix[4]arenes*. L. Kim, **A. D**. **Stancu,** A. Hamdi, J. Vicens, L. Mutihac, **10th ICC**, July 13-16, 2009, Seoul, South of Korea, poster, P-102.

5. *Aspects of recognition and transport of amino acids and peptides by synthetic receptors*, L.Mutihac, L. Kim, **A. D. Stancu**, H.-J. Buschmann, J. Vicens, **10th ICC**, July 13-16, 2009, Seoul, South of Korea, invited lecture, IL-6.

6. *Functionalized calixarenes involved in electrochemical recognition of some anions*, E. Diacu, M. M. Ceausescu, **A. D. Stancu**, L. Mutihac, **NOMARES** Juin 18-19, 2010,

Bucarest, Roumanie, Poster, p. 22.

7. *Cucurbit[n]urils in the molecular recognition of small biomolecules*, L. Mutihac, **A. D**. **Stancu**, H.-J. Buschmann, **ICCB 2011**,June 29-July 2, 2011,University of Cambridge, invited lecture, Q-27.

8. *Cyclodextrins involved in separation of amino acids by liquid membranes*, **A. D. Stancu**, M.Ceauşescu, E. Diacu, L. Mutihac, **EMS- XXVII Summer School** 14-19 June 2010, Bucharest, poster, p.12.

9. Participation: *Laboratory Seminar I,* ABL&E-JASCO România Comerț și Servicii SRL, 17 martie, 2009, București, România (Prof.dr. M. Hillebrand Spectroscopia de Dicroism Circular conference)

Research Grants

PN2- Partnership "Mobil detection, identification and biological and chemical monitoring system" **,** 81-002/2007, 2007-2010, Project leader (Project coordinator Ph.D.. V. Somoghi, CCSANBCE and Professor L. Mutihac from University of Bucharest).

PN2- Partnership "Mobil System from rapid monitoring of biological and chemical agents for strategical objectives", 31-001/2007, 2007-2010, Project leader (Project coordinator Ph.D.. V. Somoghi, CCSANBCE and Professor L. Mutihac from University of Bucharest).