UNIVERSITY OF BUCHAREST
FACULTY OF CHEMISTRY
DOCTORAL SCHOOL IN CHEMISTRY

PhD THESIS
SUMMARY

STUDIES OF INCLUSION AND SEPARATION OF SOME BIOCHEMICAL COMPOUNDS USING MACROCYCLIC RECEPTORS

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INTRODUCTION

Supramolecular chemistry has also gained considerable influence over analytical chemistry, the versatility and diversity of synthetic macrocyclic receptors asserting new limits and remarkable perfomances to the existent techniques. Recent researches of the supramolecular chemistry concerning molecular recognition principles of biological compounds using macrocyclic receptors are due to the broad spectrum of practical applications in separation, imaging , sensing, catalysis and pharmaceutical activity.

Under these aspects, the present thesis entitled „*Studies of inclusion and separation of some biochemical compounds using macrocyclic receptors*" aims the introduction of new macrocyclic receptors in processes of molecular recognition and separation (liquid-liquid extraction and liquid membrane) of a series of biologically relevant compounds.

The main objectives of the present doctoral thesis are:

a) Study of nucleobases complexation (adenine, guanine, cytosine, thymine, uracil) with the following macrocyclic receptors: hemicucurbit[6]uril, hemicucurbit[12]uril, cucurbit[7]uril, β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin and heptakis (2,3,6-tri-O-acetyl)-β-cyclodextrin using UV-Vis spectrometry and fluorescence spectroscopy.
b) Introduction of new macrocyclic receptors from cucurbituril family (hemicucurbit[6]uril and hemicucurbit[12]uril) as extractants or transporters in liquid membrane for some amino acids. It was also studied the optimization of both extraction and liquid membrane transport parameters .
c) Studies regarding the separation of some nucleobases (adenine, cytosine, uracil, thymine) by extraction and transport processes using macrocyclic receptors.
d) Determination of transport mechanisms through liquid membrane of nucleobases and amino acids using the studied macrocyclic receptors as carrier in separation processes.

This thesis is structured in seven chapters and comprises two main parts, as follows: *the literature part* is organized in three chapters and *the experimental part* in four chapters.

In the first chapter is given a general introduction regarding analytical application of supramolecular chemistry. Also, there are presented briefly the main classes of biologically relevant compounds found in host-guest chemistry.

In the second chapter and third chapter there are described the structural characteristics and molecular recognition properties of macrocyclic receptors from cucurbituril and cyclodextrin families. Versatility and potential of macrocyclic receptors from above mentioned classes is highlighted throughout the multitude of application presented briefly based on the most recent studies.

The original part of this thesis, which includes the experimental results obtained within the studies carried on, is structured in four chapters. In the first two chapters (Chapter IV and Chapter V) are presented aspects regarding the molecular recognition of nucleobases (adenine, guanine, cytosine, thymine and uracil) by macrocyclic receptor from cucurbituril family (hemicucurbit[6]uril, hemicucurbit[12]uril, cucurbit[7]uril) and cyclodextrinic receptors (β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin) using UV-Vis spectrophotometry and fluorescence spectroscopy.

In Chapter VI there are presented the results obtained in the liquid-liquid extraction and transport through bulk liquid membrane of a series of amino acid native and methyl esters using the new receptors hemicucurbit[6]uril and hemicucurbit[12]uril. It were also highlighted the transport mechanisms involved. Chapter VII is dedicated to the extraction and transport processes through bulk liquid membrane of a series of nucleobases using different synthetic receptors, considering also the membrane transport mechanisms. The last chapter describes the general conclusion based on the findings of the studies presented in this thesis.

The thesis ends with the reference and the scientific paper list based on the results from the experiments carried out.

EXPERIMENTAL PART

(numbering of the chapters, figures, tables and equations is from the thesis)

Chapter IV. Hemicucurbituril in recognition processes of some nucleobases

In this chapter are presented the experimental results concerning complexation of some nucleobases with hemicucurbit[6]uril (HemiCB[6]) and hemicucurbit[12]uril (HemiCB[12]) using UV-Vis spectrometry. Also, there are continued the experiments regarding binding affinity of cucurbit[7]uril towards adenine and cytosine [298] using fluorescence spectroscopy. Futhermore, the study is extended to the affinity of cucurbit[7]uril may exhibit to guanine using both UV-Vis spectrometry and fluorescence spectroscopy.

Chapter V. Aspects regarding the molecular recognition of nucleobases with β-cyclodextrin derivatives

V.1. Introduction

Cyclodextrins are among the most studied classes of macrocyclic receptors and are recognized for their potential applications in biological sistems due mainly to their ability to form inclusion complexes with neutral compounds in aqueous solution [191,201,205,297].

In this chapter is studied the binding affinity of native and derivatized β-cyclodextrin towards nucleobases by means of UV spectrometry and fluorescence spectroscopy. The stoichiometry and the association constants of host–guest complexes were determined using the experimental data obtained.

V.3. Experimental

V.3.1. Materials and apparatus

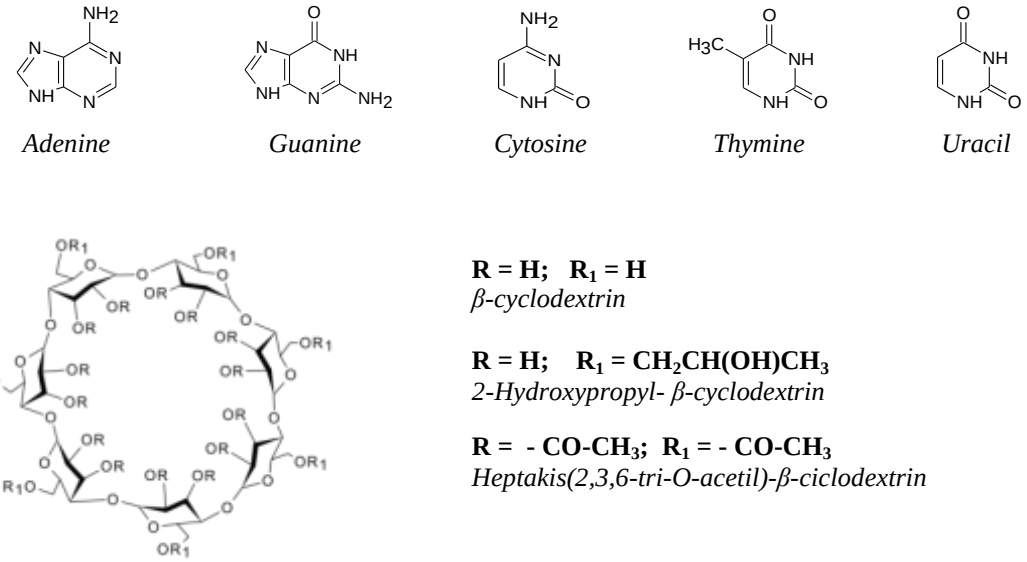


Figure 5.1. *Molecular structure of nucleobases and cyclodextrinic receptors used in the study*

V.3.2. Procedures

Titration experiments

The complexation ability of cyclodextrins towards nucleobases in aqueous solutions was evaluated by titration experiments monitored by UV spectrometry and fluorescence spectroscopy. β-CD had no UV-Vis absorption and no fluorescence emission.

Stock solutions of nucleobases was prepared with the following concentration: $C_{adenine}$ = 1,0 x 10^{-4} M, $C_{guanine}$ = 5,0 x 10^{-4} M (pH = 3,0), $C_{cytosine}$ = 1,0 x 10^{-4} M. Into a 10 mL volumetric flasks were prepared 1,0 x10^{-5} M nucleobase solution by diluting the stock solution. Stock solutions of 1,0 x 10^{-2} M β-cyclodextrin used in titration were prepared using 5 mL from the aqueous solution of 1,0 x10^{-5} M nucleobase.

V.4. Results and discussion

V.4.1. Aspects regarding complexation of guanine with β-cyclodextrin

V.4.1.1. Study of β-CD – guanine complex by UV-Vis spectrometry

Gradual addition of cyclodextrinic receptor results in a increase in guanine absorbance (Figure 5.3). The absorption maximum at 248 nm shows no shift with the increase in receptor concentration.

The induced changes in guest absorbance are often attributed to the formation of inclusion complexes [250,259,260], because they indicate that the guest is carried from the bulk solution to the hidrophobic cavity of the β-CD and consequently, the guest molecule exhibit different spectroscopic features [250, 259].

The linearity of the plot obtained by Benesi Hildebrand method (Equation 5.20) suggests a possible complex formation with a 1 : 1 stoichiometry (R^2 = 0.9913; F-stat = 1030) .

$$\frac{1}{\Delta A} = \frac{1}{(A_{11} - A_0)\, K_{11}[\beta - CD]} + \frac{1}{(A_{11} - A_0)} \qquad (5.20)$$

$$A = \frac{A_0 + A_{11} K_{11}[\beta - CD]}{1 + K_{11}[\beta - CD]} \qquad (5.21)$$

Unde A – the absorbance of guanine in (λ = 248 nm) in the presence of β-CD

A_0 – the absorbance of guanine in (λ = 248 nm) in the abscence of β-CD

A_{11} – the absorbance of the complex β-CD-guanine

K_{11} – association constant of the 1:1 complex

The relationship between the absorbance maxima of guanine and concentration of β-cyclodextrin is diplayed in Figure 5.5. By fitting the data obtained with Eq. 5.21 it is evidenced a possible complex guanine-β-CD formation 1:1 with the association constant of K_{11} = 98 ±11 M^{-1} (R^2 = 0,9929, F-stat = 1819, A_{11}/A_0 = 1,92 ± 0,07) .

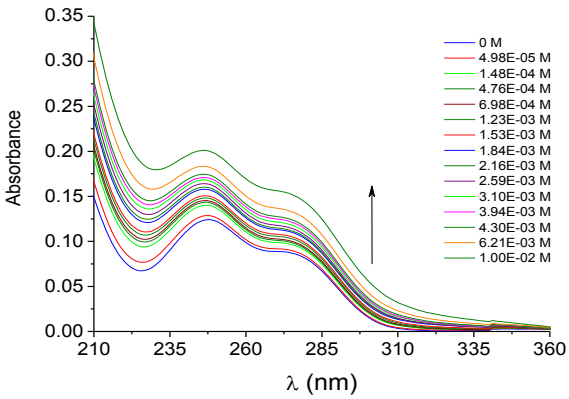


***Figure 5.3**. Absorption spectra of guanine (1,0 x 10^{-5} M) in the absence and presence of increasing amounts of β-CD (5,0 x10^{-5}–1,0 x 10^{-2} M) at pH 3.0*

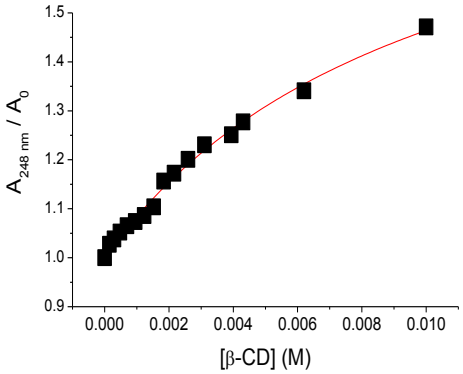


***Figure 5.5**. Curve-fitting plot for guanine-β-CD according to a 1:1 binding model (Eq. 5.21)*

V.4.1.2. Study of β-CD – guanine complex by fluorescence spectroscopy

Complexation of guanine with β-cyclodextrin was also investigated using fluorescence spectroscopy. So it was measured the fluorescence intensity in solutions containing guanine (fixed concentration, 1,00 x 10^{-5} M) in absence and presence of increasing concentrations of host (4,98 x10^{-5} M – 1,00 x 10^{-2} M). Emission fluorescence spectra were acquired in the 290-550 nm interval, at a fixed excitation wavelength of 280 nm.

Similar to UV-Vis absorption spectra, by gradual addition of β-CD the fluorescence spectra of guanine showed an increase of fluorescence signals (Figure 5.6), with the maximum emission wavelengths at λ_{em} = 350 nm. The fluorescence enhancement can be described by assuming the complex formation between guanine and β-CD.

The association constant and the stoichiometry of β-CD – guanine complex was determined by fitting the experimental fluorescence data (λ_{em} = 350 nm) using the following equations:

$$\frac{1}{\Delta I} = \frac{1}{(I_{11} - I_0)\, K_{11}[\beta - CD]} + \frac{1}{(I_{11} - I_0)} \qquad (5.22)$$

$$I = \frac{I_0 + I_{11} K_{11}[\beta - CD]}{1 + K_{11}[\beta - CD]} \qquad (5.23)$$

where I - represents fluorescence intensity of guanine in solution,

I_0 , I_{11} - represents fluorescence intensity of the free and bound guest,

K_{11} - represents association constant of the complex.

A linear plot *1/(I-I$_0$) vs. 1/[β-CD]* (Benesi-Hildebrand's method) was obtained, indicating a 1:1 stoichiometry for β-CD – guanine complex (R^2 = 0,9927). The dependence of the fluorescence enhancement of guanine with the increase of β-CD concentration is shown in Figure 5.9. By fitting the experimental data with eq. 5.23. the asociation constant was estimated at 111 ± 9 M^{-1} (R^2 = 0,9947, F-stat = 3309). The 1:1 inclusion complex stoichiometry was also studied by the Job's plot (continuous variation method) carried out using fluorescence spectroscopy.

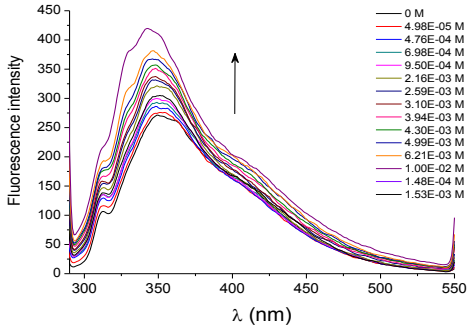


Figure 5.6. *Fluorescence emission spectra of guanine (1,00 x10^{-5} M) in abscence and increasing amounts of β-CD*
λ $_{ex}$= 280 nm; λ$_{em}$ = 350 nm

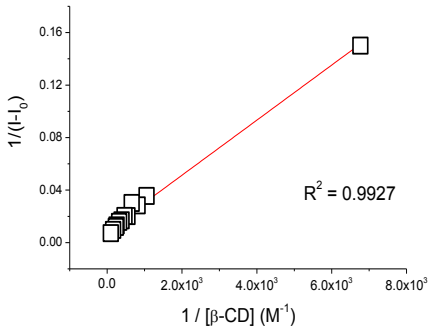


Figure 5.8. *Linear plot 1/(I-I$_0$) vs. 1/[β-CD]*

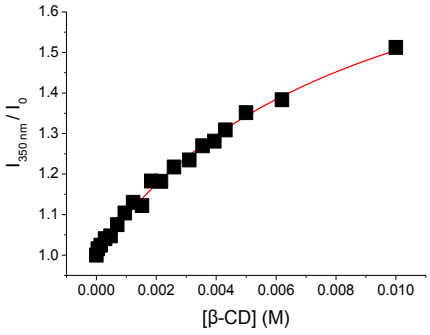


Figure 5.9. *Curve-fitting plot for the fluorimetric titration for guanine-β-CD according to a 1:1 binding model using eq. 5.23*

Considering the experimental results from the the absorption and fluorescent measurements, the formation of β-CD – guanine complex might be given by the following expression:

$$\beta - CD \; + \; Guanine \; \overset{K_{11}}{\rightleftharpoons} \; \beta - CD{:}\,Guanine$$

From tha data presented in Table 5.1. a good agreement can be observed in the case of association constants of β-CD-guanine complex obtained by both the absorption and fluorescent measurements.

Tabel 5.1 . *Fitted parameters for the β-CD- guanine complex*

	K_{11}	R^2	*F-stat*	A_{11}/A_0	I_{11}/I_0
UV-Vis Spectrometry	98 ± 11 M^{-1}	0,9929	1819	1,95 ± 0.05	-
Fluorescence spectroscopy	111 ± 9 M^{-1}	0,9947	3309	-	1,92 ± 0.07

Where K_{11}- association constants; R^2-correlation coefficient; F-stat-Fisher statistic coefficient;
I_{11}/I_0 - ratio of intensities of the bound and free guest;
A_{11}/A_0 - ratio of absorbances of the bound and free guest

V.4.2. Aspects regarding complexation of adenine with β-cyclodextrin

The absorbance and fluorescence emission spectra for adenine in aqueous solution without and in the presence of cycloclodextrinic receptor were measured. Adenine concentration was of 1,0 x 10^{-5} M.

Similar to the guanine study, it was obtained an enhancement of the both absorbance and the fluorescence intensity with the gradual addition of β-CD. The absorption maximum at 260 nm and the emission maximum at 410 nm showed no shift with the increase in receptor concentration.

In order to determine the association constant of β-CD-adenine complex, both the UV absorbance and fluorescence intensity enhancement in function of the concentration of β-CD was analysed using a non-linear models. The best fit of the intensity signal (λ_{em} = 410 nm) reveals the formation of a 1:1 β-CD-adenine complex with the corresponding association constant of 77 ± 13 M^{-1} (R^2 = 0,9927, F-stat = 1792). These findings are consistent with those from the absorption spectrometric analysis which also indicate a 1:1

stoichiometry for the β-CD-adenine complex with an association constants of 122 ± 25 M^{-1} (R^2 = 0,9918; F-stat = 1343).

The analysis of the experimental data using the Benesi-Hildebrand method confirms the possibility of formation of a 1:1 stoichiometry complex between adenine and β-CD, results consistent with those reported in the literature [304].

V.4.3. Aspects regarding complexation of cytosine with β-cyclodextrin and 2-hydroxypropyl- β-cyclodextrin

Experiments continued with the study of cytosine complexation with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin by means of UV-Vis spectrometry and fluorescence spectroscopy

V.4.3.1. Study cytosine complexation with β-cyclodextrin

The increase in UV-Vis absorbance of cytosine and in the fluorescence intensity respectively, with the gradual addition of β-cyclodextrin reveals that the host–guest interaction is achieved. The absorption maximum at 266 nm and the emission maximum at 350 nm showed no shift with the increase in receptor concentration. The complete UV-Vis absorption and fluorescence titration spectra of cytosine are provided in figure 5.19 and figure 5.22 respectively.

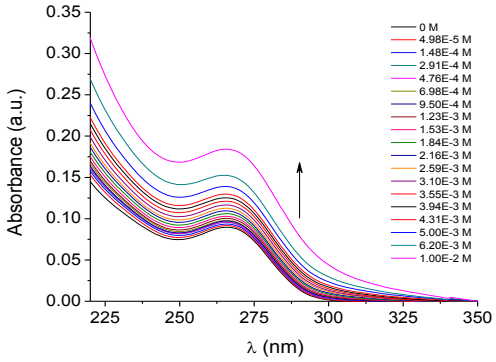


***Figure 5.19**. Absorption spectra of cytosine (1.00 x 10^{-5} M) without and in presence of different amounts of β-CD*

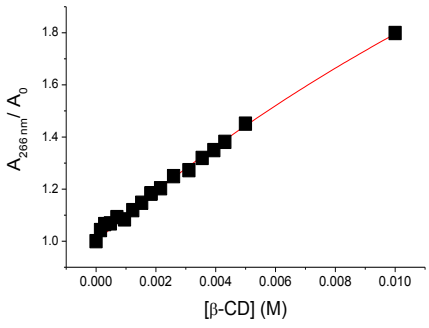


***Figure 5.21.** Curve-fitting plot for the UV-Vis titration for cytosine-β-CD according to a 1:1 binding model (R^2 = 0,9950, F-stat = 3833)*

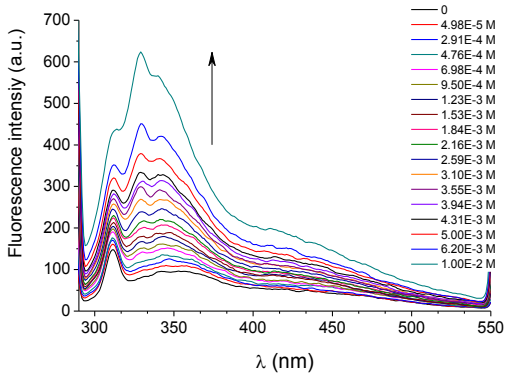


***Figure 5.22**. Emission spectra of cytosine (1,00 x 10^{-5} M) without and in presence of different amounts of β-CD*
λ_{ex} = 280 nm; λ_{em} = 350 nm

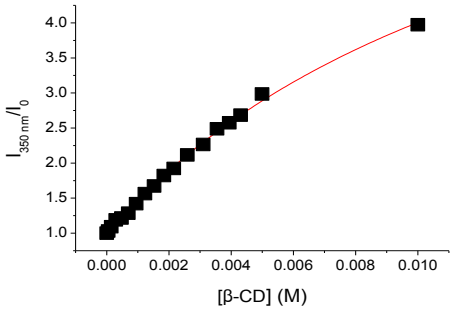


***Figure 5.25.** Curve-fitting plot for the fluorimetric titration for cytosine-β-CD according to a 1:1 binding model (R^2 = 0,9980; F-stat = 8366)*

The experimental data obtained for different β-CD concentration were fitted to both linear and non-linear equation in order to estimate the association constant for β-CD-cytosine complex. It was also indicated a stoichiometry of 1:1 for the complex formed between cytosine and β-CD. The association constant estimated at 72 ± 4 M^{-1} from the fluorescence titration measurements is larger than the value obtained from by UV-Vis measurements , 25 ± 5 M^{-1}.

$$\beta - CD \; + \; Cytosine \underset{}{\overset{K_{11}}{\rightleftharpoons}} \; \beta - CD{:}Cytosine \qquad (5.31)$$

V.4.3.2. Study cytosine complexation with 2-hydroxypropyl-β-cyclodextrin

Experiments continued with the study of the complex formed between cytosine and 2-hydroxypropyl-β-cyclodextrin in aqueous solution aiming to determine the influence of the functional groups attached to the cyclodextrinic ring on the complexation process. Fluorescence and UV–vis spectroscopic titrations measurements were carried out with a constant concentration of cytosine (1,00 x 10^{-5} M) and different amounts of receptor with a concentration range of 3,88 x 10^{-4} M – 7,80 x 10^{-2} M. In order to find the stoichiometry and the corresponding association constant of the formed complex the experimental data were fitted to the usual equations for different types of supramolecular complexes [209].

V.4.4. Aspects regarding thymine and uracil complexation with β-cyclodextrin

The complexation of thymine and uracil with β-cyclodextrin was followed by means of UV–Vis spectrometry.

Capitolul VI. Hemicucur[n]uril, n = 6, 12 in separation process of amino acids

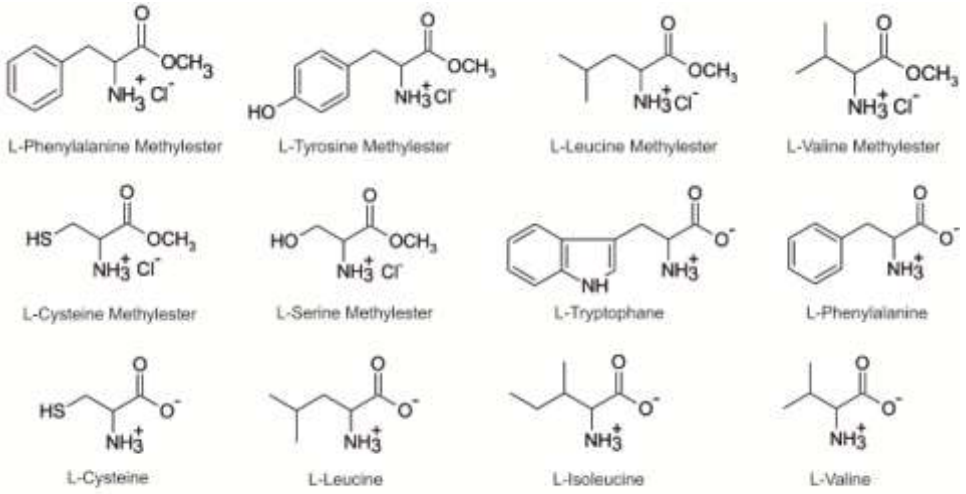
VI.1. Hemicucurbit[n]uril , n = 6, 12 as extraction agent of some amino acids

VI.1.1. General consideration

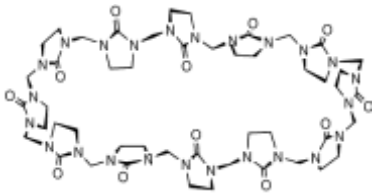
In this chapter was studied the possibility of using hemicucurbit[n]uril (n = 6, 12) and functionalized β-cyclodextrin, as extractant in liquid-liquid extraction and as carrier through liquid membrane, respectively, in order to separate a series of amino acids.

VI.1.3. Experimental

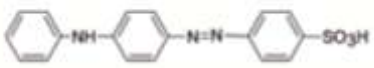
VI.1.3.1. Apparatus and materials



Hemicucurbit[6]uril (HemiCB[6])



Hemicucurbit[12]uril (HemiCB[12])



Tropeolin 00

Figura 6.1. *Molecular structure of the compounds used throughout the experiments*

VI.1.3.2. Procedure

Stock solution of amino acids were prepared in bidistilled water. Concentration of amino acid stock solution varied between 1,0 x 10^{-3} M – 2,0 x 10^{-3} M. It were also prepared stock aqueous solution of 5,0 x 10^{-4} M Tropeolin 00. Solutions of 1,0 x 10^{-4} M hemicucurbit[6]uril (HemiCB[6]) and hemicucurbit[12]uril (HemiCB[12]), respectively, were prepared in water sturated chloroform solution.

Amino acids extraction from aqueous phase in organic phase (chloroform) was made in separatory funnel as it follows:

- 5 mL of 5.0 x 10^{-4} M amino acid solution (8.0 x 10^{-5} M tropaeolin 00 at pH = 5.5) were shaken with 5 mL of 1,0 x 10^{-4} M organic solution (chloroform) of HemiCB[6] and HemiCB[12], respectively (T = 298,15 K) .
- The UV-Vis spectra of the aqueous phases was measured before and after the extraction with the receptor respectively and the maximum absorbance was read (λ = 444 nm)

The extractability was calculated according to Pedersen's procedure [307]:

$$E\,\% = \frac{A_0 - A}{A_0}\, x\, 100 \qquad (6.21)$$

where A_0, A - represent the absorbance of the initial aqueous phases and after the extraction with the receptor , respectively.

VI.1.4. Results and discussion

VI.1.4.1. Liquid-liquid extraction of amino acids with hemicucurbit[n]uril, *n = 6,12*

The ability of HemiCB[n] receptors, *n=6,12*, of being used as extraction agents in liquid-liquid extraction of a series of native amino acids (*L-phenylalanine, L-leucine, L-valine, L-isoleucine, L-cysteine, L-valine, L-tryptophan*,) and methylester amino acids (*L-phenylalanine methyl ester hydrochloride (L-PheOMe), L-leucine methyl ester hydrochloride (L-LeuOMe), L-serine methyl ester hydrochloride (L-SerOMe), L-cysteine methyl ester hydrochloride (L-CysOMe), L-valine methyl ester hydrochloride (L-ValOMe) and L-tyrosine methyl ester hydrochloride (L-TyrOMe*)) was studied at pH 5,5, in the presence tropaeolin 00 as counterion.

The receptors HemiCB[6] and HemiCB[12] present no UV-Vis absorbance in the used domain and are used for the first time in separation studies of some biologic compounds.

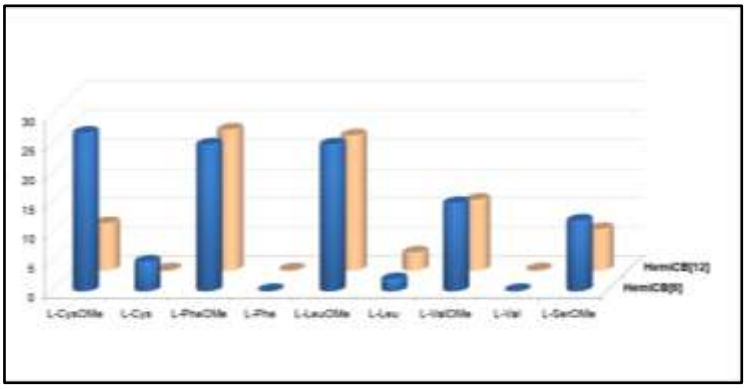


Figure 6.21. *Extraction (%) of amino acids from aqueous phase (pH = 5.5) into chloroform phase by receptors HemiCB[6] and HemiCB[12]*

În figure 6.12. are shown the values of the extraction yield from aqueous phase into chloroform phase of studied amino acids using HemiCB[6] and HemiCB[12] as extractants, in the presence of tropaeolin 00 as counterion. The extraction efficiency depends on parameters like structural properties of amino acids, the structure of the receptor, the nature of the counterion, the pH of aqueous solution and the nature of the solvent. The obtained results highlighted the good affinity of both receptors HemiCB[6] and HemiCB[12] towards hydrophobic amino acids (L-PheOMe, L-LeuOMe and L-ValOMe) [305].

VI.1.5. Conclusion

Experimental results obtained in liquid-liquid extraction of the studied amino acids using as extractans the receptors HemiCB[n], n = 6,12 highlighted the affinity of the receptors towards L-amino acids methyl ester. The extractability of the receptor HemiCB[6] towards amino acids as ion pairs is between 27% (L-CysOMe) and 3% (L-Leu), respectively, and between 25% (L-PheOMe) and 3% (L-Leu), respectively, for the receptor HemiCB[12].

Results obtained in the study regarding the influence of agitation time of the immiscible phases on extraction yield highlighted a good affinity of the receptor HemiCB[6] form the amino acids methyl ester L-CysOMe (42%) and L-PheOMe (33%).

VI.2. Aspecte prinvind transportul prin membrane lichide ale unor aminoacizi utilizând hemicucurbit[6]uril

Considering the experimental results obtained in liquid-liquid extraction that highlighted the affinity of receptor HemiCB[6] for amino acids methyl esters, the

experiments continued with the study concerning the receptor ability to be used as a carrier through bulk liquid membrane for a series of amino acids methyl esters.

VI.2.1. Experimental

VI.2.1.1. Materials and apparatus

The transport experiments were run using a U-shape glass tube given in figure 6.14.

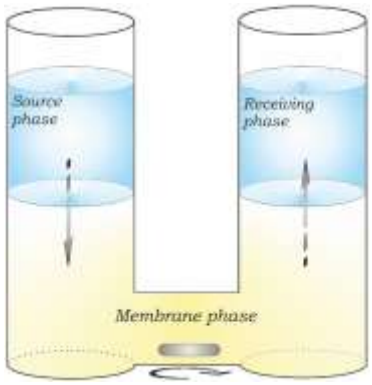


Figure 6.14. *Schematic representation of the device employed in the transport experiments*

The absorbance was determined by spectrophotometric measurements carried out by means of an UV–vis SpectrometerJASCO V-530. The pH was measured by the digital MV-870 Pracitronic pH-meter with glass electrode and saturated calomel electrode.

VI.2.1.2. Procedure

The transport experiments of amino acid methyl esters as ion pairs the presence of tropaeolin 00 as counterion through chloroform liquid membrane were carried out stirring the phases (source, organic and receiving) for 24 h at room temperature.

The phases was prepared as it follows:

- *Source phase* - 5 mL of aqueous solution of amino acid 5.0 x 10^{-4} M, and 8.0 x 10^{-5} M tropaeolin 00 as counterion at pH = 5.5
- *Organic phase (membrane)* - 10 mL of HemiCB[6] 1.0 x10^{-4} M in chloroform
- *Receiving phase* - 5 mL of aqueous solution pH = 1,5 (pH was adjusted using hydrochloric acid 0,1 N)

The concentration of amino acids in both aqueous phases (source and receiving phase) was assessed by UV–vis measurements. Blank experiments were performed for reference.

The transport yield was calculated using the following equation:

$$\eta\ \% = \frac{A_r}{A_0}\ x\ 100 \qquad (6.19)$$

where: A_r – aqueous receiving phase absorbance (after transport);

A_0 - initial absorbance of the aqueous source phase (before transport)

The transport flux was calculated according to the following equation

$$J = \frac{\Delta C_r V}{tA} \qquad (6.20)$$

unde: J – the flux (mol m^{-2} s^{-1})

ΔC_r - the concentration difference of receiving phase (M)

V – the volume of receiving phase (dm^3)

t – time (s)

A – the effective membrane area (m^2)

VI.2.2. Results and discussion

VI.2.2.1. Hemicucurbit[6]uril in bulk liquid membrane transport

The transport of amino acid methyl esters using HemiCB[6] as carrier is realised according to an active transport assisted by the pH gradient and in the presence of tropaeolin 00 as counterion respectively. The transport yields for studied amino acids were calculated acording to equation 6.19 and are given in figure 6.15.

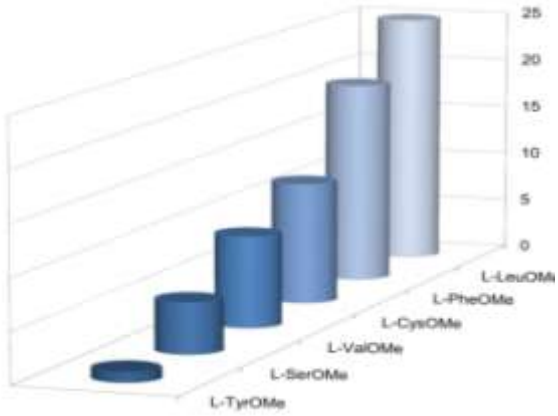


Figure 6.15*. Transport yields of some amino acids methyl esters through bulk liquid membrane using HemiCB[6] as carrier*

Source phase (5 ml): pH = 5,5; $C_{tropeolin\ 00}$ *= 8,0 x* 10^{-5} *M;* $C_{aminoacid}$ *=5,0 x* 10^{-4}*M*

Membrane (10 ml): $C_{HemiCB[6]}$ *= 1,0 x* 10^{-4} *M*

Receiving phase (5 ml): pH=1,5

t = 24h

As can be seen from Figure 6.15 and Table 6.7 the receptor HemiCB[6] exhibited good transportability towards L-LeuOMe, L-PheOMe and L-CysOMe. The transport yields of amino acids are between 1% and 25%, the sequence of transport with HemiCB[6] being realised as it follows:

L-LeuOMe (25%) > L-PheOMe (20%) > L-CysOMe (12%) > L-ValOMe (9%) > L-SerOMe (5%) > L-TyrOMe (1%).

In Table 6.7, the fluxes of amino acids through chloroform liquid membrane of studied amino acids with HemiCB[6] as carrier are presented.

Tabel 6.7. *Transport data of amino acid methyl esters through liquid membrane by HemiCB[6] as carrier in the presence of tropaeolin 00 (t = 24h)*

Amino acid	**Concentration of amino acid in source phase M x 10^{-4}**	**Concentration of amino acid in receiving phase after transport M x 10^{-4}**	**η (%)**	**J_{24} x 10^{8} *(mol m^{-2} s^{-1})***
L-LeuOMe	5,00	1,23	25	7,50
L-PheOMe	5,00	0,98	20	5,95
L-CysOMe	5,00	0,62	12	3,76
L-ValOMe	5,00	0,47	9	2,87
L-SerOMe	5,00	0,27	5	1,66
L-TyrOMe	5,00	0,03	1	0,18

VI.2.2.1.1. Aspects regarding the corelation between extractibility and transport yields of amino acids methyl esters with HemiCB[6]

In figure 6.16 are given the extraction yields (t = 30 min) and transport yields rescpectively (t = 24 h) obtained for the amino acids methyl esters using HemiCB[6] as extraction agent and as carrier.

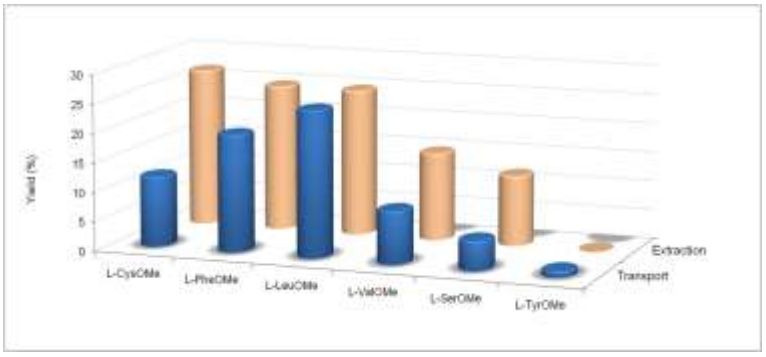


Figura 6.16. *Transport and extraction yields (%) of amino acids methyl esters through liquid membrane by HemiCB[6] as carrier and extractant*

For the amino acids L-PheOMe and L-LeuOM, HemiCB[6] showed similar affinity in both extraction and transport experiments. A large difference in extractibility and transport yield, respectively was observed for L-CysOMe.

VI.2.2.1.2. Steps of transport process through bulk liquid membranes

VI.2.2.1.3. Transport mechanism through bulk liquid membrane using HemiCB[6] as transporter

The proposed transport mechanism of amino acids through bulk liquid membrane is shown in figure 6.17.

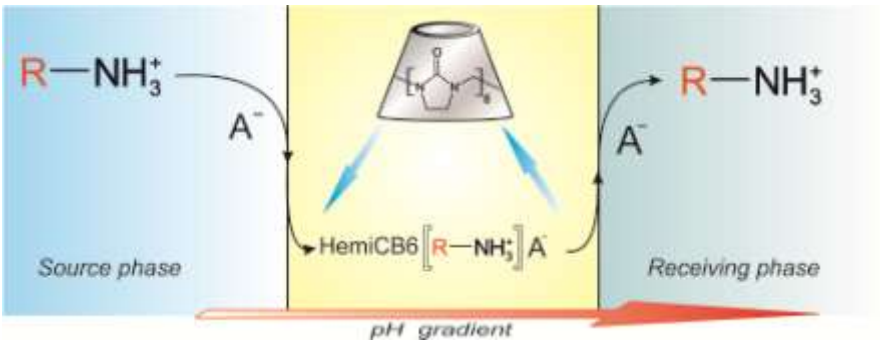


Figure 6.17. *Schematic representation of transport mechanism of amino acids through bulk liquid membrane using HemiCB[6] as carrier*
Source phase (5 ml): pH = 5,5; $C_{tropeolin\ 00}$ *= 8,0 x* 10^{-5} *M;* $C_{aminoacid}$ *= 5,0 x* 10^{-4}*M*
Membrane (10 ml): $C_{HemiCB[6]}$ *= 1,0 x* 10^{-4} *M*
Receiving phase (5 ml): pH = 1,5

It was realised an active transport of amino acids from aqueous source phase into aqueous receiving phase assisted by the pH gradient as ion pairs. The receptor HemiCB[6] forms a supramolecular complex with the amino acids and the counterion at the interface of source phase and organic phase (chloroform). The lipophilic supramolecular complex cross the membrane by diffusion, while at the interface with the receiving phase are insured the optimised condition for amino acid release. The return of the carrier HemiCB[6] to the interface with the source phase after decomplexation is also based on a diffusion process.

VI.2.3. Conclusion

The experimental results regarding the amino acid methyl esters transport through bulk liquid membrane highlighted the ability of receptor HemiCB[6] to activate as a

carrier. The affinity of receptor HemiCB[6] towards amino acic L-LeuOMe (25%) followed bye L-PheOMe (20%) and L-CysOMe (12%).

It was realised an active transport of amino acids from aqueous source phase into aqueous receiving phase assisted by the pH gradient and in the presence of tropaeolin 00 as counterion, respectively. From the liquid membrane transport experiments realised at 24h and 48 h can be noticed with the increase of agitation time an enhancement of the transport yields(L-LeuOMe 28%; L-PheOMe 28%; L-CysOMe 18%, L-ValOMe 15%) and fluxes, respectively.

Chapter VII. separation of some nucleobases by liquid-liquid extraction and transport through bulk liquid membrane

In this chapter was studied the possibility of separation of a series of nucleobases (adenine, cytosine, thymine and uracil) using different synthetic receptors as extraction agents and as carriers through bulk liquid membrane.

GENERAL CONCLUSIONS

The main objective of the present thesis entitled "*Studies of inclusion and separation of some biochemical compounds using macrocyclic receptors*" is the introduction of new macrocyclic receptors in processes of molecular recognition and separation (liquid-liquid extraction and liquid membrane) of a series of biologically relevant compounds. Therefore in these thesis was studied aspects concerning the molecular recognition of the nucleobases (adenine, guanine, cytosine, thymine and uracil) and some amino acids native and methyl esters with the following macrocyclic receptors: hemicucurbit[6]uril, hemicucurbit[12]uril, cucurbit[7]uril, β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin, and the possibility of separation by liquid-liquid extraction and transport through liquid membrane.

The experimental data obtained in the experiments carried out conduct to the following conlcusion:

- ✓ It was highlighted the possible complexation of adeninei and cytosine with cu hemicucurbit[6]uril (HemiCB[6]), receptor used for the first time in molecular recognition of biologically relevant compounds.

- ✓ The experimental data obtained through UV-Vis spectrometry and fluorescence spectroscopy regarding nucleobases complexation with β-cyclodextrin indicated the formation of 1:1 complex in the studied concentrations. The values of association constant determined through both spectroscopic methods were presented comparatively. The possible formation of a 1:1 β-CD–thymine complex was suggested by means of UV-Vis spectrometry.
- ✓ The influence of the functional groups attached to the cyclodextrinic ring on the complexation process was highlighted by the results obtained in the study of cytosine complexation with 2-hydroxypropyl-β-cyclodextrin.
- ✓ Receptors hemicucurbit[6]uril and hemicucurbit[12]uril was used for the first time in separation processes of a series od amino acids native (*L-Phe, L-Leu, L-Ile, L-Val, L-Cys, L-Trp*) and methyl esters (*L-PheOMe, L-LeuOMe, L-SerOMe, L-CysOMe, L-ValOMe, L-TyrOMe*). The experimental results obtained in the liquid-liquid extraction of the studied amino acids with HemiCB[6] and HemiCB[12] highlighted the affinity of receptors for the amino acids methyl esters.
- ✓ The ability of the receptor HemiCB[6] to act as a carrier through bulk liquid membrane was highlighted by the results obtained in the transport experiments of some amino acids methyl esters through liquid membrane. It was realised an active transport of amino acids through bulk liquid membrane assisted by the pH gradient and in the presence of tropaeolin 00 as counterion, respectively.
- ✓ The study of amino acids transport through liquid membrane at both 24 h and 48 h was observed an enhancement of the transport yield and flux of amino acids with the increasing of agitation time.
- ✓ The experimental data obtained in the transport of adenine through bulk liquid membrane using D_2EHPA as carrier highlight the influence on the transport yield of the concentration of receptor in the organic phase and of the adenine in the source phase, respectively. It was realised an active transport assisted by a pH gradient between the source phase and the receiving phase.
- ✓ In conclusion, the studied receptors : hemicucurbit[6]uril, hemicucurbit[12]uril, β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, calix[4]arena, p-tert-butyl-calix[4]arena, p-tert-butyl-calix[6]arena, 5,11,17,23-tetrakys(N-methylpiperazine) 25,26,27,28-tetrahydroxycalix[4]arena, calix[6]arena and di(2-ethylhexyl) phosphoric acid might be used in analitical application, in particular in separation processes of some biologically relevant compounds.

References list selective

1. J.-M. Lehn, *Supramolecular Chemistry. Concepts and Perspectives*, VCH, Weinheim, 1995.
2. J.-M. Lehn, *Angew. Chem. Int. Ed.* 2013, 52, 2836 – 2850.
3. E.V. Anslyn, *J. Org. Chem.* 2007, 72, 687-699.
4. L. You, D. Zha, E.V. Anslyn, *Chem. Rev*. 2015, 115, 7840−7892.
5. H.-J. Schneider, *Int. J. Mol. Sci*. 2015, 16, 6694−6717.
6. X. Ma, Y. Zhao, *Chem. Rev*. 2015, 115, 7794−7839.
7. L. Mutihac, J. H. Lee, J. S. Kim, J. Vicens, *Chem. Soc. Rev*. 2011, 40, 2777-2796.
8. O. Danylyuk, K. Suwinska, *Chem. Commun.* 2009, 5799-5813.
9. L.A. Logsdon, C.L. Schardon, V. Ramalingam, S. K. Kwee, A.R. Urbach, *J. Am. Chem. Soc.* 2011, 133,17087–17092
10. A.R. Urbach, V. Ramalingam, *Isr. J. Chem*. 2011, 51, 664–678.
11. L.A. Logsdon, C.L. Schardon, V.Ramalingam, S.K. Kwee, A.R. Urbach, J. *Am. Chem. Soc*. 2011, 133, 17087
12. M.V. Rekharsky, H. Yamamura, Y.H. Ko, N. Selvapalam, K. Kim, Y. Inoue, *Chem. Commun. 2*008, 2236-2238.
13. Y. Tauran, B. Kim, A.W. Coleman, *Journal of Nanoscience and Nanotechnology* 2015, 15, 1–19.
14. G. Ghale, W. M. Nau, *Acc. Chem. Res*. 2014, 47, 2150-2159.
15. K.I. Assaf, M.S. Ural, F. Pan, T. Georgiev, S. Simova, K.Rissanen, D. Gabel, W.M. Nau, *Angew. Chem. Int. Ed.* 2015, 54, 6852 –6856.
16. M. Sayed, M. Sundararajan, J.Mohanty, A.C. Bhasikuttan, H. Pal, *J. Phys. Chem. B* 2015, 119, 3046−3057.
17. J. Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem., Int. Ed*., 2005, 44, 4844-4870.
18. E. Masson, X. Ling, R. Joseph, L. Kyeremeh-Mensah, X. Lu, *RSC Adv.* 2012, 2, 1213−1247.
19. L. Isaacs, *Acc. Chem. Res*. 2014, 47, 2052-2062.
20. K.I. Assaf, W.M. Nau, *Chem. Soc. Rev***.** 2015, 44, 394-418.

21. F. Biedermann, V. D. Uzunova, O. A. Scherman, W. M. Nau, A. De Simone, *J. Am. Chem. Soc.* 2012, 134, 15318-15323.
22. S. J. C. Lee, J. W. Lee, H. H. Lee, J. Seo, D. H. Noh, Y. H. Ko, K. Kim, H. I. Kim, *J. Phys. Chem. B*, 2013, 117, 8855-8864.
23. L. Gilberg, M. S. A. Khan, M. Enderesova, V. Sindelar, *Org. Lett*. 2014, 16, 2446.
24. T. Minami, N. A. Esipenko, A. Akdeniz, B. Zhang, L. Isaacs, P. Anzenbacher, Jr. *J. Am. Chem. Soc*. 2013, 135, 15238−15243.
25. Y. Miyahara, K. Goto, M. Oka, T. Inazu, *Angew. Chem. Int*. 2004, 43, 5019-5022.
26. M. Sundararajan, R.V. Solomon, S.K. Ghosh, P. VenUV-Visanalingam, *RSC Advances*, 2011, 1, 1333-1341.
27. J. Svec, M. Necas, V. Sindelar, *Angew. Chem., Int. Ed*. 2010, 49, 2378-2381.
28. W. L. Mock, T. A. Irra, J. P. Wepsiec , T. L. Manimaran, *J. Org. Chem*. 1983, 48, 3619-3620.
29. M. Sěkutor, K. Molcănov, L. P. Cao, L. Isaacs, R. Glaser, K. Mlinaric-Majerski, *Eur. J. Org. Chem*. 2014, 12, 2533-2542.
30. L. P. Cao, M. Sěkutor, P. Y. Zavalij, K. Mlinaric-Majerski, R. Glaser, L. Isaacs, *Angew. Chem., Int. Ed*., 2014, 53, 988-993.
31. J. M. Chinai, A. B. Taylor, L. M. Ryno, N.D. Hargreaves, C. A. Morris, P. John Hart, A. R. Urbach, *J Am Chem Soc.* 2011, 133, 8810-8813.
32. X. Feng, X.-J. Lu, S.-F. Xue, Y.-Q. Zhang, Z. Tao, Q.-J. Zhu, *Inorg. Chem. Commun*., 2009, 12, 849-852.
33. E. A. Mainicheva, A. A. Tripolskaya, O. A. Gerasko, D. Y. Naumov, V. P. Fedin, *Russ. Chem. Bull.* 2006, 55, 1566-1573.
34. M. N. Sokolov, E. V. Chubarova, K. A. Kovalenko, I. V. Mironov, A. V. Virovets, E. V. Peresypkina,V. P. Fedin, *Russ. Chem. Bull*. 2005, 54, 615-622.
35. G. Celtek, M. Artar, O. A. Scherman, D. Tuncel, *Chem. – Eur. J.* 2009, 15, 10360-10363.
36. H. Cong, T. Yamato, Z. Tao, *J. Mol. Catal. A: Chem*. 2013, 379, 287-293.
37. R. B. Wang, L. N. Yuan, D. H. Macartney, *J. Org. Chem*. 2006, 71, 1237-1239.
38. C. Li, J. Feng, H. Ju, *Analyst* 2015, 140, 230-235.
39. L. Cao, G. Hettiarachchi, V. Briken, L. Isaacs, *Angew. Chem. Int. Ed. E*ngl. 2013, 52, 12033–12037.

40. H. Huang, Q.-H. Hu, G.-X. Song, Z. Tao, S.-F. Xue, Q.-J. Zhu, Q.-d. Zhou, G. Wei, *RSC Adv*. 2014, 4, 3348-3354.
41. D. Ma, B. Zhang, U. Hoffmann, M.G. Sundrup, M. Eikermann, L. Isaacs, *Angew. Chem. Int. Ed*. 2012, 51, 11358-11362.
42. B. Zhang, P.Y. Zavalij, L. Isaacs, *L. Org. Biomol. Chem*. 2014, 12, 2413-2422.
43. R. N. Dsouza, A. Hennig, W. M. Nau, *Chem. – Eur. J*. 2012, 18, 3444-3459.
44. G. Ghale, V. Ramalingam, A. R. Urbach, W. M. Nau, *J. Am. Chem. Soc*. 2011, 133, 7528-7535.
45. F. Biedermann, D. Hathazi, W.M. Nau, *Chem. Commun.* 2015, 51, 4977-4980.
46. T. Minami, N. A. Esipenko, B. Zhang, L. Isaacs, P. Anzenbacher, *Chem. Commun.* 2014, 50, 61-63.
47. S. J. Barrow, S. Kasera, M.J. Rowland, J. del Barrio, O. A. Scherman, *Chem. Rev*. 2015, 115, 12320−12406.
48. G. Crini, *Chem. Rev*. 2014, 114, 10940−10975.
49. H. Dodziuk, *Cyclodextrins and Their Complexes*, Wiley-VCH, Weinheim, 2006
50. Y.-L. Zhao, Z. Li, S. Kabehie, Y.Y. Botros, J.F. Stoddart, J.I. Zink, *J. Am. Chem. Soc.* 2010, 132, 13016-13025.
51. S. V. Kurkov, T. Loftsson, *Int. J. Pharm*. 2013, 453, 167– 180.
52. P. Thordarson**,** *Chem. Soc. Rev***.** 2011. 40, 1305-1323.
53. Q. Ma, X. Yuan, Y. Zhao, L. Ren, *RSC Adv*. 2015,5, 47998-48004 .
54. K. A. Connors, *Binding Constants: The Measurement of Molecular Complex Stability*, John Wiley & Sons, New York, NY, 1987.
55. K. Hirose, J. *Incl. Phenom. Macrocyclic Chem.* 2001, 39, 193–209.

DISSEMINATION OF THE RESULTS

Articles published in ISI journals:

1. *Hemicucurbiturils as receptors in extraction and transport of some amino acids(online)*
   E.I. Cucolea, H.-J. Buschmann, L. Mutihac
   Supramol. Chem. DOI: 10.1080/10610278.2015.1121267, 2016 (FI: 2.394)

2. *Interactions of cucurbit[7]uril and β-Cyclodextrin with some nucleobases*
   E.I. Cucolea, C. Tablet, H.-J. Buschmann, L. Mutihac
   J. Incl. Phenom. Macrocycl. Chem**.** 83: 103-110, 2015 (FI: 1.488)

Participations at national and international conferences

1. *Binding affinity of cucurbit[7]uril and β-cyclodextrin towards some nucleobases*
   E.I. Cucolea, C. Tablet, L. Mutihac
   10th International Symposium on Macrocyclic and Supramolecular Chemistry (ISMSC 2015), 28.06.2015 - 02.07.2015, Strasbourg, Franța (Poster)

2. *Binding properties of cucurbit[7]uril and β-cyclodextrin towards adenine and guanine*
   E.I. Cucolea, C. Tablet, L. Mutihac
   Sesiunea de Comunicări Științifice Studențești, 22 Mai 2015, Facultatea de Chimie, Universitatea din București (Oral presentation)

3. *Cyclodextrin –based supramolecular complexes*
   E. Diacu, E.I. Cucolea, L. Mutihac
   XXXIII-a Conferință națională de chimie 1-3 Octombrie, 2014 Călimănești-Căciulata, Vâlcea, Romania (Poster)

4. *Fluorescence and UV-Vis studies of the host-guest complexes of nucleobases with cyclodextrins and cucurbituril*
   E.I. Cucolea, C. Tablet, L. Mutihac
   2nd International Conference on Analytical Chemistry RO - ICAC'2014 - Analytical Chemistry for a Better Life, 17-21 septembrie 2014, Târgoviște, Romania (Poster)

5. *Functionalized calixarene used in bioanalytical applications*
   L. Mutihac, E.I. Cucolea, J. Vicens
   2nd International Conference on Analytical Chemistry RO - ICAC'2014 - Analytical Chemistry for a Better Life, 17-21 septembrie ,Târgoviște, Romania (Invited lecture)