JAN DLUGOSZ UNIVERSITY IN CZESTOCHOWA

Faculty of Mathematics and Natural Sciences

Tomasz Girek, Ph.D.

Summary of professional accomplishments

Cyclodextrin polymers crosslinking by dicarboxylic acid anhydrides – structure and application

Czestochowa, 2014

Spis treści

1. Given name and surname

Tomasz Girek

2. Diplomas, scientific degrees held – stating name, place and the year in which they were acquired

I acquired a Master's degree in chemistry with teaching specialization in 1987 at the Faculty of Mathematics and Natural Sciences of the Higher Teacher Education School in Czestochowa. The title of my Master's thesis was: „**N-substituted derivatives of azaaromatic systems and their reactivity**". Supervisor - dr Teresa Zujewska, Ph.D.

I acquired my Ph.D. degree in Chemistry in 1994 at the Faculty of Mathematics, Physics and Chemistry of the Silesian University, on the grounds of my doctoral dissertation on: **"Cyclization reactions of diazaphenanthrene derivatives"** prepared under the supervision of Professor Wanda Śliwa, D.Sc. (Habilitated)

3. Information about employment in scientific establishments to date

1994.10.01 – to date	Assistant Professor in the Department of Organic Chemistry of the Institute of Chemistry, Environmental Protection and Biotechnology in the Higher Teacher Education School in Częstochowa (from October 1, 2004 – The Jan Dlugosz University in Częstochowa)
1987.10.01 – 1994.09.30	assistant leader in the Department of Organic Chemistry of the Institute of Chemistry of the Higher Teacher Education School in Częstochowa
1987.03.06 – 1987.09.30	intern in the Department of Organic Chemistry of the Institute of Chemistry of the Higher Teacher Education school in Czestochowa

4. Presentation of a scientific accomplishment, resulting from Article 16 (2) of the Act on University Degrees and the University Title and on University Degrees and the University Title in the Field of Fine Arts of March 14, 2003 (Dz. U. [Law Gazette] No 65, item 595, with later amendments):

4.1. The title of the scientific/artistic accomplishment:

Cyclodextrin polymers cross-linking by
dicarboxylic acid anhydrides – structure and application.

4.2. Publications connected with the scientific accomplishment:

The list underneath includes a series of 14 publications that fall within the scope of this habilitation dissertation [H1-H14]. They are papers devoted mostly to cyclodextrin polymers crosslinking by carboxylic acid anhydrides, whereas one paper frames a further direction of my research devoted to cyclodextrin-protein conjugates, which is the subject I am working on at present.

The papers are listed in the sequence of discussing them in this presentation.

[H1] **Tomasz Girek**, Dong-Hoon Shin, Seung-Taik Lim; *Polymerization of β-cyclodextrin with maleic anhydride and structural characterization of the polymers*, Carbohydrate Polymers, 2000, 42, (1), 59–63. (IF=1,184)
My input of work in the implementation of this project included planning of the research, unassisted making of all the syntheses, unassisted carrying out of all HPLC-SEC-RI-MALLS chromatography measurements of molecular weights of the obtained samples, making complete analyses of the HMNR spectra, interpretation of results, and writing the manuscript. I assess my percentage share of the work to be 80%.

[H2] J.-K. Choi, **T. Girek**, D.-H. Shin, S.-T. Lim; *Structural and physical characterization of octenylsuccinyl β-cyclodextrin*; Carbohydrate Polymers 2002, 49, 286-296 (IF=1,655)
My input of work in the implementation of this project included planning of the research, help in carrying out syntheses and HPLC-SEC-RI-MALLS chromatography measurements of molecular weights of the obtained samples, making analyses of the HNMR spectra, interpretation of the results of the study and co-editing the manuscript. I assess my percentage share of the work to be 40%.

[H3] W. Śliwa, **T. Girek**; *Metallocyclodextrins and related species*; Heterocycles, 2003, 60, 2147 (IF=1,082)
My input of work in the implementation of this project included reviewing the resources, partial analysis of the collected material, writing a number of subchapters of the manuscript and making all the diagrams. I assess my percentage share of the work to be 50%.

[H4] **T. Girek**, C.A. Kozłowski, J. J. Kozioł, W. Walkowiak, I. Korus, *Polymerisation of β-cyclodextrin with succinic anhydride. Synthesis, characterization, and ion flotation of transition metals*, Carbohydrate Polymers, 2005, 59, 211-215 (IF=1,583)
My input of work in the implementation of this project included planning part of the research, unassisted making of all the syntheses, help in carrying out HPLC-SEC-RI chromatography measurements of molecular weights of the obtained samples, and help in carrying out experiments connected with making use of the obtained polymers in the processes of ion flotation, partial interpretation of the results of chromatography, carrying out analysis of the NMR spectra partial interpretation of the results of the study, and co-editing of the manuscript. I assess my percentage share of the work to be 45%.

[H5] W. Śliwa, **T.Girek**, J.J.Kozioł; *Cyclodextrin oligomers*; Current Organic Chemistry, 2004, 8, 1445-1462 (IF=2,775)
My input of work in the implementation of this project included reviewing the resources, partial analysis of the collected material, writing a number of subchapters of the manuscript and making all the diagrams. I assess my percentage share of the work to be 45%.

[H6] C. A. Kozłowski, T**. Girek**, W. Walkowiak, J. Kozłowska, *The effect of β-CD polymers structure on the efficiency of copper (II) ion flotation*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2006, 55 (1-2), 71-77 (IF=1,251)
My input of work in the implementation of this project included planning part of the research, unassisted making of all the syntheses, carrying out HPLC-SEC-RI chromatography measurements of molecular weights of the obtained samples and participation in carrying out experiments connected with making use of the obtained polymers in the processes of ion flotation, interpretation of the results of chromatography, carrying out analysis of the NMR spectra, partial interpretation of the results of the study, and co-editing of the manuscript. I assess my percentage share of the work to be 45%.

[H7] W. Sliwa, **T. Girek**; *Noncovalently-bound cyclodextrin Dimers and related compounds,* Chemistry of Heterocyclic Compounds, 2005, 41, 1343-1361 (IF=0,134)
My input of work in implementation on this project included reviewing the resources, partial analysis of the collected material, writing a number of subchapters of the manuscript and making all the diagrams. I assess my percentage share of the work to be 50%.

[H8] C.A. Kozłowski, **T. Girek**, W. Walkowiak, J. J. Kozioł, *Application of hydrophobic β-cyclodextrin polymer in separation of metal ions by plasticized membranes*, Separation and Purification Technology, 2005, 46, 136-144. (IF=1,752)
My input of work in the implementation of this project included planning part of the research, unassisted making of all the syntheses of polymer samples, carrying out HPLC-SEC-RI chromatography measurements of molecular weights of the obtained samples and participation in carrying out experiments connected with making use of the obtained polymers in the processes of obtaining the membranes, interpretation of the results of

chromatography, carrying out analysis of the NMR spectra, and co-editing of the manuscript. I assess my percentage share of the work to be 45%.

[H9] C.A. Kozlowski, W. Walkowiak, **T. Girek**, *Modified cyclodextrin polymers as selective Ion carriers for Pb(II) separation across plasticized membranes*, Journal of Membrane Science, 2008, 310(1+2), 312-320 (IF=3,247)
My input of work in the implementation of this project included planning part of the research, unassisted making of all the syntheses of polymer samples, carrying out HPLC-SEC-RI chromatography measurements of molecular weights of the obtained samples and participation in carrying out experiments connected with making use of the obtained polymers in the processes of obtaining the membranes, interpretation of the results of chromatography, carrying out analysis of the NMR spectra, partial interpretation of the results of the study, and co-editing of the manuscript. I assess my percentage share of the work to be 45%.

[H10] **T. Girek**, W. Ciesielski, *Polymerization of β-cyclodextrin with maleic anhydride along with thermogravimetric study of polymers*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2011, 69(3-4), 445-451
My input of work in the implementation of this project included planning the research, unassisted making of all the syntheses of polymer samples, participation in thermogravimetric analyses with the use of the NETSCH STA 409 thermal analyzer, interpretation of the results of the DSC-TG-DTG studies, and co-editing of the manuscript. I assess my percentage share of the work to be 50%.

[H11] **T. Girek**, W. Ciesielski, *Polymerization of β-cyclodextrin with succinic anhydride and thermogravimetric study of the polymers*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2011, 69(3-4), 439-444
My input of work in the implementation of this project included planning the research, unassisted making of all the syntheses of polymer samples, participation in thermogravimetric analyses with the use of the NETSCH STA 409 thermal analyzer, interpretation of the results of the DSC-TG-DTG studies, and co-editing of the manuscript. I assess my percentage share of the work to be 50%.

[H12] **Girek, T**.: *Cyclodextrin-based rotaxanes*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2012, 74(1-4), 1-21
My input of work in implementation on this project included reviewing the resources, analysis of the collected material, writing the manuscript and making all the diagrams. I assess my percentage share of the work to be 100%.

[H13] **Girek, T**.: *Cyclodextrin-based polyrotaxanes*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2013, 76, 237-252

My input of work in implementation on this project included reviewing the resources, analysis of the collected material, writing the manuscript and making all the diagrams. I assess my percentage share of the work to be 100%.

[H14] **Girek T.**, Goszczynski T., Girek B., Ciesielski W., Boratynski J., Rychter P.: *β-Cyclodextrin/protein conjugates as innovative drug systems: synthesis and MS investigation*, Journal of Inclusion Phenomena and Macrocyclic Chemistry., 2013, 75 293-296

My input of work in the implementation of this project included planning part of the research, unassisted making of all the syntheses of cyclodextrin derivatives, analyzing the NMR and MS spectra, partial interpretation of results of the study and co-editing of the manuscript. I assess my percentage share of the work to be 30%.

4.3. Discussing academic objectives of the listed publications and the obtained results and discussing their potential use:

Papers marked as H1-H14 in this presentation refer to the works listed in 4.2.

Introduction

After my return from an internship in Japan, I started to search for new areas of the science of chemistry in which I could develop my scientific interests. I decided to take advantage of the acquired experience in the research on products of natural origin. In order to acquire proper knowledge and experience in working on cyclodextrin systems I took advantage of an opportunity to go for a one-year scientific internship to the Graduate School of Biotechnology, at the Korea University in Seoul, in South Korea, to work in Professor Seung-Taik Lim's research group. My work in Professor Lim's team enabled me to gain experience in biotechnological research on natural carbohydrate polymers, particularly in acquiring knowledge of numerous chromatographic techniques, including the technique of determining molecular weights of starch and cyclodextrin polymers with the use of HPLC-SEC-RI-MALLS chromatography.

Since then, cyclodextrins, their modifications and possibility of polymerization and application of cyclodextrin polymers have been my dominant scientific interest.

Cyclodextrins – basic facts

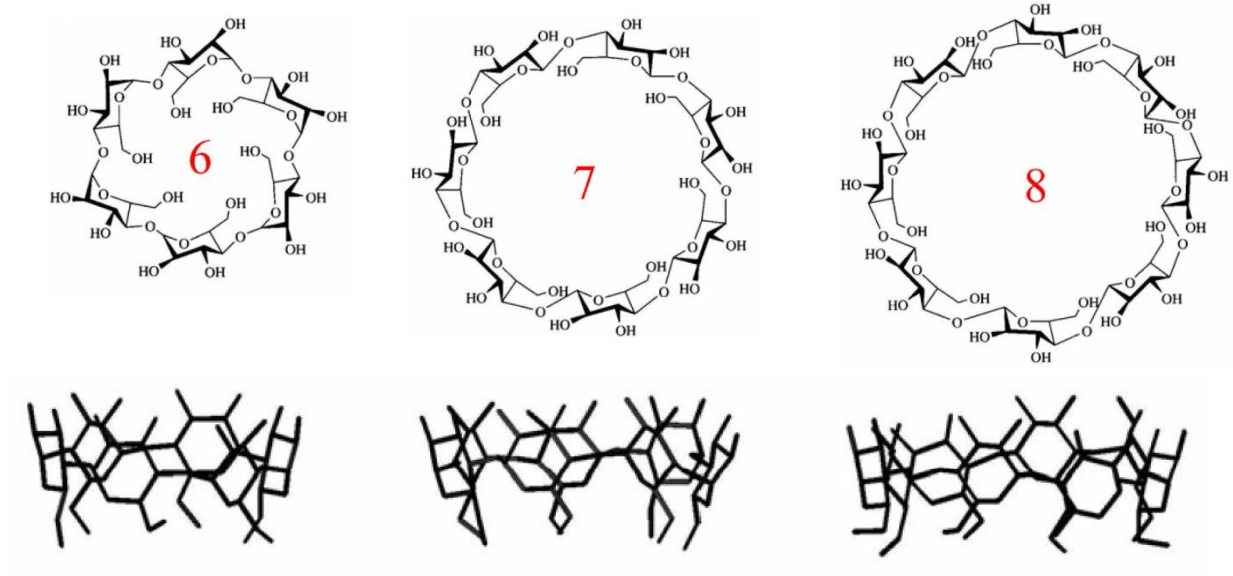
Cyclodextrins are molecules highly interesting for science and all sorts of industry; they are interesting to be studied and they find numerous applications in different fields of science and technology, *e.g.*: pharmacy and medicine, food industry, cosmetology and agriculture, in different fields of environmental protection and biotechnology. They are

semi-natural products, produced from natural material, starch, by means of simple enzymatic conversion. At present, they are produced in large quantities (thousands of tons per year) by means of environmentally friendly technologies. Their chemical properties can be easily and significantly modified. Any of their toxic effects are of secondary importance and can be eliminated by selecting the appropriate CD type or derivative or mode of application. CDs can be consumed by humans as ingredients of drugs, foods, or cosmetics. There are many commercial products on the market in which CDs are used.

Cyclodextrins comprise a family of three well known, industrially produced major, and several rare, minor cyclic oligosaccharides.

The three major cyclodextrins are crystalline, homogeneous, nonhygroscopic substances, which are torus-like macro-rings built up from glucopyranose units.

- α-cyclodextrin, (Schardinger's α-dextrin, cyclomaltohexaose, cyclohexaglucane, cyclohexaamylose, α-CD, ACD, C6A) comprises six glucopyranose units,
- β-cyclodextrin (Schardinger's β-dextrin, cyclomaltoheptaose, cycloheptaglucane, cycloheptaamylose, β-CD, BCD, C7A) comprises seven glucopyranose units,
- γ-cyclodextrin (Schardinger's γ-dextrin, cyclomaltooctaose, cyclooctaglucane, cyclooctaamylose, γ-CD, GCD, C8A) comprises eight glucopyranose units.

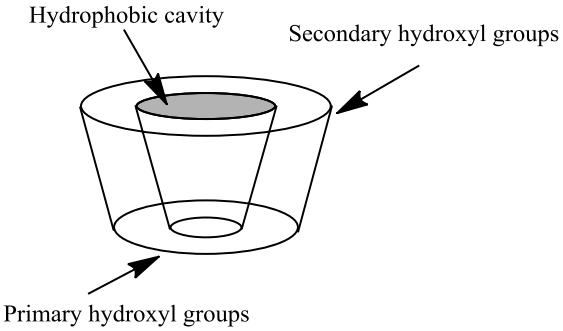


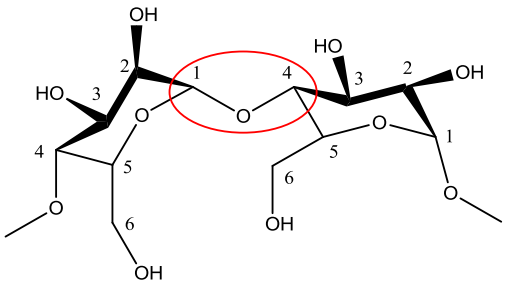
α-cyclodextrin **β-cyclodextrin** **γ-cyclodextrin**

The cyclodextrin ring is in fact a cylinder or, to be more precise, a conical cylinder, which is frequently characterized as a flowerpot-, doughnut- or wreath-shaped truncated cone. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges, respectively. The nonbonding electron pairs of the glycosidic oxygen bridges are directed

toward the inside of the cavity producing a high electron density there and lending to it some Lewis base characteristics. It is very important for creating inclusion complexes, where cyclodextrins are used as host molecules.

Individual glucopyranose molecules are joined by 1-4-glycosidic links. At the same time, all secondary hydroxyl groups are situated on one of the two edges of the ring (the wider one), whereas all the primary hydroxyl groups are placed on the other edge (the narrower one)**.**





Physical chemical properties of cyclodextrins are well known and widely described in all sorts of sources [1-18].

There is an untypical solubility of cyclodextrins in water that deserves attention, however. At room temperature, solubility of individual cyclodextrins in water is, as follows: α-CD = 14,5g/100ml; β-CD = 1,85 g/100ml; γ-CD = 23,2 g/100ml. Explanation for this untypical solubility of β-CD can be found in the ability of individual CDs to form intra- and intermolecular hydrogen bonds.

The hydroxyl group C-2-OH of one glucopyranose unit can form hydrogen bonds with the hydroxyl group at C-3-OH neighboring glucopyranose units. By this mechanism, a belt of hydrogen bonds is formed around the cyclodextrin molecule, which gives rigidity to the whole molecule. These intra-molecular bonds are probably responsible for the low solubility of β-CD. The belt of hydrogen bonds in α-CD molecule consists of only four bonds, which makes it impossible for one glucopyranose unit to create a bond, so the OH groups can participate in the process of dissolution in water by forming intermolecular hydrogen bonds. Whereas, the most flexible due to its size γ-CD, is also the most soluble. [3]

β-cyclodextrin dissolves relatively well in glycerin (43 g of β-cyclodextrin in 100 ml of glycerin at 25^{o}C) as well as in some aprotic solvents, such as dimethylformamide (DMF) or dimethyl sulfoxide (DMSO).

Application of β-CD (the most popular and, at the same time, the cheapest) can be extended by modifications of primary and secondary hydroxyl groups. This can result in higher "flexibility" of a molecule, and above all it is also a simple way to solve the problem of poor solubility. For example, any –OH group can be subjected to methylation or hydroxyalkilation. The developed β-CD derivatives display a much higher solubility than their mother cyclodextrins [18].

Cyclodextrin modifications

The process of cyclodextrin modifications is a challenge for a chemist and an opportunity to obtain new and interesting derivatives. However, it can be, due to the presence of a hydrophobic cavity and a large number of hydroxyl groups, quite complicated.

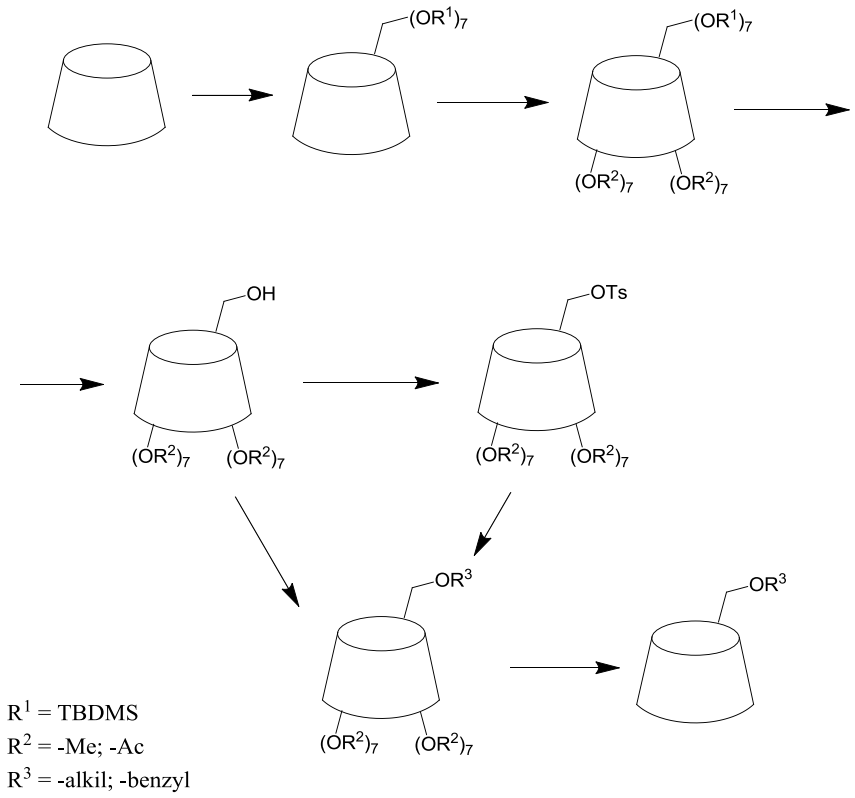
The –OH group placed on positions "2", "3" and "6" compete with one another in reacting with different substances, which makes selective modification extremely difficult. On the other hand, the hydrophobic cavity tends to complex reacting substances, which unpredictably changes their reactivity [14].

There are many reasons for carrying out cyclodextrin modifications, *e.g.* achieving solubility in a desired solvent, or facilitating observation of enzymatically catalyzed model reactions. A strategy of modification depends on the planned use of the final product. Chemical modifications of cyclodextrins contribute to changes in their properties, which on many occasions increase their usefulness in industry and particularly in studies conducted on new pharmaceutical preparations [19-22] or artificial enzymes [23-25].

There are many methods for selective modification of cyclodextrins, yet the methods for selective modification of cyclodextrin can be divided into three categories: [14]:

The "**clever**" method; where the chemistry of cyclodextrin is exploited to get the desired product by the shortest route. It is a "clean" method and very successful, but it is very rarely used, because only a few substrates used in modifications can create stable and well oriented host-guest type complexes with a cyclodextrin molecule. An example of such a reaction is tosylating of the secondary hydroxyl groups in position "2". The product was obtained by reacting β-cyclodextrin with m-nitrophenol tosylate. In this synthesis, the complexation property of cyclodextrin is taken advantage of, which resulted in the tosyl group being sent to the secondary side. This avoids the natural tendency of cyclodextrin to react on its primary side and predominantly gives cyclodextrins substituted at the 2-position.

The "**long**" method; where a series of reactions involving protection and deprotection of individual –OH groups take place in order to selectively reach the selectively accessible positions. The method is mainly used for alkylation of the primary hydroxyl groups [26]



The third method used for selective modification of cyclodextrins is called the "**sledgehammer**" method, where cyclodextrin indiscriminately reacts with various compounds and gives a mixture of products and then the desired product is painstakingly separated out from other isomers and/or homologues by means of chromatographic methods. A very good example here is ditosylation of the secondary hydroxyl groups of cyclodextrin. In this case, tosyl chloride is reacted with cyclodextrin to give a mixture of products. This mixture is separated using reverse phase HPLC.

Creating appropriate reaction conditions and making use of insignificant differences in chemical properties of the primary and secondary hydroxyl groups it is possible in a way to predict and due to this in a way monitor the course of modification of cyclodextrins.

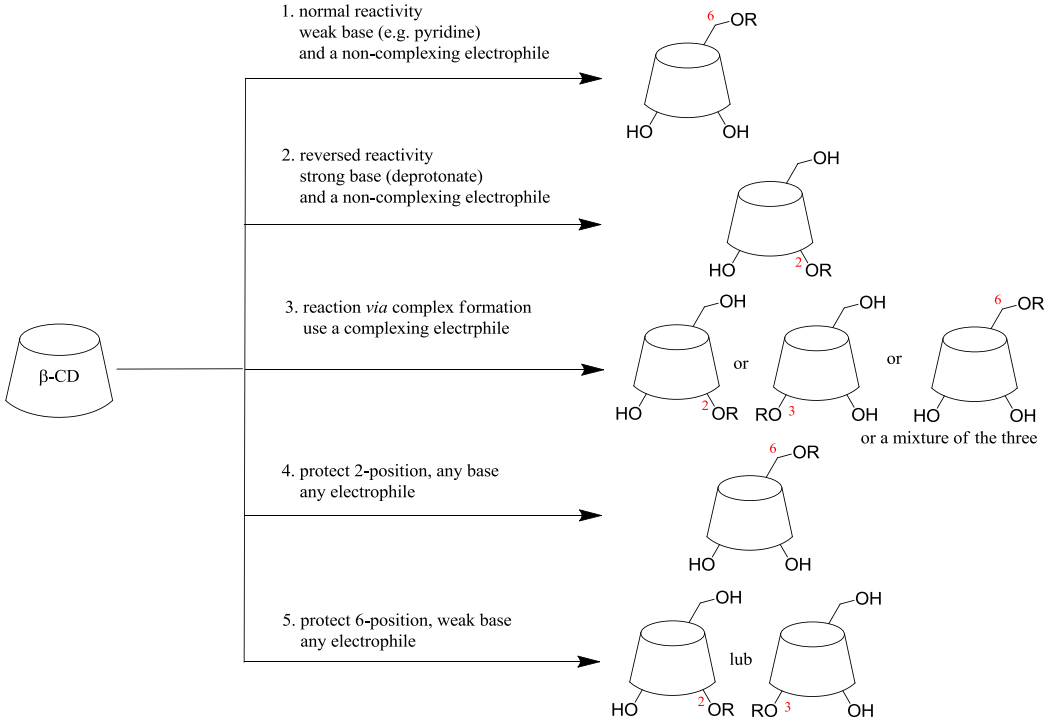
Of the three types of hydroxyl groups present in cyclodextrins, those at the 6th-position are the most basic (and often most nucleophilic), those at the 2-position are the most acidic. Those at the 3-position are the most inaccessible. In normal conditions the electrophile attacks positions "6".

An interesting trait of the CD is its complexation property, which can be used for its modification. If an electrophile forms a complex with the cyclodextrin, the orientation of the reagent within the complex will result in the necessity to introduce an additional agent, which will allow to determine the character of a product.

In order to avoid complications connected with "trapping" of the reagent in the cyclodextrin cavity it is necessary to protect some –OH groups and send the reagent only to the other –OH groups.

For example, one reagent is used to protect position "2", and another reagent is used to the drive the electrophile to position "6".

Similarly, the protection of the primary rim makes it possible for the attacking electrophile to direct its action only at the hydroxyl group set in position “2”.
The general diagram of the possibilities of cyclodextrin modifications is presented in the Figure below:



Cyclodextrin polymers

Cyclodextrin polymers are cyclodextrin derivatives characterized by high molecular weight, exceeding 3000Da. During polymerization of certain cyclodextrin derivatives, homopolymers are created. Whereas, copolymers are created as a result of reactions with bi- and poly-functional compounds, characterized by a possibility to couple with cyclic dextrin hydroxyl groups. The latter ones are also called “cyclodextrin resins”. Up to a definite molecular weight, polymers are water soluble. Increase of molecular weight and of the networking results in creation of a gel structure, insoluble in water [27-31].

The model of cyclodextrin polymers is based on the reactivity of the –OH groups, or on their properties to form complexes of the host-guest type with polymer chains or with side chains of the polymer skeleton due to hydrophobic forces. It can be predicted that such systems can be used for specific applications, *e.g*. in the processes of separation, catalysis, and controlled release of biologically active compounds.

In recent years, CDs and their derivatives have been frequently used as monomers to build various polymer networks and their complexes. Polymer materials, including hydro-gels, nano-materials and micelles, are often examined by pharmaceutical and biomedical concerns in terms of their application for release and directed supply of bioactive substances (*e.g*. drugs of low molecular weight, peptides, proteins, and genetic materials, such as pDNA

and siRNA), in tissue engineering and medical diagnostics [32-34]. Many new polymer networks, which have been designed recently, are either chemically (permanently) cross-linked or physically bonded which gives them exceptional mechanical properties and appropriate characteristics of drug release.

Polymer networks containing CD were initially used as fillings in chromatography, whereas since 1980 they have found their application in pharmaceutics. The first polymers were synthesized by chemical cross-linking of CD (α, β or γ) with epicholrohydrin (EPH) used as a bi-functional cross-linking agent in the alkaline media, which leads to formation of polymer hydro-gels. Numerous studies have revealed that these networks have the property of complexation of many different drugs of low water-solubility [27,35].

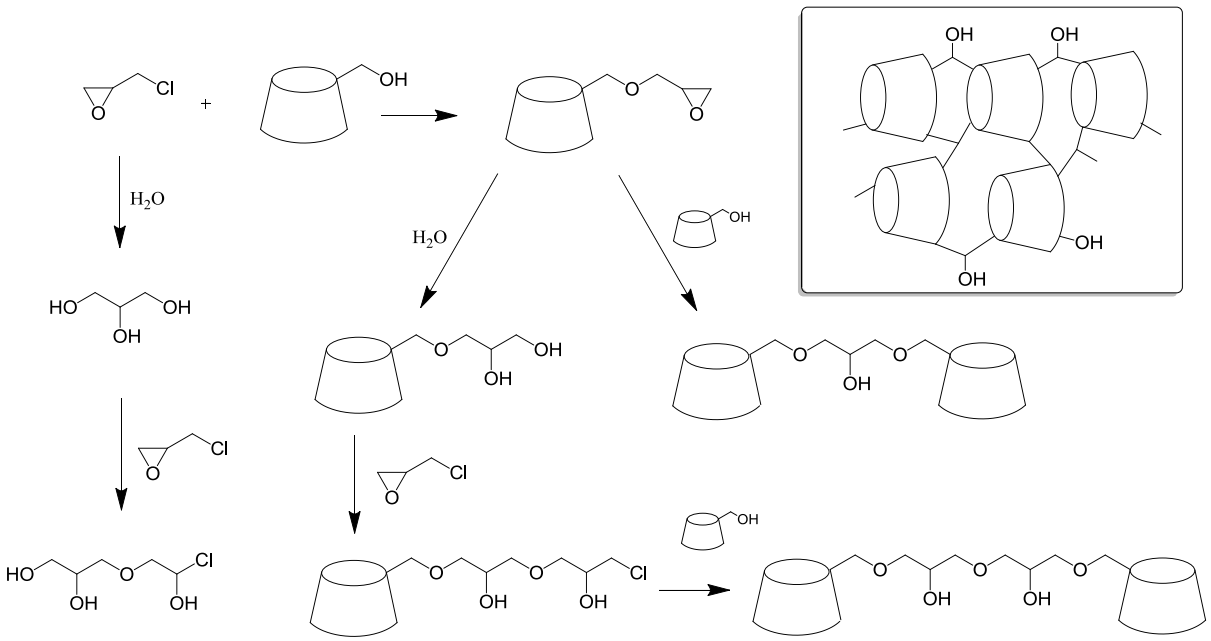


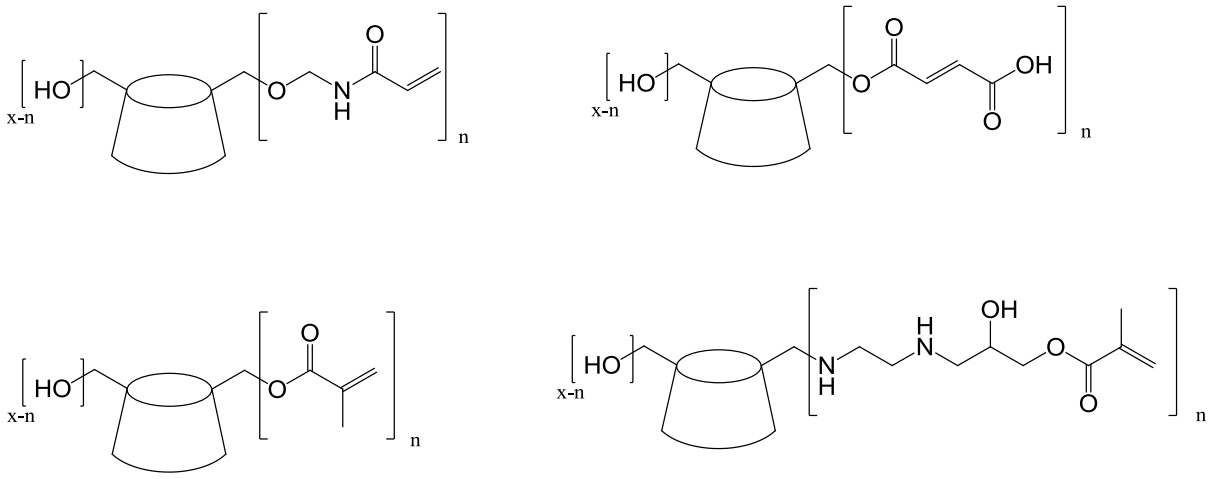
Diagram of CD cross-linking by means of EPH [30].

In order to adapt mechanical properties of polymerized CD networks, cross-linked with the use of EPH, diepoxide or diisocyanate, cross-linking often takes place in presence of water-soluble polymers, such as poly(vinyl alcohol) (PVA) or hydroxypropyl methylcellulose (HPMC) [36,37].

Apart from polymerization of CD with the use of low molecular mass molecules, the covalently cross-linked systems were also developed by crosslinking CD with numerous polymers. For example, many cyclodextrins containing polymer networks were prepared by using CD as a crosslinking agent. This method allows to create polymer networks developed by heating water solutions containing poly(acrylic acid) (PAA) and β-CD in the temperature range 90-120°C [38].

The most frequently used strategy of preparing the CD/polymer network consists in copolymerization (chemical or by means of the radical method) of vinyl derivatives or (met)

acryloil CD with other universally applied vinyl monomers, such as acrylic acid (AA), 2-hydroxyethyl methacrylate (HEMA) and N-isopropyl acrylamide (NIPAAm)[39]. These methods allowed to obtain hydro-gels based on functionalized cyclodextrins with the use of poly(hydroxyethyl methacrylate) (pHEMA), which are considered to be useful as permanently releasing drugs in soft contact lenses [40].



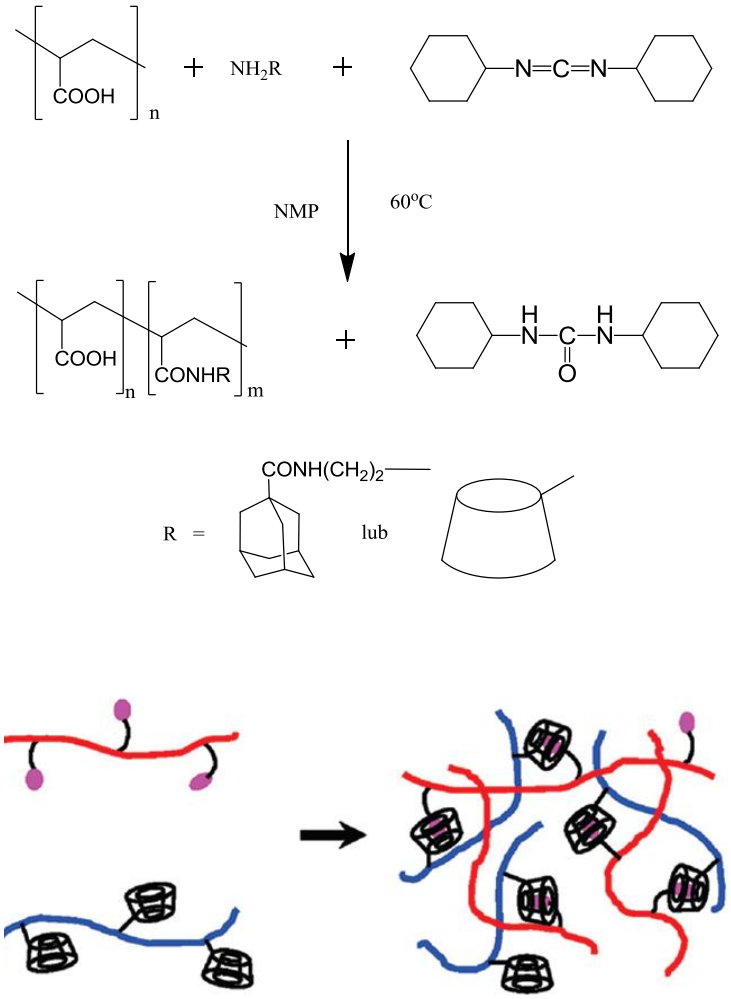
CD monomer polymerization [30].

The CD networks show above, as well as many other networks, were connected with the polymer skeleton by means of numerous polymer reactive places. In many cases it may result in decrease of CD rotation and limit their availability to other molecules. Therefore, polymers or conjugates prepared from mono-derivative CDs containing only one reactive group seem much better in terms of availability.

Another interesting and promising method of synthesizing cyclodextrin polymers is the ability of cyclodextrins to create inclusion complexes, which provides numerous opportunities to create supramolecular polymers [41].

An often discussed example of this type of the system is creating inclusion complexes between the adamantane (ADA) and β-CD derivatives often used for assembly of polymer networks because of high bond constants (*e.g*. Ka for carboxyl adamantine (ACA) and β-CD= 3.2 10^4 M^{-1}). For example, Li et al. carried out a polymer synthesis by means of two additional graftings: either with a 6-monoamino-β-CD or with amine derivative of adamantane (N-(2-aminoethyl)adamantane-1-carboxamide) to poly(acrylic acid) (pAA), using dicyclohexylcarbodiimide (DCC) as a coupling reagent. Mixing of both polymers in water solution resulted in creation of a reversible polymer networks, which can be disconnected by adding a competitive non-bounded β-CD or by increase of temperature (> 40°C). The largest networks of the type were obtained at equimolar quantities of the grafted β-CD and ADA

groups (1:1), which suggests that the binary interactions between these functional groups are responsible for creating these networks [42].



Formation of polymer networks with the use of matching polymers [42].

4.4. Objective of the research and presentation of its most important results.

The objective of my research initiated in professor Seung-Taik Lim's group at Korea University was studying possibilities of synthesis of cyclodextrin polymers with the use of dicarboxylic acid anhydrites. To do this, I developed an original method of cyclodextrin crosslinking based on the possibility of activation of the secondary hydroxyl groups in the in the anhydrous dimethylfomamide (DMF) solution with the use of sodium hydride. In these conditions, like in the case of the cyclodextrin reactions in the highly alkaline media, there is a nucleophilic substitution of difunctional compounds, which results in development of a polymer network with various cyclodextrin substitution. However, as opposed to the reaction in concentrated water solution of NaOH, where the reaction of deprotonation occurs nonspecifically, the direction of preferred deprotonation in the anhydrous DMF is in position "2" [43]. At the same time, it can be observed in these conditions that the created oxoanion in position "3" is converted into 2,3-cyclodextrin epoxide (44).

The maleic anhydride was used as a model system for cross-linking. The reaction between an oxo-anion and the anhydride was carried out and examined in a wide range of temperatures and molar ratios of the reagents. In order to check the conditions of cross-linking at the assumption of activating mainly position "2" in β-cyclodextrin I used various molar ratios of β-CD to NaH (1:1; 1:2; 1:4; 1:7).It was meant to activate a definite number of secondary hydroxyl groups in cyclodextrin. Maleic anhydride (MA) was added in the molecular ratios from 1:1 to 1:11. The reactions of cross-linking were conducted in a wide range of temperatures, from room temperature to 130 ^{o}C. Several dozen samples of different cross-linking degree were obtained, which allowed to determine the best conditions for formation of the cyclodextrin polymer. During the first stage, I carried out measurements of molecular weights of the obtained systems using the HPLC-SEC method aided by a system of chromatography equipped with SEC type columns and two Wyatt Technology detectors: RI and MALLS [H1].

B-CD:MA	B-CD:NaH			
	1:1	1:2	1:4	1:7
1:1	1300 (23%) 1700 (10,3%) 1970 (10,3%) 3560 (6,1%)	1250 (11,6%) 2160 (3,7%) 3880 (18%)	1200 (18,3%) 2100 (13,1%) 2300 (10,5%)	1260 (20,3%) 1780 (7,9%) 1830 (8,4%) 6550 (1,1%)
1:2	1400 (27,7%) 1600 (19,4%) 2770 (12,8%) 5330 (10,4%)	1300 (38,8%) 1500 (18,2%) 2800 (19,6%)	1650 (22,8%) 2100 (12,9%) 4200 (8,5%)	1300 (21,7%) 1600 (16,6%) 2000 (20,3%) 5500 (7,1%)
1:4	1400 (20,6%) 1500 (27,5%) 2300 (14,7%) 5100 (24,8%)	1500 (24,6%) 1700 (15,4%) 2700 (10,7%) 6330 (35,3%)	1600 (14%) 1800 (14,6%) 2100 (14,5%) 7270 (21,3%)	1350 (12,1%) 1500 (24,6%) 2700 (29,2%) 6800 (17,8%)
1:7	1800 (31%) 2600 (16,9%) 3900 (19,3%) 13.500 (17,4%)	2300 (24,9%) 3500 (10,4%) 5900 (28,7%) 19.950 (17,8%)	1800 (25,7%) 3450 (6,1%) 6200 (34,3%) 27.300 (15,5%)	2500 (7,9%) 3500 (8,7%) 5400 (63,4%) 60.000 (15,8%)
1:11	1800 (26,9%) 2600 (15,7%) 3400 (25,1%) 12.600 (22,4%)	4000 (17,2%) 9400 (54,9%) 42.000 (22,5%)	3800 (19,3%) 18.700 (43,8%) 104.000 (21,7%)	4100 (8,4%) 20.000 (55,5%) 170.000 (31,6%)

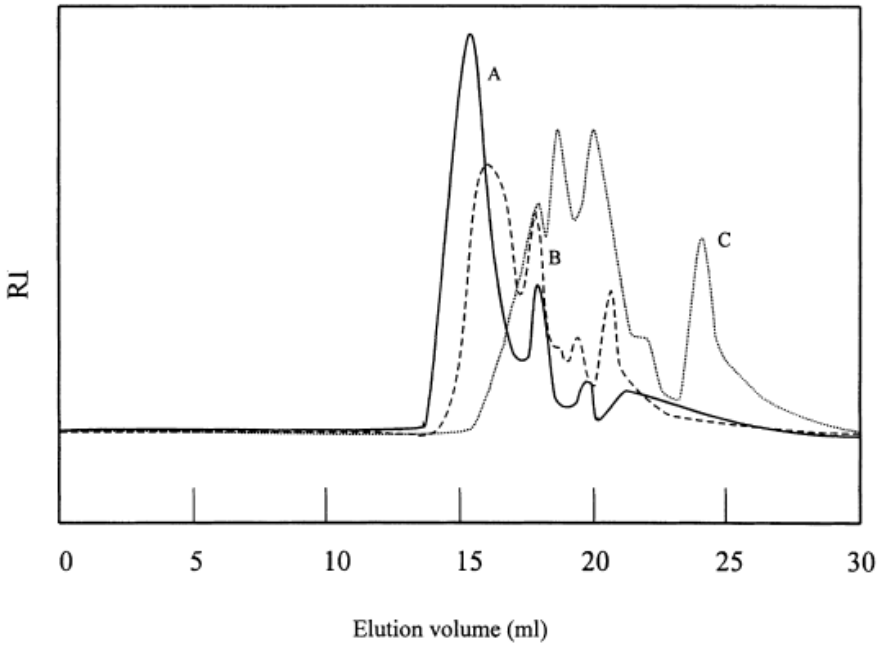
Table 1: Weight-average molecular weights (M_w) of the systems prepared at different molar ratio of β-CD:NaH:MA (reaction time and temperature were 12 h and 60°C, respectively [H1].

	4 h	12 h	24 h
25°C	1300 (13%) 6500 (67%)	Not studied	1300 (47,5%) 2300 (40,7%)
60°C	2700 (15%) 9100 (80%) 43.000 (2,4%)	Not studied	1900 (12%) 3800 (50%) 17.000 (20%) 46.000 (5,3%)
100°C	4200 (17,5%) 12.000 (43%) 18.000 (17%) 88.000 (19%)	12.000 (8,4%) 31.000 (15%) 47.000 (24%) 88.000 (24%) 288.000 (14%)	17.000 (5,3%) 28.000 (11%) 78.000 (49%) 380.000 (18%)
130°C	225.000 (17,2%) 466.000 (40,6%)	250.000 (14,6%) 530.000 (39%)	Not studied

Table 2: Weight-average molecular weights (M_w) of the systems prepared at different reaction temperatures and times (molar ratio of β-CD:NaH: maleic anhydride was 1:7:7) [H1].

A fraction of the weight-average molecular weights (M_w) and percent ratio of each chromatographic fraction of the polymerization products under different reaction conditions are presented in Tables 1 and 2. Weight-average molecular weights of the systems prepared at the reaction time and temperature of 12 h and 60^{o}C, respectively, are listed in Table 1. In these conditions, irrespective of the applied molar ratio of the reacting substances, only a slight amount of the prepared samples with *M*w greater than 20,000 Da was detected. Such systems were created only when the highest of the examined reactant molar ratios were applied. The analysis of results revealed that in these conditions the major products are CD derivatives containing from one to several butenedioic acid ester substituents, or CD dimers or oligomers with small molecular weight.

Weight-average molecular weights of the systems prepared at different molar ratio of β-CD:NaH:maleic anhydride 1:7:7 and at different reaction temperatures and times are presented in Table 2. Increase of the reaction temperature to 100^{o}C and 130^{o}C caused that no ester monomers with M_w lower than 1500 Da were detected in the prepared products. In these conditions of the reactions, the main products were systems with average molecular weights higher than 10 000 Da. Actually, majority of the prepared samples contained systems of molecular weights higher than 100 000 Da. At the same time, the systems were characterized by insignificant polydispersity, which was evidence of their high homogeneity.

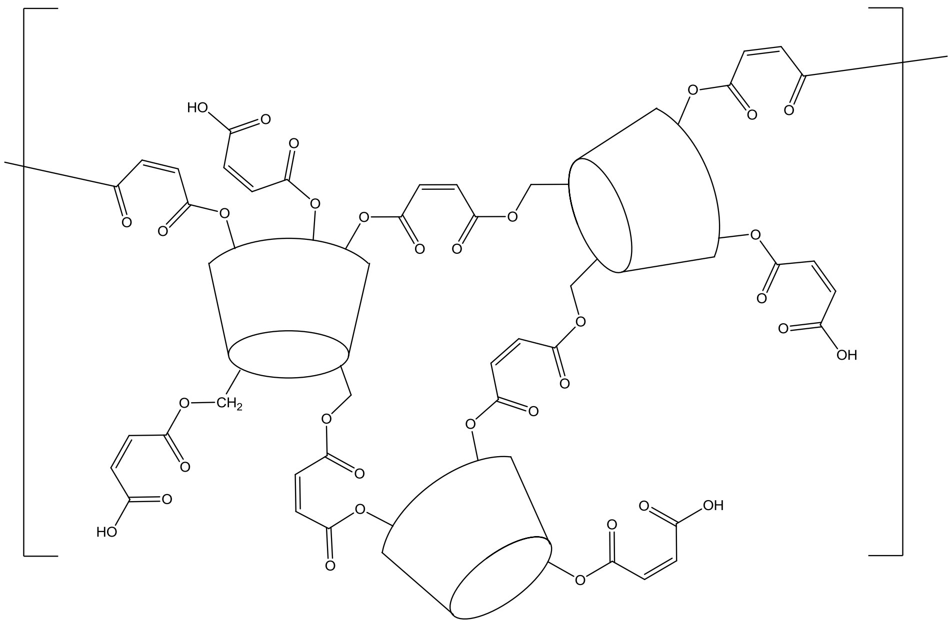


Scheme 1: Typical chromatogram of the CD polymer products: the molar ratio of the β-CD:NaH:MA substrate is 1:7:7, reaction temperature - 100 ^{o}C,, reaction time A-24h; B-12h; C-4h) [H1].

Detailed analysis of the above chromatogram for the reaction carried out at the temperature of 100^{o}C allows to draw interesting conclusions as to the course of the reaction in time. After 4 hours of the reaction, it was possible to notice a relatively high polydispersity in the samples and measurements of average molecular weights clearly show occurrence of small such systems as CD derivatives containing from one to several butenedioic acid ester substituents, or CD dimers or oligomers of small molecular weight. Further heating result in disappearance of small CD oligomers, which combine into more complex structures of average molecular weights from 20 000 to 90 000 Da. At the same time, no CD ester derivatives can be found. As the reaction time proceeds, polydispersity of the prepared samples diminishes, while the fraction molecules with high molecular weight over 100 000 keeps growing.

As early after 4-hour-long reaction, the systems obtained at the temperature of 130^{o}C, (in a tightly closed vessel placed in an autoclave) showed average molecular weights higher than 200 000 Da. In this case, further extension of the time of reaction did not result in obtaining systems of higher molecular weights.

The reaction between β-CD and NaH results in formation of CD oxo anions. These systems react with more than two particles of the maleic anhydride forming polymer systems in which the β-CD particles are connected by ester bridges of the butenedioic acid.



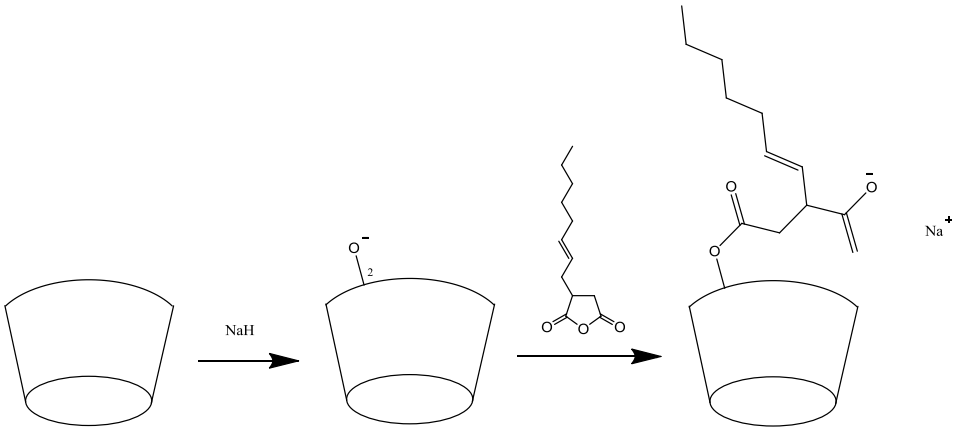
The above deliberations are confirmed in the proton NMR analysis of the prepared products of polymerization. Extending the reaction time from 4 to 12 and then to 24 hours causes slow fading of signals attributed to the products of esterification of β-cyclodextrin, whereas the signal corresponding to vinyl protons in the symmetric diester bond linking the individual β-CD particles. Similar observations were made for products prepared at higher and higher temperature.

Due to the possibility of future studies on application of the prepared cross-linked polymers, there were tests for water solubility of the studied polymers carried out. The systems whose substrate molar ratio was lower than 1:7:7 (β-CD:NaH:maleic anhydride) and whose temperature of reaction was lower than 130^{o}C, irrespective of its reaction time, were water soluble (more than 20%). But the water solubility dropped sharply to 4.2% for the systems prepared in the temperature 130^{o}C and at the highest substrate molar ratios (1:7:7 and 1:7:11), whereas the systems heated over 12 hours became practically water insoluble.

The possibility of easy modification of the secondary cyclodextrin hydroxyl groups by means of dicarboxylic acid anhydrides in presence of NaH, allows a wide range of modifications of the cyclodextrin complexing properties. During our studies, we tried to answer the question in what way the presence of a long, hydrophobic chain (one or a few) affects the complexing properties of systems of similar construction. The 2-octenylsuccinic anhydride was used as a model compound. It is often used to modify the starch surface in order to enhance their sorption and emulsifying properties [45].

The structure of the 2-octenylsuccinic anhydride demonstrates amphiphilic properties by the presence of a hydrophobic octenyl chain and hydrophilic succinate moiety in its structure. In order to make a test of using modified β-cyclodextrin in the food industry, apart from the synthesis and determination of the structure of the prepared product, there was also a test of using the product in the process of emulsifying fatty acid carried out.

Synthesis of the octenylsuccinyl β-cyclodextrin was carried out in conditions similar to those of the reaction of the β-CD with the maleic anhydride, however, on the basis of the earlier acquired knowledge, the time of reaction, temperature of the process and the substrate molar ratio were so well-matched as to make the reaction yield only mono- and poly-substituted esterification products and prevent formation of possible oligo- and polymer systems [H2]



Molar ratio	Temperature and time of reaction		
β-CD/NaH/OSA*	Deprotonation	Addition reaction	DS**
1:3:3	25°C; 6 h	25°C; 3 h	0,518
1:1:1	25°C; 6 h	25°C; 3 h	0,178
1:1:1	25°C; 3 h	25°C; 3 h	0,179
1:1:1	25°C; 1 h	25°C; 3 h	0,150
1:1:1	25°C; 6 h	100°C; 3 h	0,147
1:1:1	100°C; 3 h	100°C; 3 h	0,115
1:1:1	100°C; 1 h	100°C; 3 h	0,123

*OSA = 2-octenyl-succinic anhydride

**DS = Degree of substitution

Table 3: Conditions of the reaction of synthesizing octenylsuccinyl β-cyclodextrin [H2].

Changing conditions of the course of the reaction (reaction temperature and time) as well as use of the proper ratio of the reacting substances allows to change the degree of β-cyclodextrin substitution. During the conducted tests it was revealed that temperature does not have a fundamental effect on the course of the process. It is only the extension of the reaction time that allows to achieve a slightly higher degree of substitution. Whereas, the fundamental effect on the degree of cyclodextrin substitution is effected by the molar ratio of used reacting substances, particularly using a larger amount of sodium hydride, as well as of the 2-octenylsuccinic anhydride, which contributes to the growth of the degree of substitution. Analysis of proton NMR spectra for individual fractions confirms that the reaction proceeds on the secondary hydroxyl groups deprotonated by means of NaH. The degree of substitution (DS) was calculated on the basis of the integration ratio for the area corresponding to the methyl group of the octenyl substituent and integration of the area of glucose anomeric proton (H_1). It allowed to make a statement that the number of substituents linked to one β-CD molecule in individual samples varies from 5 to 1. In order to confirm the obtained results, there was a measurement of molecular weights carried out with the use of HPLC-SEC-RI-MALLS chromatography system. The obtained results do not allow explicit conformation of the degree of substitution calculated on the basis of spectroscopic data. The sample of the highest degree of substitution showed its average molecular weight to be c. 5500 Da, and the others ranged from 3800 to 2200. The molecular weight of the mono-octenylsuccinyl β-cyclodextrin is 1345. The difference between the spectroscopic method and the SEC analysis results from a relatively low peak resolution of the MALLS detector for small molecules. However, assuming that the data is correct, it is possible to suggest two solutions of the existing problem of variance of the experimental data. One of them is the possibility of creating a cyclodextrin dimer linked by a butane bridge. Another solution can be obtaining a complex in which the octenyl chain is a guest for the second molecule of β-cyclodextrin.

The obtained octenylsuccinyl β-cyclodextrins of different degree of substitution (DS c. 5; and DS c. 1) were tested for solubility in the water-ethanol solution and in the citrate buffer solution. Knowledge on solubility of the cyclodextrin derivatives is indispensable to find their specific uses, particularly in pharmaceutical, cosmetic and food industries.

The octenylsuccinyl β-cyclodextrins show much higher solubility in the ethanol-water solution than pure cyclodextrin. Non substituted β-cyclodextrin has low solubility in pure water (c. 1.8g/100ml H_2O), which increases a little when ethanol is added to the solution, only to drop practically to zero in the 75% ethanol solution after reaching maximum solubility in the 25% solution (c. 4g/100ml) [3]. The poly-substituted derivative shows an exceptionally high solubility in the 75% ethanol solution (over 40g/100ml). Whereas mono-octenylsuccinyl β-CD has a relatively low solubility at the level of 3.9g/100ml, but its solubility in the 25% ethanol solution is very high and exceeds 40g/100ml. These observations seem consistent with the difference in hydrophobicity displayed by poly- and mono-substituted cyclodextrins containing long alkyl chains [1].

Very interesting and useful information can be obtained when analyzing results of solubility of the prepared samples in citrate buffer solution of pH=5 or pH=3 and in diluted HCl solution of the same pH. Higher solubility of the mono-octenylsuccinyl β-CD in the citrate buffer than that in HCl solution of the same pH results from the ability to form complexes in the case of the buffer solution. However, poly-substituted β-CD shows definitely lower solubility in the buffer, which is connected with presence of a large number of free acid groups, whose presence does not contribute to increase of the ion strength [18]. Low solubility can be advantageous in case of an attempt to use the prepared system *e.g*. as a drug carrier, because in this case solubility of a complex can be controlled through changing the pH solution.

In order to determine the possibility of stabilizing fat emulsions by means of the prepared cyclodextrin derivatives, water-oil (lionoleic acid) solution was prepared to which non substituted β-cyclodextrin was added and β-cyclodextrin derivatives mono- and poly-substituted with the octenylsuccinyl group. Thus prepared mixtures were subjected to the process of homogenization and left in room temperature in order to observe stability of the prepared emulsion. The sample containing the native β-CD displayed almost complete separation of the oil already after 30 minutes, but microscopic observation shows that even in this case we do not deal with as completely clean separation as in the water-oil system. It is connected with partial complexation of the lionoleic acid and accumulation of complex molecules in the layer between water and oil. The emulsions created with added octenylsuccinyl β-CD display perfect stability without observing any separation of layers, even after 24 hours. Stability of this type of emulsion taking advantage of the amphiphilic properties of the β-CD derivatives can be of great importance in many industrial areas, starting from food industry and ending on different methods of sewage purification.

The 2-octenylsuccinic anhydride was used earlier to prepare amphiphilic derivatives of β-cyclodextrin. Now, I decided to make use of the non-substituted succinic anhydride as a cross-linking agent to obtain cyclodextrin polymers of large molecular weights, and test the possibility of application of the prepared systems in the process of ion flotation of the transition metals, which can be of colossal importance in the process of production of rare-earth elements, or in sewage purification from compounds dangerous for the environment.

B-CD:NaH:SA*	**Reaction temperature (°C)**		
	25	60	100
1:1:1	Polymer A 1759 (6,27%) 2155 (10,43%) 2641 (15,51%) 3237 (21,43%) 4861 (9,99%)	Not studied	Not studied
1:7:7	Not studied	Polymer B 3237 (7,30%) 3967 (6,45%) 4861 (5,83%) 55,789 (4,93%) 68,369 (5,70%) 102,681 (6,23%) 125,837 (5,09%)	Polymer C 2641 (5,35%) 3237 (8,54%) 3967 (6,08%) 4861 (6,37%) 5958 (5,50%) 83,787 (3,19%) 102,681 (3,67%) 154,213 (4,37%) 188,989 (4,24%)

*SA = succinic anhydride

Table 4: Weight-average molecular weight for the systems prepared at different molar ratio of the reacting substances and in different temperatures and time of reaction [H4].

Its resemblance in structure and reactivity to the maleic anhydride allowed to assume that in this case it is possible to obtain similar results in cyclodextrin cross-linking. Using similar reaction conditions, a number of products were obtained whose average molecular weights were examined with by means of HPLC-SEC-RI chromatography with the use of pullulan standards as models of molecular weights. As in the case of earlier reactions, the strongest effect on preparation of samples containing fractions of average molecular weight higher than 50 000 Da is exerted by the temperature of the process. As a result of reactions conducted at room temperature, irrespective of the used ratio of the reacting substances, only mono- and multi-succinyl were obtained, with a slight share of cyclodextrin dimers and oligomers. The average molecular weights of the prepared compounds were within the range 1000 to 5000 Da. Yet, during the reactions conducted at the temperature of 60°C and 100°C, and the molar ratio of the reacting substances 1:7:7 (β-CD:NaH:SA), we managed to obtain a large fraction of polymers with average molecular weights ranging from 50 000 to over 200 000 Da [H4].

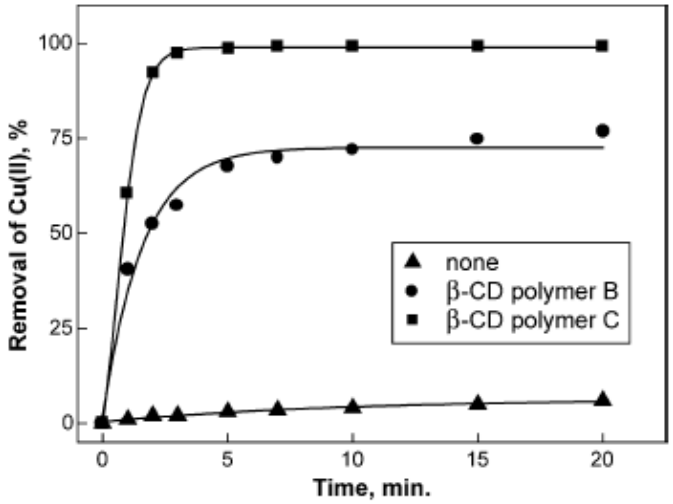
The analysis of proton NMR spectra is a serious support for determining the structure and the degree of substitutions in cyclodextrin polymers. In this way it was possible to demonstrate that in samples prepared at room temperature it is possible to find systems containing only succinic groups linked to a β-cyclodextrin molecule. Whereas the spectra of samples prepared in higher temperatures show the presence of a signal responsible for the presence of two identically surrounded methylene groups in a diester butanedioic bridge linking adjacent β-CD molecules. The degree of substitution (DS) was calculated on the basis of the integration ratio for the area corresponding to methylene groups and integration of the anomeric proton of glucose (H_1). This allowed to find that in case of monoester systems prepared at room temperature, we deal with a monosubstitution, as only one molecule of the succinic anhydride is attached to a cyclodextrin molecule. The systems prepared at higher temperature have on average 2 diester links between individual CD molecules as well as 3 to 5 free succinic groups. The systems with such a structure can display interesting complexing properties resulting not only from the presence of cyclodextrin molecules, but also from the presence of many free acidis groups.

Simultaneously with conducting original research on cross-linked cyclodextrins and looking for their potential applications, I conducted extensive literature research aimed at learning about the state of knowledge on cyclodextrins, their polymers, and the reaction of complexing them with metals, focusing on the future application of cyclodextrin polymers in different process applied in the environmental protection. As a result of my literature research, I published a number of review papers in major scientific periodicals and prepared, together with professor Wanda Śliwa, a book “Chemistry of Cyclodextrin” [46], which was published by the Jan Dlugosz University in Czestochowa Publishing House.

The first review work, which was part of the basic research conducted at that time was on metallocyclodextrins. The first part of the paper discussed cyclodextrin complexes with copper (II) and platinum (IV), which was important from the point of view of the research conducted at that time. Cyclodextrin complexes with ferrocene and ruthenium connected with heterocyclic systems , known as ligands. In the next part of the said review work I focused on cyclodextrin complexes with ions of various metals connected with porphyrin and phthalocyanine ligands. Cyclodextrin systems modified with the use of gold, silver and palladium nanoparticles are discussed in the last chapter [H3].

Conducting research on environmental protection at our faculty, and particularly on the techniques of metal ion separation and post-industrial sewage resulted in an interesting cooperation on the possibility of using cyclodextrin polymer systems in these processes. Polymer foaming processes and ion flotation methods are widely recognized methods used in the mining industry in the ore concentration processes. There are many works discussing cyclodextrin inclusion complexes with metals [24,47-49]. CD derivatives can also form this type of complexes, which was described in the review paper, which I am a co-author of [H3].

Although interrelations between cyclodextrin derivatives and ions are weak, I made an attempt at using cross-linked hydrophilic cyclodextrin derivatives in the process of ion flotation. It was the first original application of such compounds in the processes of this type. In order to make a broadly conceived study of the effect of the presence of the polymer system on the process of ion flotation, there was a measurement of copper ions (II) recovery from the solution by means of the potentiometric method [50]. The highest recovery of copper ions (II) from the studied solution was obtained in samples containing polymers of the highest molecule weights. At the same time, the maximum recovery took place in a short period of conducting the process, *i. e*. after about 5 minutes. It also turned out that the bond of the studied metal ions with the cyclodextrin polymer is closely related to pH of the solution and takes place only when pH is lower than 4.5. In solutions of pH in the range 4.5-5.6 it is actually constant and is c. 70%, however, with the increased acidity of the solution, bonding increases and practically total removal of copper ions (II) from the solution by means of the process of ion flotation is possible then.



Scheme 2: Removal of copper ions (II) from the water solution in the process of ion flotation by means of cyclodextrin polymers [H4].

Because there were interesting results obtained concerning ion flotation in the case of copper, an attempt was made at examining selectivity of removal of metal ions from solutions. For this purpose, the solution containing Zn(II), Cd(II) and Cu(II) ions was prepared and, in the first place, a confirmation was obtained that application of polymers of the highest average molecular weights creates favorable conditions for removal of metal ions from solutions. It was found that there is indeed a competition in removal of the studied ions from the solution. For the polymers with the highest average molecular weights the sequence of selectivity is as follows: Zn(II)>Cd(II)>Cu(II). The simplest explanation of the fact seems to be compatibility of the size of individual metal ions and the size of the CD cavity. It was the Zn(II) ion that was the most compatible for the size of the cavity, which may explain the highest degree of Zn(II) ion removal from the solution in the process of ion flotation [H4].

Another theoretical paper, on which I cooperated with the employees of the Institute of Chemistry and Environmental Protection of the JDU in Czestochowa, was a work devoted to synthesis and properties of cyclodextrin oligomers. The up-to-date state of knowledge on cyclodextrin dimers and larger oligomer systems, such as cyclodextrin trimers and tetramers. Linkage of two cyclodextrin molecules with the use of a molecular connecting link results in formation of a dimer which has two complexing centers in one molecule. The closer affinity of the dimer in comparison to the cyclodextrin free molecule results from co-linkin of the guest molecule by two free cyclodextrin cavities, which are part of the dimer. It also results in higher selectivity of the dimer. Higher complexing properties of dimers linked with secondary rims in comparison to dimers linked to primary rims were also revealed. The structure of cyclodextrin dimers and their complexing properties make it possible to find their numerous potential applications, such as the chemical processes in which dimers can act as centers active for active artificial enzymes influencing the rate and direction of chemical reactions. They include *e.g*. dimers in which two cyclodextrin molecules are linked by a bipyridyl system, the dimer which facilitates reaction of benzoin condensation or increases the rate of phosphodiester hydrolysis. Many dimers containing an alkenyl bond can be used as photosensitizers in photodynamic anticancer therapy. Many dimers, as well as cyclodextrin oligomers can be applied in chiral recognition, as well as in construction of nano-scale molecular machines [H5].

Apart from applications of simple dicarboxylic acid anhydrides, there were also studies carried out on phthalic and 3-nitrophthalic anhydrides as the systems cross-linking cyclodextrins [H6]. They were also examined in terms of their application in the process of ion flotation. As in the earlier reactions of the type, it was the possibilities of cross-linking cyclodextrins in different temperature conditions and the reaction time at different molar ratios of the reacting substances that were paid special attention to. The use of dicarboxylic anhydrides having aromatic systems in their structure yielded surprising results. In the case of applying molar ratio of the reagents 1:1:1 and room temperature of the process, samples having average molecular weights 1000-7400 Da were obtained in the general share 53-79%. As the temperature of the process rose to 65^{o}C, polymer fractions of average molecular weights exceeding 100 Da appeared in the prepared samples, but their percentage share was small. In this case, it is a surprise that as the temperature kept rising to 100^{o}C no polymer fractions over 10 kDa were observed. In the case of using the molar ratio of the reagents 1:7:7 (β-CD:NaH:phthalic anhydride), the fractions containing the highest molecular weights were observed when the reaction was carried out at room temperature, however as many as 10% of the fractions had average molecular weights over 400 kDa. Conducting the process at the temperature of 65^{o}C resulted in dramatic plummeting of the average molecular weight to c. 50-65 kDa, but their percentage share in the entire sample was very small (less than 5%). In the case of the process carried out at the temperature of 100^{o}C, only negligible fractions of average molecular weights exceeding 30 kDa (c. 2%) could be

observed. Only small systems, which looked like substituted cyclodextrins rather than larger oligomer or polymer systems were observed in the samples.

β-CD:NaH:PA*	Reaction temperature (^{o}C)		
	25	65	100
1:1:1	<1000 (5%) 1000-6000 (58%) 6000-46.000 (36%) >46.000 (1%)	<1000 (1%) 1000-6000 (62%) 6000-240.000 (30%) >240.000 (1%)	<1000 (1%) 1000-7400 (86%) >7400 (13%)
1:7:7	<1000 (20%) 1000-6000 (16%) 6000-430.000 (54%) >430.000 (10%)	<1000 (35%) 1800-30.400 (21%) >65.000 (5%)	<1000 (56%) 1000-6000 (25%) 6000-38.000 (17%) >38.000 (2%)

*PA = Phtalic Anhydride

Table 4: Weight-average molecular weights of the systems prepared at different molar ratio of the reagents and at different temperature [H6].

Whereas very narrow ranges of molecular weights were found in the prepared samples in the reaction of the 3-nitorphthalic anhydride used as a cross-linking agent. Polydyspersity of the examined systems was c. 1.5 in comparison to 6.4, which value occurred as an average value in the case of systems cross-linked by means of phthalic anhydride. In the systems prepared at the reagent molar ratio 1:1:1, irrespectively of the temperature of the process, the samples displayed high homogeneity and low average molecular weights, mainly in the 1000-5000 Da range. In this case, taking into consideration inaccuracy of the measurement taken with the use of the HPLC-SEC-RI chromatography system, it is possible that in the samples appeared mainly cyclodextrin derivatives with a low percentage of cyclodextrin dimers, or possibly trimers. Measurement of molecular weights of the samples prepared at the reagent molar ratio of 1:7:7 showed the presence of a small share of molecular weights c. 20-30 kDa, however, as in the case of the systems cross-linked with the non substituted phthalic anhydride, here again it was possible to observe that together with the increase of temperature a lower share of the higher average molecular weights fraction could be observed.

β-CD:NaH:3N-PA*	Reaction temperature (°C)		
	25	65	100
1:1:1	<1000 (3,74%) 1000-5000 (92,62%) >5000 (1,9%)	<1000 (3,4%) 1000-6000 (91,32%) >6000 (3,77%)	<1000 (2,55%) 1000-5000 (83,05%) >50400 (8,26%)
1:7:7	<1000 (1%) 1000-5000 (41,38%) 5000-20.000 (29,5%) >20.000 (25%)	<1000 (2,25%) 1800-5000 (42,17%) 5000-20.000 (27,1%) >20.000 (11%)	<1000 (7%) 1000-5000 (79%) 6000-38.000 (11%)

*3N-PA = 3-Nitrophtalic Anhydride

Table 5: Weight-average molecular weights of the systems prepared at different molar ratio of the reagents and at different temperature [H6].

The results listed above were confirmed by the analysis of the ^{1}H NMR spectra of the prepared polymer samples. In the case of both anhydrides accessibility and, at the same time, non overlapping of the aromatic proton region and the cyclodextrin proton region allows precise calculation of the degree of substitution of β-cyclodextrin by phthalic substituents. The degree of substitution was calculated by the integration ratio between the region of aromatic protons and the H_1 anomeric protons present in each β-CD molecule. For the systems where the reagent molar ratio was 1:1:1 the number of phthalic and 3-nitrophthalic connecting links per 1 β-CD molecule is less than 1, and it additionally decreases with as the temperature grows. Whereas, at the molecular ratio 1:7:7, when a larger number of the secondary hydroxyl groups was activated, the number of links per 1 β-CD molecule increases to 4, and when the reaction is conducted at the temperature of 100°C, to almost 6, in the case of reaction with the phthalic anhydride. The results are very well consistent with the measurement of the average molecular weights for individual, prepared systems.

Reaction conditions (molar ratio/temperature)	β-CD	Moieties	
		phthalic	3-nitrophthalic
1:1:1; 25°C	1	0,58	0,6
1:1:1; 65°C	1	0,52	0,66
1:1:1; 100°C	1	0,2	0,34
1:7:7; 25°C	1	4,53	2,44
1:7:7; 65°C	1	4,56	3,35
1:7:7; 100°C	1	5,92	3,10

Table 6: The degree of substitution of linkers in cyclodextrin polymers cross-linked with phthalic and 3-nitrophthalic anhydrides [H6].

The use of phthalic and 3-nitrophthalic anhydrides gave a very interesting result in comparison the alkylic anhydrides that had been used until then. The presence of a fragment of an aromatic ring in the structure effectively upset the possibility of creating large polymer structures of high molecular weights. At the same time, it is possible to state that in this case the reaction was preferred towards preparing cyclodextrin derivatives with the substituted phthalic groups, and not towards creating large spatial structures. It probably results from a certain kind of stiffening of anhydrides containing an aromatic system, contrary to aliphatic anhydrides, particularly in the case of the succinic anhydride, having a much higher ability of changing their conformation, particularly in higher reaction temperature. The presence of the aromatic ring effectively blocked possibilities of cyclodextrin cross-linking, and at the same time closeness of the aromatic ring in cyclodextrin phthalic derivatives does not allow to form an inclusion complex with another cyclodextrin molecule which might significantly speed up the reactions of dimerization or even oligomerization in the reactions of this type [1,4].

Taking into consideration the possibility of using this type of systems in the process of ion flotation, similarly to the previously studied polymers, the effect of the prepared systems on the process of ion flotation of Cu(II) ions was examined. As in the previous studies, here too, only the fractions containing polymer systems of high molecular weights show significant possibility of removing Cu(II) ions from water solutions. In the case of samples prepared at the molar ratio of 1:1:1, *i.e*. containing mainly cyclodextrin phthalic derivatives, the degree of copper ion removal in the flotation process is on the average 35-50%. Using polymers, even a small share, in the prepared samples, or studying samples prepared with the use of molar ratio of 1:7:7, allowed to determine the degree of copper ion removal at 70-97%, depending on the used anhydride. In the case of using the 3-nitrophthalic anhydride as a cross-linking agent a higher share of the fraction of higher average molecular weights created favorable conditions for increasing effectiveness of copper Cu (II) ion removal in the process of ion flotation. It should be noted, however, that low molecular weights of the prepared systems do not create favorable conditions for using this type of systems in the flotation processes [H6].

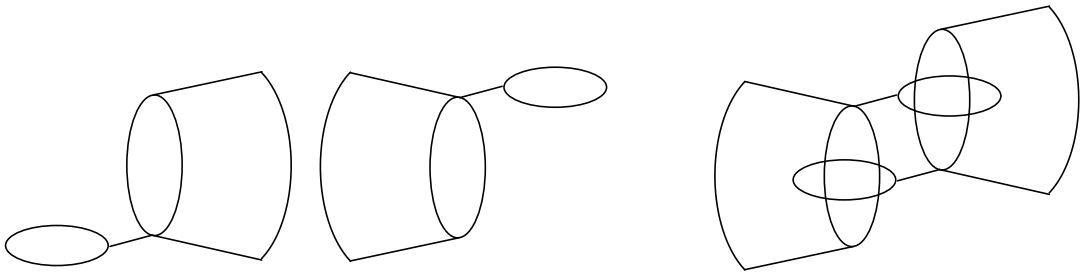
Connecting link	Reaction conditions			
	20°C		100°C	
	1:1:1	1:7:7	1:1:1	1:7:7
Phthalic	35%	68%	40%	73%
3-nitrophthalic	38%	85%	49%	93%

Table 7: Copper Cu (II) ion removal from the water solution in the process of flotation with the use of cyclodextrin polymers cross-linked with phthalic and 3-nitrophthalic anhydrides [H6].

It seems that using systems of high molecular weights, as in the case of polymers cross-linked with succinic anhydride creates favorite conditions for effective ion flotation process. In this case, a change in the surface tension of the non-ionizable surfactant used in the processes of flotation in the presence of a cyclodextrin polymer demonstrating the traits of a micellar system, though of irregular shape, may be of great importance [51-53].

The previous review paper concerning cyclodextrin oligomers described mainly the CD dimers covalently bonded with the use of various connecting links, yet, remarkable properties of the systems of this type cause that studies on supramolecular systems similar in their construction to cyclodextrin dimers and oligomers. My interest in cyclodextrin polymers having not only the structure of a linear system, but also a probable 3D structure, resulted in literature studies on the non-covalently bonded cyclodextrin dimers, cyclodextrin complexes of the 2:1 type, and mutually penetrating cyclodextrin oligomers. Cyclodextrin complexes of the 2:1 type (2 molecules of the β-cyclodextrin, one molecule of a guest) allow to complex a guest of insignificant dimensions in relation to the size of a cyclodextrin cavity. Threading two or more CD molecules is possible, when the guest's molecule permits. Rotaxan formation is possible, in which the CD molecules move freely along the guest's chain changing its physical and chemical properties. This curious property is widely studied at present in order to build machines, particularly molecular switches. However, complexes of the 2:1 type are created most frequently when a guest molecule is too large to make a complex of the 1:1 type and, at the same time, a certain part of it shows close affinity to the cyclodextrin cavity. There are many well-known complexes of the type. They may be, *e.g.* steroid antibiotics, which as a result become more effective drugs. Large molecules, like porphyrins, or even fullerens, can create complexes with cyclodextrins totally changing their properties, *e.g.* it is possible to create a 2:1 fullerene complex with 2 molecules of γ-cyclodextrin, which is water soluble. This type of complex can decompose DNA in the oxygen saturated water solution [H7].

Other systems described in the paper are the self-organizing cyclodextrin dimers. They can take two kinds of positions with regard to each other. They may be contact complexes, and they can be self-penetrating dimer complexes. The way the dimer molecules are situated against each other depends mainly on the side chain attached to the cyclodextrin, and, first of all, on its affinity to the cavity. At the same time, such complexes can be arranged in a "head to tail" position or a "tail to tail" position. In this way other self-organizing system can be created, or even cyclodextrin tetramers in which long-chain mono-derivatives penetrate each other. This type of molecule self-organization results, first of all, in increased water solubility of the systems [H7].



contact dimers inclusion dimers

The results obtained up to date fully confirm the possibility of synthesis of cyclodextrin polymers based on β-CD oxoanions formed as a result of the process of deprotonation of the secondary hydroxyl groups by means of sodium hydride (NaH) in the anhydrous media. At the same time, it was confirmed that there are large possibilities to control the cyclization process by matching appropriate media for conducting reactions (time and temperature of the process) and by using an appropriate molar ratio of the reagents. Using 2-octenylsuccinyl anhydride to prepare amphiphilic derivatives was also fully successful. Continuous searching for new materials of interesting properties and using them in the processes of separation of metals and transportation through liquid polymer membranes turned my attention to the possibility of synthesis of polymers containing long hydrophobic chains. It appears that this type of materials can be used as macrocyclic carriers in the processes of separation, which are used in our laboratories in order to find the best possible methods of purification of the council and industrial sewage of heavy metal ions. In order to synthesize this type of materials, I used succinic anhydride derivatives containing even longer alkyl chains than the 2-octenylsuccinyl anhydride used so far.

In order to prepare new polymer systems of increased hydrophobic properties, I used 2-nonenylsuccinic anhydride and 2-dodecenosuccinic anhydride as cross-linking agents. In both cases, bearing in mind earlier experiences and the need to prepare water insoluble polymers, but soluble in non-polar solvents, such as methylene chloride, I assumed from the very beginning that the reactions shall be conducted in high temperature and at the highest molar ratios of the reagents. The prepared polymers were to find application in the studies on metal ion separation in their transport through polymer inclusion membranes. The products prepared as a result of the those reactions were analyzed by HPLC-SEC-RI gel chromatography system with the use of pullulan standards as molecular weight standards. In the case of these systems, an interesting irregularity, which has not been fully explained, was observed and possibly requires further studies. A fraction of average molecular weight of over 1 000 000 Da was observed for the first time in a polymer system prepared as a result of a reaction of β-CD with the 2-nonenylsuccinic anhydride (Polymer A). At the same time, the studied anhydride contained only a small share of the fraction with average molecular weight smaller than 1 000 000 Da (c. 15%) and just traces of the 2-nonenylsuccinyl β-cyclodextrin (0.4%). The prepared polymer system is soluble in methyl chloride and shows

amphiphilic properties due to the presence of the alkyl chains in connecting links. Analyses of the average molecular weights and ^{1}H NMR spectra allow to draw conclusions concerning the supposed structure of the prepared cyclodextrin polymer system. The NMR analysis of the spectrum confirmed the presence of nonenylsuccinic moieties in the examined system, apart from linkers connecting individual cyclodextrin molecules. Measurement of integration allows to maintain that the ratio of CD to linkers is 1:3. It is this very value of the DS and the very large value of the average molecular weight allows a statement that the proposed macrocyclic system is more likely to have a three-dimensional structure than a linear one. Most probably, long alkyl chains penetrate hydrophobic interiors of cyclodextrin molecules causing preparation of a more condensed and intertwined structure [H8].

Ranges of average molecular weight M_w (Da)	Polymer A (%)	Polymer B (%)	Polymer C (%)
>1.000.000	84,60	1,70	-
100.000-1.000.000	9,24	4,39	-
11000-100.000	2,31	26,51	3,77
2000-11000	3,45	6,95	-
<2000	0,45	60,45	96,23

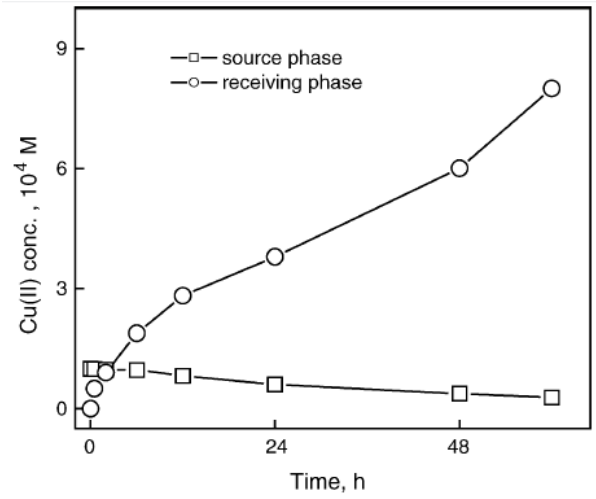
Table 8: Distribution of average molecular weights for the polymers prepared in reactions with the 2-nonenylsuccinic anhydride (polymer A) [H8], 2-dodecensuccinic anhydride (polymer B) [H8], and 2-docosenylsuccinic anhydride (polymer C) [H9].

A completely different situation takes place in the case of the reaction with the 2-dedecylsuccinic anhydride (Polymer B). Using the same conditions of carrying out the proves, the main fraction (c. 60%), had an average molecular weight lower than 2000 Da. However, a fraction with the average molecular weight of over 1 000 000 Da (c. 1.7%) was also found in the sample. Such a composition of the prepared sample suggests the presence of mainly cyclodextrin derivatives substituted with dodecenyl succinic moieties, which only to a small extent underwent the reaction of cross-linking with the CD molecules. Measurement of integration in ^{1}H NMR spectra shows that the average degree of substitution is 1:4.45. At the same time, the NMR spectroscopy confirms the earlier assumption that the sample contains mainly cyclodextrin systems substituted with 4-5 dodecenyl succinic moieties.

Such a large number of free acidic groups makes molecules assume a more hydrophilic nature. Systems of this type, in spite of containing long alkyl chains are more likely to be soluble in water and not hydrophobic in nature. Difficulties in mutual penetration of alkyl groups with cyclodextrin molecules probably result from the structure of the prepared polymer system and mutual positioning of the substituents in space.

The prepared polymers were used in studies concerning separation of heavy metal ions in the transfer through the polymer inclusion membranes. Membranes of this type are usually built of polymer (usually cellulose triacetate), plasticizer (*e.g*. *O*-nitrophenyl alkyl ether) and ion transfer carrier (our case – a cyclodextrin polymer) [54]. In order to study ion transfer in diluted solutions containing metal ions, a number of polymer inclusion membranes, containing different amounts of cyclodextrin polymer was prepared. At the same time, membranes not containing polymer were prepared for comparison. In this case, no ion transfer was observed.

The obtained results of measuring the content of metal ions, *e.g*. Cu(II) allowed to determine the best conditions for preparing a membrane. Concentration of polymer in a membrane should not exceed 0.02 M. No increase in transfer tempo has been observed above this value. It results, first of all, from low solubility of the polymer in the plasticizer. In optimum conditions for carrying out the process of transfer, it was observed that after 24 hours of the experiment 96% of the entire ion (Cu(II) content was transferred from the source solution to the receiver at first containing only distilled water. The conducted studies of stability of a polymer membrane containing 0.01 M of the cyclodextrin polymer showed that a membrane can be used many times without losing its transfer properties and not undergoing significant structural changes. After 10 processes, the standard deviation in the measurement of Cu(II) ion concentration in the recovery phase was only 0.009 mM, which is evidence of an extraordinary stability of this type of system and gives rise to the hope of using this type of membranes for purification of environmental solutions from heavy metal ions.



Scheme 3: Changing Cu (II) concentrations in the source phase and in the receiving phase of the transfer through PIM [H8].

Solution containing Cu(II), Co(II), Ni(II) and Zn(II) ions was used for studying selectiveness of the transfer process through inclusion membranes containing different amounts of cyclodextrin polymer. In this case, selectiveness of the process decreases in the

series: Cu(II)>Co(II)>Ni(II)>Zn(II). Moreover, the highest selectiveness can be observed in the case of lower polymer concentration in a membrane [H8].

In order to verify the hypothesis concerning the influence of the length of a side chain in a substituted succinic anhydride on possibilities of creating cyclodextrin polymers, reactions of cross-linking β-cyclodextrin by means of the docosenylsuccinic anhydride (Polymer C) were carried out. Similarly to the previously studied and described cases, here again attention was focused on the samples prepared at the molar ratio of 1:7:7. The obtained results are in principle consistent with the previously discussed diagram saying that lengthening of the side chain does not favor creation of a fraction of higher molecular weight. In the case of the reaction carried out at room temperature, mainly a fraction of average molecular weight of c. 2300 Da was observed. Only about 4% of the examined sample had the average molecular weight higher than 10 000 Da. The obtained result fully informs that in these conditions only the cyclodextrin dimer was formed. In the case of the reaction carried out at the temperature of 100^{O}C, dimer creation was not observed, just higher oligomer and polymer fractions of higher molecular weights. Yet the highest observed average molecular weights for the sample prepared in this way was about 34 kDa at the percentage share in the sample below 40%. At the same time, the samples prepared at both temperatures displayed low polydispersity and were relatively homogeneous. Analysis of the H NMR spectra of the prepared samples fully confirmed the results obtained in HPLC-SEC-RI chromatography. Measurement of integration in the case of the fraction containing the CD dimer confirmed that there is one docosenyl moieties to two cyclodextrin molecules, which confirms formation of the cyclodextrin dimer. In the sample prepared at a higher temperature, the measurement of the methyl proton integration ratio and anomeric proton in cyclodextrin shows that in this type of polymers there are no more than three docosenyl groups to one cyclodextrin molecule[H9].

The obtained cyclodextrin dimer was used in synthesis of new polymer inclusion membranes, and then the prepared membranes were used in studies concerning separation of metal ions in transfer through this type of membranes. Despite a small number of the docosenyl groups in individual molecules, the prepared systems showed higher solubility in a plasticizer, which affected increase of transfer effectiveness and higher transfer selectivity than in the case of systems with a shorter alkyl chain. Transfer selectivity of different heavy metal ions was also studied and in this case it could be observed that this type of membranes can be particularly useful for removal of Pb(II) ions, which were the fastest and most effectively removed from diluted water solutions.

The use of succinic anhydride with a long, containing 21 atoms alkyl substituent, confirmed that lengthening of the alkyl substituent does not create favorable conditions for creation of polymers of high and very high molecular weights. The highest molecular weights (over 1000 KDa) were observed in the polymers cross-linked with the use of the 2-nonenylsuccinic anhydride. In the case of the 2- dodecensuccinic anhydride the share of

such large systems in the studied samples was only symbolical, and in the case of the 2-docosenylsuccinic anhydride no trace of any systems of medium molecular weight over 35 KDa was found. In this case, the major prepared system was the cyclodextrin dimer. It seems that the existence of a long alkyl connecting link in the linker results in steric difficulties in creating larger polymer systems. It may be a confirmation that the cyclodextrin polymers prepared by means of cross-linking β-cyclodextrin with anhydrides of the dicarboxyl acids are rather spatial, not linear, systems. This type of polymers can find numerous applications not only in the processes connected with environmental protection, but also in pharmacology in studies on finding new methods of oncologic drugs transfer through biological membranes, and even through using them for crossing the blood-brain barrier [55].

Due to participation of water in the processes of separation with the use of cyclodextrin derivatives, a number of DSC-TG studies were carried out with the use of the NETZSCH STA 409C simultaneous DSC-TG studies analyzer owned by the Faculty of Mathematics and Natural Sciences of the JDU in Czestochowa. In order to do that, a number of selected samples of polymers cross–linked with the maleic anhydride and succinic anhydride. Irrespective of the selected cross-linking factor, as well as the applied reaction conditions and the molar ratio of the reagents, the thermogravimetric studies allow to say that there are two general forms of the prepared polymers: anhydride polymers, or the ones non-containing in their structure included water molecules, and hydrated polymers [H10,H11]. Generally speaking, in the case of this type of cyclodextrin polymers the performed thermogravimetric studies do not provide much additional knowledge on the structure of the prepared systems. The course of TG and DSC temperature distribution curves shows many similarities with the non-bounded cyclodextrin, but at the same time one can say that the structure of the anhydrous polymer systems seems to be more cross-linked and, therefore, more thermally stable than that of hydrated polymers.

I have recently started cooperation with the team of professor Janusz Boratynski, Head of the Laboratory of Biochemical Chemistry at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wroclaw in order to obtain drug-carrier conjugates based on active proteins, modified with the use of cyclodextrin.

Target treatment, with the use of the newly discovered mechanisms of the proliferating tumor cells, stops development of the tumor without damaging healthy cells of the system. Along with the dynamically developing molecular research, there are intensive studies going on in oncology on searching for new molecules, which could be of therapeutic use [57].

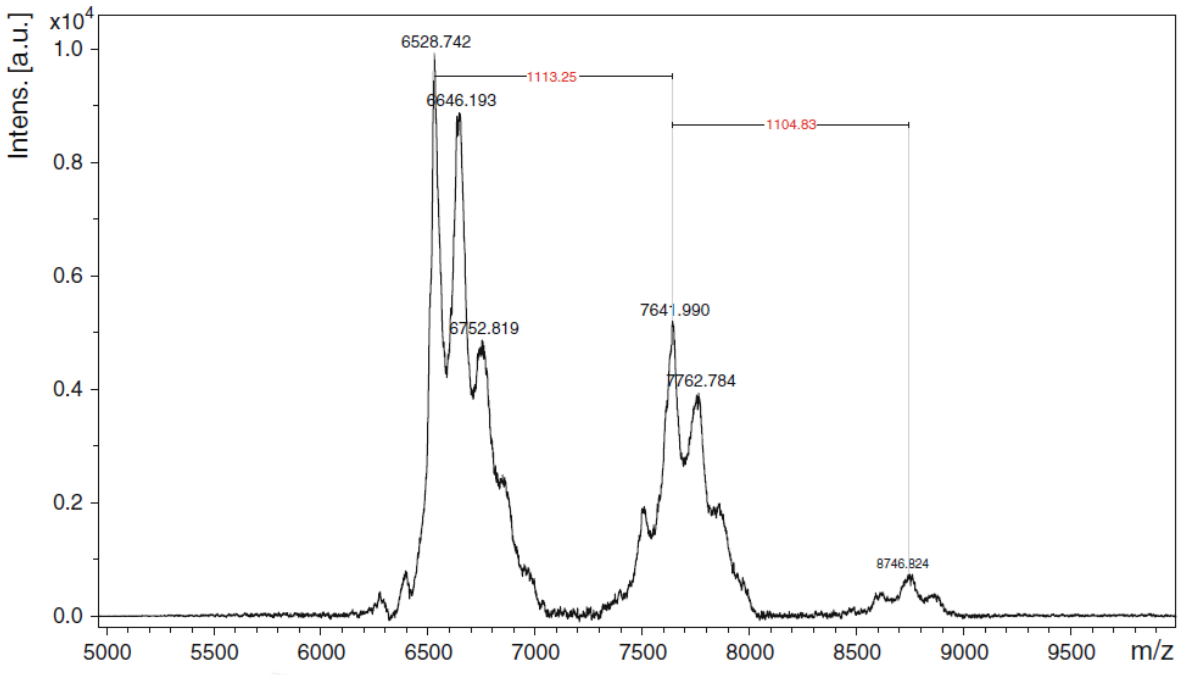
In many cases, small molecules of anti-cancer drugs are excellent therapeutics, but because of lack of specificity and unfavorable physical and chemical properties, such as solubility, change of properties as a result of pH, fast metabolism in the system, there are serious problems in using the drugs in many anticancer therapies.

Binding cyclodextrins with carriers allows to include a drug into hydrophobic cavities of a conjugate. Taking advantage of the typical properties of a carrier will probably allow selective impairment of life functions of the target cells.

The main reaction of linking CD to a protein molecule is the reaction between the terminal amino acid containing a free amine group and the monoformyl β-CD derivatives. As a result of the reaction of the aldehyde group with protein amine groups in high temperature, stable chemical bonds are formed.

CD/protein conjugates were synthesized with the use of an original high-temperature method [58,59]. The methods involves synthesizing the conjugate in high temperature (90-125 ^{o}C) lyophilized mixture of substrates. The applied method allows to obtain the conjugate without losing the biological and enzymatic activity of the protein used in the reaction, and without disarrangement of chemical structure of the cyclodextrin molecule. The method is simple, inexpensive and can be used to synthesize products on a large scale.

Mono-6-*O*-formyl-β-CD was obtained during the studies and was synthesized with the proteins: lizozym and BPTI (Bovine Pancreatic Trypsin Inhibitor). Analysis of the MALDI-TOF spectra explicitly indicates formation of a conjugate in which one or two β-CD molecules were linked to a protein molecule. The proposed method of synthesis is characterized by high repeatability which makes it useful for pharmaceutical purposes [H14].

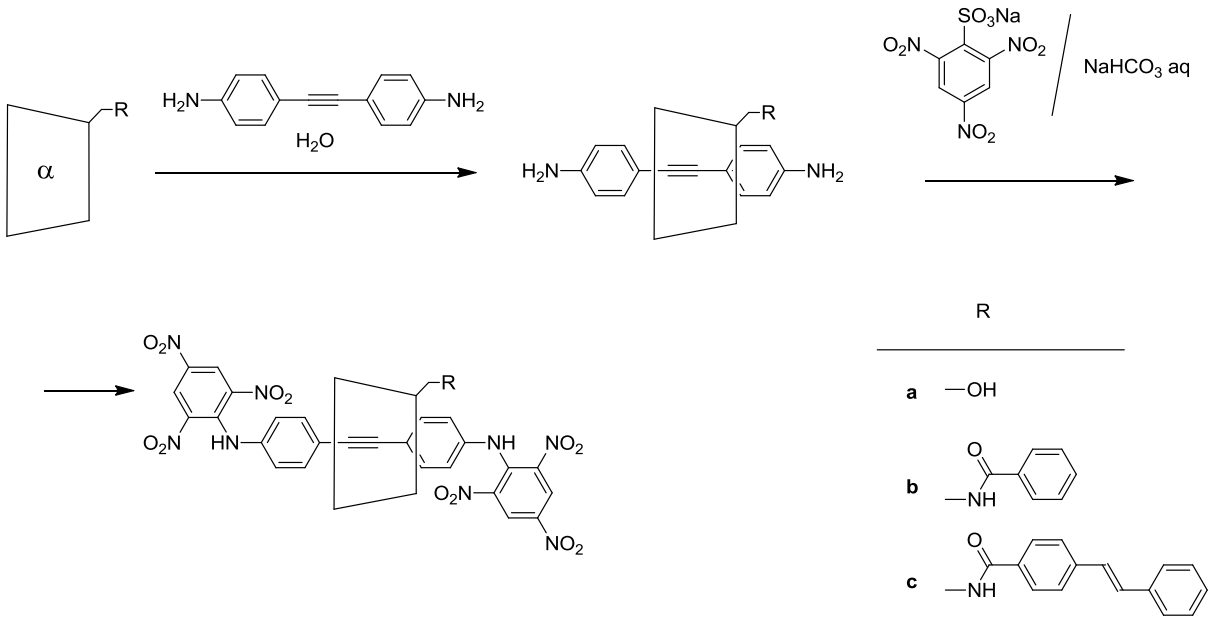


Scheme 4: MALDI-TOF for the cyclodextrin-protein (BPTI- (Basic pancreatic trypsyn inhibitor)) conjugate [H14].

Works concerning cyclodextrin conjugate have become part of the widely understood research concerning pharmaceutical chemistry, which may result in the future in finding practical applications of the studied molecules. On the other hand, gaining closer knowledge on structure and specific effects of such large, biologically active systems extends the knowledge in the field of very interesting and rapidly developing supramolecular chemistry.

On account of my previous theoretical and practical interest in polymer cyclodextrin systems and biologically active cyclodextrin-protein conjugates I started theoretical and survey studies on the latest studies and applications of cyclodextrins in supramolecular chemistry. The effect of my studies were two survey works discussing the latest results in application of cyclodextrins in the structure of rotaxanes and polyrotaxanes. Extending knowledge on the practical use of cyclodextrins in supramolecular chemistry will make it possible to continue the work connected with applications of cyclodextrins in medical chemistry and biotechnology.

Rotaxanes are supramolecular systems, which due to their possible applications in the synthesis of molecular machines and switches, are currently very widely studied in different scientific centers all over the world. It was the cyclodextrins which, due to their macrocyclic structure, are most often used in the synthesis of rotaxanes. A typical synthesis of [2]rotaxane looks like as it is shown on the diagram below. A molecule of mono-substituted α-cyclodextrin is threaded onto a diphenylacetylene chain fitted to its cavity and blocked in this form by means of two large blocking groups, *e.g*. 2,4,6-trinitrobenzene. Depending on the size of the R-substituent, it is possible to observe the change in free rotation of the substituted α-cyclodextrin in relation to the chain passing through its interior.



The properties of rotaxanes discussed above do not use all the possibilities of their applications. They can be, *e.g*. used to build sensors used in chemical analysis and in biological and environmental studies [H12].

Just like rotaxanes, polyrotaxanes using in their structure molecules of cyclodextrins are at present studies intensively due to their possible uses. Polyrotaxanes are formed as a result of using the long polymer chain, such as PEG (polyethylene glikol), PEO(polyethylene oxide), PANI (polyaniline) or PNIPA (poly(*N*-isopropylacrylamide) as a thread, which can be freely used by cyclodextrin molecules to transfer. It is due to this specific structure that they can be used as molecular switchers, insulated molecular wires, light emitting diodes, smart materials. In the field of biomedical studies, polyrotaxanes can become promising supramolecular materials in the nearest future. There are many literature reports concerning possible uses of polyrotaxanes as biosensors, as well as their uses as drug carriers, particularly in the anticancer target therapy and gene therapies [H13].

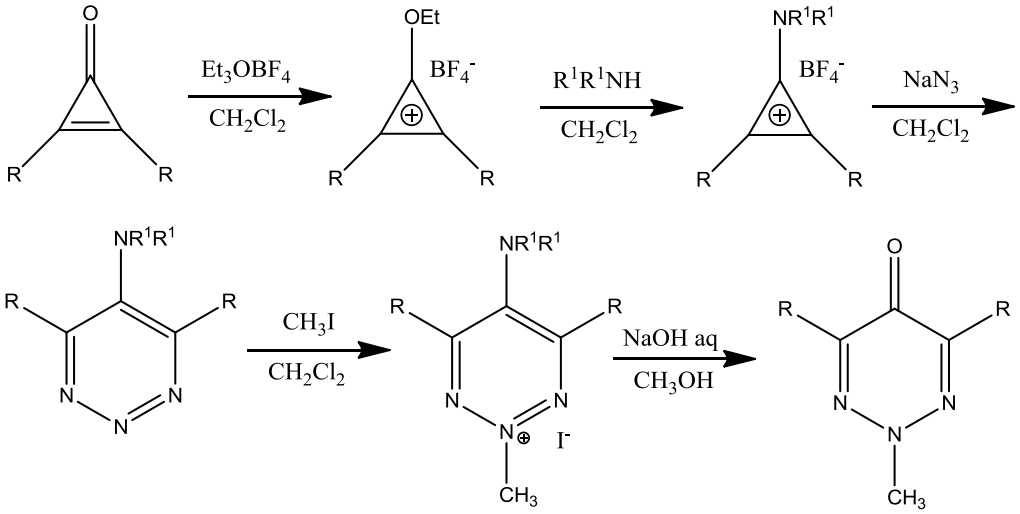
4.5. Résumé of the most important research achievements in the works connected with the habilitation process

As part of the studies connected with the habilitation works, I carried out reactions of synthesis of polymeric cyclodextrin systems with the dicarboxylic acid anhydrides. An original method of cross-linking cyclodextrins, based on the possibility of activation of the secondary hydroxyl groups in the dimethylformamide anhydrous media with the use of sodium hydride. In these conditions, as in the cyclodextrin reactions in the highly alkaline media, a nucleophylic substitution of difunctional compounds takes place, which results in creation of a polymer network with different degrees of cyclodextrin substitution. In my opinion, one of the most important achievements in my experimental work was a study of the reaction of cyclodextrin cross-linking in full, acceptable range of reaction conditions and applied reagent molar ratios. The results of these studies, described in detail in each publication, provided a large amount of important information on the media in which the reaction of cross-linking takes place, and allow designing polymer cyclodextrin systems with the desired molecular weights and physicochemical properties. It allowed to extend the range of scientific research on finding applications for the systems prepared in the processes of ion flotation and metal ion penetration of the polymer inclusion membranes.

4.6. Presentation of other scientific research achievements.

My other scientific research achievements are connected with the studies on:

- chemistry of heterocyclic compounds:
  - synthesis and physicochemical studies of the quaternary 4,6-diazaphenantrene derivatives and their application in the reactions of 1.3-dipolar cycloaddition [60,61]. Although the reactions of this type were in my field of interest before my doctorate, they still remain within the field of my research interest. At present I am working on preparation of cyclodextrins (azides and propagryl derivatives used in the reactions of dimerization and trimerization), which are precursors in the cyclization reactions.
  - Synthesis and physicochemical studies of symmetrical derivatives of diphenylcyclopropenone and their use in the synthesis of 1,2,3-triazines as potential herbicides [62-65]. I worked on these papers mainly during my double stays with professor Kiyoshi Matsumoto's group at Kyoto University.



  - As part of extending my knowledge on the chemistry of the heterocyclic compounds, particularly the compounds containing atoms of sulfur, selenium, and tellurium, I prepared 3 chapters in the Science of Synthesis, co-authored with professor Józef Drabowicz and others, concerning mainly synthesis of dialkilo derivatives of sulfur, selenium, and tellurium and their derivatives [66-68].
- polysaccharide and cyclodextrin chemistry
  - Synthesis and physicochemical studies of β-cyclodextrin pyridine derivatives, conventional synthesis and microwave field synthesis [69]. An attempt at combination of the studies on heterocyclic compounds with cyclodextrin chemistry resulted in one original paper and a conference announcement. The works were performed during supervision of a Master's thesis.

- Thermogravimetric studies of β-cyclodextrin and their complexes with metal ions [47]. Apart from thermogravimetric studies of the systems prepared during the projects that form the basis of my habilitation, I also conducted research on the possibility of preparing β-cyclodextrin complexes with metal ions. The projects were an interesting supplement of theoretical knowledge of preparation of this type of complexes in view of making use of cyclodextrin polymers in the process of ion flotation and metal ion transfer through polymer inclusion membranes.
- The studies concerning application of β-cyclodextrin propargyl derivatives (mono-substituted and per methylated) in the reactions of cyclotrimerization catalyzed with metal (RuII) complexes. Synthesis and studies on physicochemical products of cyclotrimerization. As a result of these studies I prepared one of the [2+2+2] propargyl derivatives of β-cyclodextrin with 1.6-dyine, at the same time setting the conditions of the process of carrying out the reaction. The project was implemented during my one-month stay in the Laboratory of Molecular and Tioorganic Chemistry (Laboratoire de Chimie Moléculaire et Thio-organique), ENSICAEN, CAEN, France. Cooperation with professor Bernard Witulski within a scholarship of the French government.
- Part of extending my knowledge on the cyclodextrin chemistry included preparation of a monograph, in co-authorship with professor Wanda Śliwa. In the book I made a review of the latest literature reports of the time on cyclodextrin inclusion complexes. Modified cyclodextrins in the aspect of their syntheses, reactivity and complexing properties were also discussed. I also presented cyclodextrin dimers and polymer-cyclodextrin systems. Diverse possibilities of application of cyclodextrins were also characterized [46].

- Supramolecular chemistry:
  - When extending my knowledge on the supramolecular chemistry, I prepared, in co-authorship with professor Wanda Śliwa and other members of staff of the Institute of Chemistry and Environmental Protection, a series of articles devoted mainly to supramolecular chemistry. Among the more extensively discussed compounds were viologens, *i.e*. systems containing in their structure a 1,1'-dimethyl-4,4'-bipiridine ion. As the methyl viologens assume the form of dication and are colorless in solution, and at the same time they strongly absorb in the UV region, they can find numerous applications in construction of molecular machines, optoelectronic devices, or logic gates [70,73]. Other systems of great importance in supramolecular chemistry are porphyrins, especially the compounds having quaternary azaaromatic moieties built-in their structure. Physicochemical properties, as well as porphyrin systems showing biological properties were also described. Finally, I also presented possibilities of covalent and non-covalent linking of porphyrin systems containing quaternary azaaromatic moieties with fullerenes and cyclodextrins [71]. Other works on supramolecular chemistry were connected with calixarene complexes with ion metals. I focused in them on

describing syntheses of that type of complexes and their possible applications in constructing sensors used in environmental protection [72].

- Environmental chemistry:.
  - Synthesis and physicochemical studies of β-cyclodextrin complexes with herbicides from the chlorophenoxyacetic acids group. A solid state complex with 2,4-dichlorophenoxyacetic acid using one of the typical methods of creating CD complexes (kneading) was prepared, the physicochemical properties of which were determined by thermogravimetric studies and IR spectroscopy. The complexes prepared in this way were examined for release of the herbicide from the complex, obtaining a sustained-release of the compound. It gives hope as to possible use of this type of complexes in applied pesticides characterized by the sustained-release, which can result in limitation of the negative effect of the commercial preparations on the environment. The work is continued in our Institute and the results obtained to date have been presented at national and international conferences and were submitted to be published.
  - Synthesis and physicochemical studies of β-cyclodextrin complexes with environmentally friendly selenoorganic compounds. Within the frames of the ERASMUS program it was possible to establish not only a didactic, but also scientific cooperation the result of which is a small scientific program concerning complexes of selenoorganic compounds. The cooperation was established with professor Claudio Santi from the Faculty of Pharmacy at the University of Perugia.

4.7. List of reference quoted in the above chapter (except the works included in the habilitation proceedings – [H1-H14]

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