SELF-ASSEMBLY BEHAVIOR OF AMPHIPHILIC OLIGOMERS

Ph.D. Theses

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I. Introduction and objectives:

Nowadays, there is a constantly increasing demand for polymers with special properties. This kind of special polymers is easily obtained when a hydrophobic and hydrophilic block is present within the same macromolecule. Amphiphilic block copolymers consisting of hydrophilic and hydrophobic parts have attracted great consideration from both academic and practical points of view. When dissolved in water (preferred for the hydrophilic block) or in organic solvents (preferred for the hydrophobic block) the self-assembly of amphiphilic block copolymers is expected into various types of polymer aggregates, such as micelles, vesicles, and rod-like associates.

The control of the aggregation process (by chemical and physical parameters) is essential when the preparation of planned structures in micro and nanometer size range come into sight. These amphipilic block copolymers can be used for drug delivery in the human body. This is a new way in human therapy and it improved very quickly in the last 20 years. In the current medical therapy drug molecules need to go through the whole human body before exerting the wanted biological effect on the desired spot. On the contrary if the dug molecule is “packed” into a micelle in this case the micelle “carries” the active ingredient to its desired destination within the human body. It improves the efficiency of the therapy while at the same time improves the physical characteristics of active ingredients including stability and solubility.

The solubilization of water insoluble drugs has been the focus of surfactant chemistry. Those compounds which consist of a hydrophobic and hydrophilic part are called surfactants. In the past soap molecules fell in this class but nowadays a number of new surfactants were synthesized from simple tenzides to complex amphiphilic block copolymers. One of biggest part of surfactants is the non-ionic tensides which are used mainly in household and personal care, pharmaceutical and oil industry.

The formed aggregates determine the application field of these materials.

The amphiphilic block copolymers consist of a hydrophobic and a hydrophilic part and form aggregates in block selective solvents. The same phenomenon is expected when one of the blocks is exchanged to bulky substituents. Cyclodextrines (CD) and their derivatives mean a cheap alternative for both types of blocks. If all but

one hydroxyl groups of the cyclodextrine ring are replaced by methoxi groups we receive an apolar ring. The CD ring can be linked to another molecule through the unchanged hydroxyl group. By the combination of a hydrophilic polymer chain and a modified hydrophobic CD ring, one can profit from the benefit of the formation of supramolecular structures (i.e. micelles and/or vesicules) and inclusion complexes at the same time. The core of the micelles/vesicules may be doped independently from the CD cavity therefore the preparation of new types of multifunctional medicines becomes within reach.

The aim of our research was to synthesize new types of amphiphilic block copolymers and investigate their self-assembly behavior as well as already existing surfactants in aqueous solutions. Our aims were to find correlation between the HLB number and critical micelle concentration of those surfactants where the carbon chain lengths were the same but the ethylene oxide chain varied in aqueous solution.. Furthermore we wanted to investigate the self-association properties and determine the basic thermodynamic parameters of those systems as well.

Our goal was to synthesize, characterize and investigate the self assembly behavior of such a block copolymer where both the base polymer and formed aggregates can be doped independently of each other that is why we chose an modified CD derivative which can be obtained by linking (permethyl-6-amino-6-deoxy-β-Cyclodextrin, PMe-β-CD) to poly(ethylene-glycol, PEG).

Our aim was the synthesis of PIB-PVA diblock copolymers having the same size PIB block but varying PVA block. The effect of the diblock architecture on the micellar properties, as well as the solubilization ability of the polymers, were meant to be studied in detail, focusing on the length ratios of the two blocks. In addition, the aggregates formed in aqueous media were tested as possible drug carrying nanoparticles.

II. Investigation and synthetic methods

II.1. Materials and synthetic methods:

The Neodol 91-2.5E, 5E, 6E and 8E alcohol ethoxylate samples were obtained from Shell Chemicals LTD, permethyl-6-amino-6-deoxy-β-cyclodextrin was received from CycloLab Cyclodextrin Research & Development Laboratory Ltd. for our synthesis.

The polyisobutylene and poly(tert-butyl-vinylether) preparation were carried out using the so called dry box method. The polymers prepared were purified by column chromatography and dialysis method. The polyisobutylene-block-poly(tert-butyl-vinilether) was hydrolyzed to poly-isobutylene-block-poly-vinyl alcohol by hydrogen-bromide at 0 ºC, in nitrogen atmosphere in dry CH_2Cl_2 solution.

II.2. Instruments and softwares

The MALDI-MS measurements were performed with a Bruker BIFLEX III mass spectrometer equipped with a time-of-flight (TOF) mass analyzer. The spectra were externally calibrated with a poly(ethylene glycol) standard (M_n=1450 g/mol, M_w/M_n=1.02) using linear calibration.

The M_n and molecular mass distribution (MWD) of the polymers were measured by SEC in THF at 35 °C with a Waters chromatograph equipped with four gel columns (7.8 x 300 mm, 7 μm Ultrastyragel columns: 500, 10^3, 10^4, 10^5 Å), a Waters 600 HPLC pump, and Waters 490E UV and Waters 410 refractive index detectors. The M_n and M_w/M_n values of the oligomers were calculated relative to polyisobutylene**.**

The micellar properties were investigated by Light Scattering experiments on a Brookhaven light scattering instrument equipped with a BI-9000 digital correlator and temperature-controlled goniometer was used. The light source was a solid-state vertically polarized laser operating at λ= 533 nm.

The ^{1}H-NMR spectra were recorded in $CDCl_3$ at 25 °C on a BRUKER AM 360 spectrometer with tetramethylsilane as the internal standard (δ=0 ppm).

Steric correlations were obtained in NOESY experiments while the 2D DOSY experiments were run for molecular weight and solvent state behavior determination.

The NMR measurements were carried out with a Bruker DRX-500 spectrometer at 300K temperature, in D_2O solution and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt was used as internal standard.

The UV-Vis spectra were recorded on a HP 8453 diode array spectrometer at 25 °C in a quartz cuvette of 1 cm optical path length. The absorbance at peak maximum of 321 nm was used for indomethacin concentration calculations.

III. New scientific results

III.1 Characterization and self-assembly behavior of Neodol samples

The self-assembly of polydisperse industrial-type ethoxylate based nonionic surfactants was studied. Important solution parameters such as the critical micelle formation concentration, micelle size, and size distribution were measured. It was found that the cmc value increases with the HLB number, and a linear correlation was found between ln(cmc) and the average number of EO units. From the slopes of the ln(cmc) versus average number of EO units the contribution of a single EO unit to the total free energy of micellization was deduced and found that ΔG_{EO}°=0.9 kJ/mol was at 25 ºC and 0.3 kJ/mol was at 35 ºC . The samples were characterized by MALDI-TOF-MS and ^{1}H-NMR methods, and the number average molecular weight and HLB numbers were determined from the corresponding spectra. There were significant differences between the values of both M_n and HLB determined by means of the two methods.

III.2. Synthesis and self-assembly behavior of a new β-cyclodextrin conjugate

The synthesis and detailed study of a new amphiphilic molecule, namely the PMe-β-CD-PEG conjugate was accomplished. According to light scattering studies the substance forms aggregates in aqueous media. The 2D NOESY experiment showed the absence of inclusion complex type aggregation while the 2D DOSY measurements suggest a flexible anisotropic structure. The aggregates can serve as the starting materials for novel drug carrying bioconjugates as well as graft amphiphilic copolymers using the appropriate rotaxanate-forming polymer chain.

III.3. Poly(vinyl alcohol)-based amphiphilic copolymer aggregates as drug carrying nanoparticles

The synthesis of amphiphilic PIB-b-PVA copolymers of varying hydrophilic segment lengths was carried out. The self-assembly behavior was investigated in aqueous media by dynamic and static light scattering methods. Direct dissolution was excluded as a proper method for solution preparation, and instead the dialysis method is suggested. The cmc values behaved as expected but micelle sizes and aggregation numbers were found to be much higher than in the case of regular amphiphilic copolymers. The micelles have a more or less spherical shape according to their Rg/Rh ratios, but considering their sizes they cannot be classical core-shell type micelles. Important micellar properties such as the volume of the apolar micelle core were calculated. The hydrophobic cavity of the aggregates was tested as a drug carrying compartment by doping the aqueous copolymer solutions with indomethacin. Each copolymer solution increased the solubility of the water insoluble drug but a large dependence of segment length ratio was found. The best result was obtained in the case of PIB-b-PVA2 where the PIB:PVA length ratio was 1:8 where a tenfold increase in indomethacin solubility was observed. From the solubility data a partition constant, K_v, was calculated and was found to be dependent upon the aggregation number. The cmc of the doped copolymers remained unchanged, although the micelle size was reduced significantly.

IV. Possible application of the results

The comprehensive overview of the ethylene oxide base nonionic surfactants promote the better understanding of these kind of systems. Our results can be used in. pharmaceutical, household or personal care industry at the beginning of the product planning phase. It would accelerate the selection of surfactants and reaction or process conditions. The MALDI technique can be used to investigate the composition of these kinds of surfactants parallel with other existing analytical methods.

The PMe-β-CD-PEG can be used for the preparation of new types of multifunctional medicines as drug delivery compounds because the CD ring and formed aggregates

can be doped by two different drug molecules independently of each other. In this case both drug molecules can be transferred to the desired destination within the human body.

The apolar core of the formed PIB-b-PVA aggregates can be used to water insoluble drug delivery as nano container. The solubilization of active ingredients can be controlled by the modification of the PVA length.

V. Scientific publications and Lectures

V.1. Publications in the field of the dissertation

1. Miklós Nagy, **László Szöllősi**, Sándor Kéki, and Miklós Zsuga: Self-Assembly Study of Polydisperse Ethylene Oxide-Based Nonionic Surfactants, *Langmuir* **23**, 1014-1017 (2007), IF:3,9

2. Miklós Nagy, **László Szöllősi**, Sándor Kéki, Pál Herczegh, Gyula Batta, László Jicsinszky, Miklós Zsuga: Synthesis and Self-Assembly Behavior Study of α,ω-Dicarboxyl-Poly(ethylene-glycol)–Permethyl-6-Amino-6-Deoxy-β-Cyclodextrin-Monoamide: A New b-Cyclodextrin Conjugate, *Journal of Polymer Science: Part A: Polymer Chemistry,* Vol. **45**, 5149–5155 (2007), IF:3,4

3. Miklós Nagy[1], **László Szőllősi[1]**, Sándor Kéki[1], Rudolf Faust[2], Miklós Zsuga[1]: Poly(vinyl alcohol)-based amphiphilic copolymer aggregates as drug carrying nanoparticles; beküldve

   IF (összes): 7,3

V.2. Lectures and posters in the field of the dissertation

1. **Szöllősi László**, Deák György, Kéki Sándor, Zsuga Miklós: Etilén-oxid bázisú nemionos tenzidek; VIII. Nemzetközi Vegyészkonferencia 2002. november 15-17. Kolozsvár, Románia

2. Kéki Sándor, Nagy Miklós, **Szőllősi László**, Batta Gyula, Herczegh Pál, Zsuga Miklós: Self-assembly of amphiphilic telecelics with bulk functionality*, 1st European Chemistry Congress***,** Budapest, Hungary (27-31 August 2006).

3. Miklós Nagy, **László Szőllősi**, Sándor Kéki, György Deák, Pál Herczegh, Gyula Batta, László Jicsinszky, Miklós Zsuga: An amphiphilic polyethylene glycol cyclodextrin conjugate, *MTA Szénhidrátkémiai Munkabizottsági Ülés***,** Mátrafüred 2006

4. Nagy Miklós, **Szöllősi László**, Kéki Sándor, Herczegh Pál, Batta Gyula, Zsuga Miklós: α,ω-Poli(etilén-glikol) permetil amino β ciklodextrin konjugátum szintézise és vizsgálata, *XII. Nemzetközi Vegyészkonferencia, Csíkszereda, Románia (2006. október 03-08.)*

5. Nagy Miklós, **Szőllősi László**, Kéki Sándor, Herczegh Pál, Batta Gyula, Zsuga Miklós: α,ω-Poli(etilén-glikol) permetil amino β ciklodextrin: szintézis és karakterizáció, *Zilele Academice Aradene,* Arad, Románia (2007. május 11-13.)

6. **Szőllősi L**., Nagy M., Kéki S., Faust R., Zsuga M.: *Micelles from polyvinyl alcohol - polyisobutylene (PVA-PIB) block copolymers,* 10th International Symposium on Particle Size Analysis, Environmental Protection and Powder Technology, Debrecen, Hungary (August 27—29 2008)

7. Nagy M., **Szőllősi L.,** Pazurik I., Kéki S., Deák Gy., Herczegh P., Batta Gy., Jicsinszky J., Zsuga M.: Amphiphilic polyethylene glycol cyclodextrin conjugates; 10th International Symposium on Particle Size Analysis, Environmental Protection and Powder Technology, Debrecen, Hungary (August 27—29 2008)

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