UNIVERSITY OF PÉCS

Doctoral School of Chemistry

# ENCAPSULATION OF SULFAMETHAZINE DRUG BY *β*-CYCLODEXTRINS AND ITS ADSORPTION ON CARBON NANOTUBES

**PhD Thesis**

**Hiba Mohamedameen**

Supervisor:

**Dr. Beáta Lemli**

Assistant professor


**Pécs**
**2020**

## 1. Introduction

Sulfonamides are preventive and therapeutic agents for certain infections caused by gram-positive and gram-negative microorganisms. At the same time, their widespread application is hindered by their low solubility in aqueous media. Since the water solubility of sulfonamide drugs is increased in the presence of cyclodextrins (CDs), the host-guest type complex formation of these antibiotics with CDs is an extensively studied field.

Sulfamethazine (SMT) is a representative member of the sulfonamide antibiotic drugs, it is still used in human and veterinary therapy. Previous studies described that the aqueous solubility of SMT has been increased by its encapsulation with *β*-cyclodextrin (BCD) and methyl-*β*-cyclodextrin. Comparing the efficiency of solubility improvement under acidic and basic conditions with buffer-free solutions, the ionization of the SMT does not support the increase of its solubility. Although experimental results verified the formation of 1:1 inclusion complexes of SMT with both CDs, the affinity constant derived from phase solubility studies is higher in the case of the native BCD. At the same time, the functionalization of the host molecule does not affect the complexation mechanisms and the inclusion modes of the guest molecule. The spatial conformations of the complexes determined using NMR and molecular modeling studies show that SMT included the substituted pyrimidine ring into the CD cavity. Contradicting to this, the result of another combined NMR and molecular modeling study revealed that the encapsulation of SMT by BCD is favorable with the inclusion of the guest's aniline moiety through the host's cavity. In this latter work, the association constant of SMT-BCD complex (logK ~ 2.9) in buffer-free aqueous solutions was also determined based on ultraviolet and visible spectroscopic studies.

Sulfonamide antibiotics are widely used in veterinary medicine all over the world, but most of the drugs fed to animals cannot be metabolized, therefore livestock feces and urine containing these antibiotics have created serious environmental pollution. Therefore, research extensively focuses not only on the possible risk of the pollution, but on the possible strategies for removing these veterinary antibiotics. One of the possible removal techniques is adsorption. Based on the inclusion complexation of the small bioactive molecules with CDs the CD-containing polymers can effectively decrease the pollutant content of aqueous solution. Furthermore, carbon nanotubes

(CNTs) are reported to be promising adsorbents readily applicable in water and wastewater management. The optimization strategies of the removal of SMT from water using CNTs have been also reported

## 2. Aims

Previous studies described the capability of BCD for increasing the solubility of SMT in aqueous solution and investigated the structure of the complexes by experimental and molecular modeling techniques. However, contradictory results of the complex conformation have been reported. The aims of the present work:

- To get a deeper look into the biologically important interactions of SMT drug with native and randomly methylated BCDs to explain the inclusion complex stability and the stoichiometry in aqueous solutions under different environments.
- The effects of temperature on the host-guest system will also be analyzed. The thermodynamic parameters will be investigated both with experimental methods and molecular modeling.

The interactions between sulfonamides and CNTs have attracted great attention, due to the serious ecological risks of these antibiotics. Since SMT is one of the serious environmental pollutants, in this work, the removal of SMT from water also will be tested. The aims of the present work:

- Based on the interaction between SMT and BCD, a CD containing insoluble polymer will be investigated to extract SMT from aqueous solution.
- CNTs, earlier successfully used for the adsorption of several antibiotics, will also be investigated as possible sorbents to remove this drug from aqueous solution. Systematic analysis will be done to describe the effect of the number of layers of walls and the effect of the functionalization of CNTs.

## 3. Materials and methods

### 3.1 Materials

SMT was purchased from Alfa Aesar. BCD, randomly methylated $\beta$-cyclodextrin (RAMEB) and β-cyclodextrin bead polymers (BBP) (BCD content in the BBP: 50 m/m%) were obtained from CycloLab Cyclodextrin Research & Development Laboratory Ltd (Budapest, Hungary). Single-walled carbon nanotubes (SWCNTs,), double-walled carbon nanotubes (DWCNTs), multi-walled carbon nanotubes

(MWCNTs), hydroxyl functionalized multi-walled carbon nanotubes (H-MWCNTs) and carboxyl functionalized multi-walled carbon nanotubes (C-MWCNTs) were purchased from Guangzhou Heji Trade Co. (China). The tubes' average diameters are 1-2, 1.3-3, < 8, < 8 and < 8 nm and they are about 50, 15, 50, 50 and 50 microns in length with the purity of more than 90%, 50%, 95%, 95% and 95% in the case of SWCNTs, DWCNTs, MWCNTs, H-MWCNTs and C-MWCNTs, respectively. Methanol (spectroscopic grade) was purchased from Reanal. All the other analytical grade chemicals were purchased from VWR International Ltd (Debrecen, Hungary). Phosphate buffers with different pH (2- 10) were prepared in ultrapure water (conductivity < 0.1 μS/cm, Adrona water purification system).

### 3.2 UV–Vis spectrophotometry

Specord plus 210 spectrophotometer (Analytik Jena, Germany) was used to record the UV-VIS spectra. Photon counting method with 0.1 s integration time at 3 nm bandwidths was used for data collection. The measurements were carried out at 298.2 K. Quartz cuvettes (Hellma, path length 1.0 cm) were used in the measurements. Phosphate buffers with different pH (2, 5, 7 and 10) were the solvent.

### 3.3 Fluorescence spectroscopic studies

Highly sensitive Fluorolog tau3 spectrofluorometer (Jobin-Yvon/SPEX, Longjumeau, France) was used to investigate the fluorescence spectra of the different solutions. For data collection, the photon counting method with 0.1 s integration time was used. Excitation and emission bandwidths were set to 4 nm. A 10 mm thickness of the fluorescent probes with right-angle detection was applied. Temperature-dependent steady-state fluorescence spectroscopic measurements were carried out at different temperatures: 298.2 K, 303.2 K, 308.2 K, and 313.2 K. The fluorescence emission spectra of SMT (30 μM) was recorded in the absence and presence of increasing concentration of BCD or RAMEB (0–3 mM) in different phosphate buffers, using 280 nm excitation wavelength. Similarly, to our previous studies, overall and stepwise association constants of the complex formation were calculated by non-linear fitting, based on the fluorescence emission data obtained, employing the HyperQuad2006 program package.

To determine the thermodynamic parameters, temperature dependence of the complex stabilities was examined. According to the van’t Hoff equation (1) the temperature-

dependence of the association constants offers possibility to determine the thermodynamic parameters related to the formation of the SMT-BCD and SMT-RAMEB complexes:

$$ln\,K = -\frac{\Delta G}{RT} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (1)$$

where the ΔH and ΔS stand for the enthalpy and entropy changes of the complex formation, while ΔG is the Gibbs free energy change. R stands for the gas constant, while T is the temperature in Kelvin.

### 3.4 Infrared Spectroscopy

Fourier transform infrared spectra of SMT, BCD, RAMEB and SMT-BCD and SMT-RAMEB complexes were recorded on Platinum Alpha T FT-IR Spectrometer (Bruker, Ettlingen, Germany). Droplets of samples is used for these measurements. Average of ten scans with 5 $cm^{-1}$ resolution is applied.

### 3.5 Modeling Studies

The thermodynamic parameters of the SMT-BCD or SMT-RAMEB complexes were determined at 298 K as follows: The enthalpy change considered as the energy change calculated by subtracting the total energies of the reactants from the total energies of the products. Similarly, the entropy changes were calculated by subtracting the entropy terms of the reactants from the entropy terms of the products. The entropy terms of the species interacted were calculated applying Boltzmann statistics. The higher contribution to the entropy comes from the vibrational motions. Therefore, after calculating the vibrational frequencies using the harmonic approximation, the entropy was then determined as the following equation implemented in the HyperChem code:

$$S_{vib} = R\sum_i \left\{ \frac{h\nu_i/kT}{e^{(h\nu_i/kT)}-1} - \ln[1-e^{(-h\nu_i/kT)}] \right\} \qquad (2)$$

where the $\nu_i$ is the frequency of vibration and T is the temperature (298.16 K).

The total energies of the species interacted have been calculated at semi-empirical MINDO/3 level using HyperChem 8 code. After the geometry optimization at MINDO/3 level the vibrational-rotational analysis was performed in harmonic approximation using AM1 approximation. Neutral aqueous environment was considered by the TIP3P solvation model implemented in HyperChem code.

Considering that in the present studies ionic species are interacted, the ionic strength of the buffer were considered by the additional $PO_4^{3-}$, $K^+$, $Na^+$ and $H_3O^+$ ions as described in an earlier study. Accordingly, the final cube for representing solvents has 30 Å × 30 Å × 30 Å sizes and contained water, $PO_4^{3-}$, $HPO_4^{2-}$, $K^+$, $Na^+$ and 9 $H_3O^+$ according to the composition of the buffer solution while the pH varied from 7, 5 and 2. After 10 ps MD simulation to equilibrate the system at room-temperature at MM+ level, the calculations for the complexes and the separated species interacted were performed at MINDO/3 level. To reduce the huge computational time, the random-methylated CD derivative (RAMEB, which have electron-rich cavity) was considered as negatively charged species of the native BCD.

### 3.6 Adsorption of SMT on BBP and CNTs

The removal of SMT by CNTs or by BBP under different environmental conditions was tested as follows: 20 µM SMT was mixed with 0-0.5 mg of adsorbents in phosphate buffer solution at pH 2, 5 or 7 for 60 minutes. Then the insoluble adsorbents were sedimented by pulse centrifugation and the supernatant was gently collected. SMT contents of these samples were quantified by HPLC (see below). Using the same experimental conditions increasing concentration of SMT (2.5-100 µM) mixed with a constant mass of adsorbents (0.1 mg), then the SMT content of the supernatant was determined. Using the latter data, the adsorption of SMT onto adsorbents was analyzed based on the Langmuir and Freundlich isotherms:

$$q_0 = (Q_0 \times K_L \times C_e)/(1 + K_L \times C_e) \quad (3)$$

$$q_0 = K_F \times C_e^{(\frac{1}{n})} \quad (4)$$

where $q_e$ is the amount of bound SMT (mg) by adsorbents (g) at the equilibrium, while $C_e$ is the amount of unbound SMT (mg) in the solution phase at equilibrium. $Q_0$ is the maximum adsorption capacity, i.e. the calculated maximum amount of SMT (mg) bound per g of adsorbents; $K_L$ (L/mg) and $K_F$ (mg/g)(L/mg)1/n) denote the Langmuir equilibrium constant and Freundlich constant, respectively. Furthermore, $n$ is the adsorption constant or heterogeneity index as an indicator of isotherm nonlinearity.

HPLC system (1100 HPLC, HP) equipped with a UV detector at 263 nm and reversed-phase C18 column (HP, 5 µm, dimensions 4.6 mm inner diameter x 150 mm length) was used to

determine SMT in the gently collected supernatant. The injection volume was 20 μL, the flow rate was 1 mL/min and the mobile phase was methanol and deionized water with the volume ratio of 50:50. The retention time for SMT was 2.6 min and the concentration was determined by the working curve method from 0.1 μmol/L to 100 μmol/L.

### 3.7 Statistical Analyses

Representing mean ± SEM values of the data were calculated depending on three independent experiments. The One-Way ANOVA test was applied with Microsoft Excel used for statistical analyses. The significant level was set as 99% and $p < 0.01$.

## 4. Thesis points

1. Sulfamethazine formed weak but stable complexes (log$K$ ~ 3) with native and randomly methylated $\beta$-cyclodextrin under different environmental conditions. Although the functionalization of the $\beta$-cyclodextrin host molecule by methyl groups does not affect considerably the complex stabilities, the association constants differ significantly only between acidic conditions (pH 2) and at elevated pH (5-10). Based on this finding the relationships between the estimated complex stabilities at room temperature are the following: $SMT^+$ – BCD ~ $SMT^+$ – RAMEB < $SMT^0$ – BCD ~ $SMT^0$ – RAMEB ~ $SMT^-$ – BCD ~ $SMT^-$ – RAMEB.

2. From the thermodynamic point of view both the spectroscopic measurements and the molecular modeling studies highlight the importance of the reorganization of the solvent molecules. Furthermore, the values of thermodynamic parameters are affected not only by the desolvation of the species interacted but also by the different kinds of noncovalent interactions (electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding). The presence of competition of the noncovalent interactions and hydration/dehydration processes was confirmed by the analysis of enthalpy – entropy compensation.

3. pH-affected structures of the complexes have been found: the sulfamethazine molecule enters into the cyclodextrin cavity with its aromatic amine moiety or with its methyl substituents at low or higher pH, respectively. This result explains the contradictory findings of the complex structures published earlier.

4. Molecular modeling calculations indicate the possible tautomerization of sulfamethazine molecule after entering into the randomly methylated $\beta$-cyclodextrin cavity. The formation of zwitterionic sulfamethazine in the host cavity has been supported experimentally by FT-IR measurements.

5. The insoluble β-cyclodextrin bead polymer is not applicable to remove SMT from aqueous solution effectively. The sulfamethazine-binding ability of this polymer was significantly lower than it was expected on the basis of stability of SMT – BCD complex.

6. Carbon nanotubes are capable of effectively decreasing the SMT content of aqueous solution in pH 2, 5, and 7. According to the pH the following order of efficiency for the removal of sulfamethazine from aqueous solution was found: pH 2 > pH 5 > pH 7. The electrostatic interaction between the SMT molecule and carbon nanotube surface is known to be the driving force of the adsorption, therefore the adsorption of cationic and neutral forms of the drug molecule are higher than the anionic form.

7. Both functionalization and the increase of the side wall of the nanotubes decrease their adsorption capacity. Therefore, among the 5 different types of carbon nanotubes investigated, the SWCNT is the most suitable to extract SMT from aqueous media under the range of the environmental conditions investigated.

These observations might be relevant for the preparation of orally administered products of sulfonamide-cyclodextrin complexes and they can be useful remove these drugs from contaminated beverages e.g. from drink water.

# 5. Publication List

## 5.1 Publications in refereed journals related to this thesis

1. Mohamed Ameen, H., Kunsági-Máté, S., Szente, L., Lemli, B. Encapsulation of sulfamethazine by native and randomly methylated β-cyclodextrins: the role of the dipole properties of guests, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 225 (2020) 117475. (IF: 2.931)

2. Mohamed Ameen, H., Kunsági-Máté, S., Szente, L., Bognár, B., Lemli, B. Thermodynamic characterization of the interaction between an antimicrobial drug sulfamethazine and two selected cyclodextrins, Molecules, 24 (2019) 4565. (IF: 3.060)

3. Mohamed Ameen, H., Kunsági-Máté, S., Noveczky, P., Szente, L., Lemli, B. Adsorption of sulfamethazine drug onto the modified derivatives of carbon nanotubes at different pH (under review)

## 5.2 Publications in refereed journals not related to this thesis

1. E. AL-Mukhtar, S., F. AL-Katib, H., A. AL-Nuamimy, L. Preparation and characterization of some transition metal complexes with crotyl xanthate ligand and their adducts with nitrogen bases, J. Al-Rafidane , 26(1) (2017) 49-55

2. F. AL-Katib, H. Aseries of M-M heterometallic coordination polymers: syntheses structures properties. KUJSS, 1992 (2016) 0849

3. Kiss, L., Mohamed Ameen, H., Lemli, B., Kunsági-Máté, S. Determination of solubility of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and its sodium salt in acetonitrile and voltammetric investigation of sulphonamide drugs in different solvents in their absence and presence (under review)

## 5.3 Posters and presentations related to the thesis

1. Mohamed Ameen, H., Kunsági-Máté, S., Lemli, B. The effect of pH on the interaction between sulfamethazine and β-cyclodextrin, 3rd Symposium on Weak Molecular Interactions, Poland, Opole, (2017) p11

2. Mohamed Ameen, H., Lemli, B. pH dependent encapsulation of sulfamethazine antibiotic by cyclodextrin derivatives, Med Pecs conference, Hungary, Pecs, (2018) p 41

3. Mohamed Ameen, H., Kunsági-Máté, S., Lemli, B. Encapsulation of selected sulfamethazine by beta cyclodextrin and random methylated beta-cyclodextrin, 3rd International Symposium on Scientific and Regulatory Advances in Biological and Non-Biological Complex Drugs: A to Z in bioequivalence conference, Hungary, Budapest, (2018) p 13