J Incl Phenom Macrocycl Chem (2010) 66:417–421
DOI 10.1007/s10847-009-9632-8

SHORT COMMUNICATION

Host–guest complexation of a nitroheterocyclic compound with cyclodextrins: a spectrofluorimetric and molecular modeling study

Renato Grillo · Nathalie F. S. de Melo · Daniele R. de Araújo · Gustavo H. G. Trossini · Elaine F. F. da Cunha · Teodorico de Castro Ramalho · Leonardo Fernandes Fraceto

Received: 29 April 2009 / Accepted: 1 July 2009 / Published online: 24 July 2009

Abstract Nitroheterocyclic compounds (NC) were candidate drugs proposed for Chagas disease chemotherapy. In this study, we investigated the complexation of hydroxymethylnitrofurazone (NFOH), a potential antichagasic compound, with α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), Hydroxypropyl-β-cyclodextrin (HP-β-CD), Dimethyl-β-cyclodextrin (DM-β-CD) and γ-cyclodextrin (γ-CD) by fluorescence spectroscopy and molecular modeling studies. Hildebrand–Benesi equation was used to calculate the formation constants of NFOH with cyclodextrins based on the fluorescence differences in the CDs solution. The complexing capacity of NFOH with different CDs was compared through the results of association constant according to the following order: DM-β-CD > β-CD > α-CD > HP-β-CD > γ-CD. Molecular modeling studies give support for the experimental assignments, in favor of the formation of an inclusion complex between cyclodextrins with NFOH. This is an important study to investigate the effects of different kinds of cyclodextrins on the inclusion complex formation with NFOH and to better characterize a potential formulations to be used as therapeutic options for the oral treatment of Chagas disease.

Keywords Nitroheterocyclic compound · Cyclodextrins · Fluorescence spectroscopy · Molecular modeling · Inclusion complex

R. Grillo · L. F. Fraceto (✉)
Universidade Estadual Paulista Júlio de Mesquita Filho, Campus Sorocaba, Av. Três de Março, 511, 18087-180 Sorocaba, São Paulo, Brazil
e-mail: leonardo@sorocaba.unesp.br

N. F. S. de Melo · D. R. de Araújo · L. F. Fraceto
Departamento de Bioquímica, Instituto de Biologia, Unicamp, Cidade Universitária Zeferino Vaz, s/n, Campinas, SP, Brazil

D. R. de Araújo
Universidade Federal do ABC, Santo André, SP, Brazil

G. H. G. Trossini
Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brazil

E. F. F. da Cunha · T. de Castro Ramalho
Universidade Federal de Lavras, Campus Universitário da UFLA, Lavras, MG, Brazil

Introduction

Chagas disease is a serious health problem for people living in Latin America. The nitroheterocyclic hydroxymethylnitrofurazone (5-nitro-2-furaldehyde N-(hydroxymethyl)-semicarbazone) (NFOH) has been shown to be active against *T. cruzi*, the causative agent of Chagas disease and in this way, this compound has been proposed for a new alternative of chemotherapy [1].

Cyclodextrins (CDs) are cyclic oligosaccharides that can be used as a carrier systems for drugs [2, 3] because of their ability to form inclusion complex with different kinds of compounds [4]. Previous works reported some characterization of the inclusion complex formed between nitroheterocyclic compounds (NC) and CDs [5–9]. In this study we have been investigated the formation of inclusion complex between NFOH with different cyclodextrins (α-cyclodextrin (α-CD), γ-cyclodextrin (γ-CD), β-cyclodextrin (β-CD), Hydroxypropyl-β-cyclodextrin (HP-β-CD), Dimethyl-β-cyclodextrin (DM-β-CD)) in order to obtain better information about the inclusion phenomena and the rationale for CD selection as an excipient for generate a delivery system for future therapeutic options for the oral treatment of Chagas disease. In order to

investigate the inclusion complex formation of NFOH with cyclodextrins we used fluorescence measurements (measurements of an association constant by Benesi–Hildebrand plot) and molecular modeling studies.

Experimental

α-Cyclodextrin (α-CD), γ-cyclodextrin (γ-CD) were obtained from Sigma Chem. Co. β-cyclodextrin (β-CD), Hydroxypropyl-β-cyclodextrin (HP-β-CD), Dimethyl-β-cyclodextrin (DM-β-CD) were a gift from (Roquette, France). Hydroxymethylnitrofurazone (NFOH) was synthesized as previously described in literature [10]. The solutions were filtered through 0.22 μm Millipore® nylon membrane filter (Belford, USA). A Hitachi F-4500 spectrofluophotometer (Hitachi, Japan) with matched 10-mm quartz cell was used to measure the fluorescence.

General experimental procedure

An aliquot of the stock solution of NFOH was transferred into a 10 mL volumetric flask to reach 50×10^{-6} mol/L as function of different CDs (α-CD, γ-CD, β-CD, HP-β-CD, DM-β-CD) concentrations. The concentrations of CDs ranged from 0 to 10×10^{-3} mol/L at room temperature (25 °C). Fluorescence emission spectra of NFOH were acquired with an excitation wavelength at 375 nm and the emission intensities were monitored from 450 to 600 nm. Excitation and emission bandwidths were set at 5 nm.

Affinity constant (K) values of NFOH complexation with CDs (α-CD, β-CD, HP-β-CD, DM-β-CD and γ-CD) were calculated assuming a 1:1 (CDs: NFOH) inclusion model as described in the literature [7, 8]. The K values can be calculated from fluorescence data using the Benesi–Hildebrand equation [11]:

$$\frac{1}{\Delta F} = \frac{1}{K \cdot k \cdot Q[\text{drug}]_0} \times \frac{1}{[\text{CD}]_0} + \frac{1}{k \cdot Q[\text{drug}]_0}$$

where $[\text{CD}]_o$ and $[\text{drug}]_o$ were the initial concentrations of CDs and NFOH, respectively. K was the formation constants for CDs-NFOH complex. Q was the quantum yield for the complex and k was an instrumental constant. F was the change in NFOH fluorescence caused by the addition of CDs. The straight lines obtained by plotting ($1/\Delta F$) versus $1/[\text{CD}]_o$ for each CDs-NFOH system were utilized to evaluate K values from the intercept and the slope.

Molecular modeling studies were realized using crystal coordinates of CD were used to the optimization procedure [12–14]. In addition, we optimized the gas-phase structure of the cyclodextrins as a standard for the docking calculation [15]. After the optimization strategy, the compounds were docked into the DM-β-CD, β-CD, α-CD, HP-β-CD and γ-CD binding sites using the ArgusLab 4.0.1 [16], a program for suggesting the most likely conformation and interaction of how a ligand will bind to a cyclodextrin molecule. The ligand and CD molecules are considered flexible during the docking simulation. Thus, a candidate solution is encoded by an array of real-valued numbers representing ligand position, orientation, and conformation as cartesian coordinates for the ligand translation, four variables specifying the ligand orientation (encoded as a rotation vector and a rotation angle), and one angle for each flexible torsion angle in the ligand.

The active site exploited in docking studies was defined as a subset region of 10.0 Å around the center of the cyclodextrin. The interaction modes of the ligand with the CD cavity were determined as the highest energy scored protein–ligand complex used during docking strategy [22]. Thus, we selected the conformers of each compound mostly associated with bioactive conformations, which were represented by the structure with the most favorable binding affinity (ΔE_{bind}) and interaction energy between the pose of ligand and the macromolecule, like CD. Finally, the structures selected from the docking procedure were used for the single-point energy from QM calculations. The QM calculations were performed at Hartree-Fock level with the basis sets 6-31G* in the Gaussian98 A.11 software package [17].

Results and discussion

Figure 1 shows the fluorescent spectra of NFOH in aqueous solution containing DM-β-CD at different concentrations.

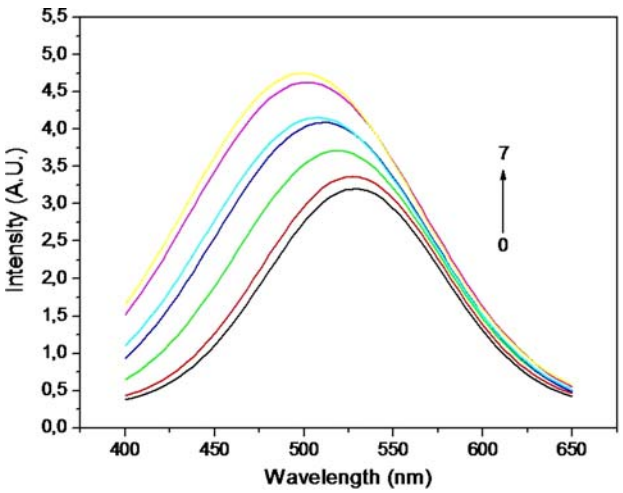

Fig. 1 The fluorescence spectra of NFOH (50×10^{-6} mol/L) containing various concentrations of DM-β-CD. Concentration of DM-β-CD (1) 0×10^{-3} mol/L; (2) 1.0×10^{-3} mo/L; (3) 2.0×10^{-3} mol/L; (4) 4.0×10^{-3} mol/L; (5) 6.0×10^{-3} mol/L; (6) 8.0×10^{-3} mol/L; (7) 10.0×10^{-3} mol/L

NFOH can emit relatively weak fluorescence in the absence of cyclodextrin, however, with the addition of DM-β-CD, the fluorescence intensity was enhanced and the maximum excitation wavelength slightly shifted to a shorter wavelength, from 530 to 500 nm. This bathochromic shift occurs due to the change in polarity environmental of NFOH caused by the addition of cyclodextrins and can indicate that this molecule probably interacts with the cyclodextrins cavity and changes their chemical environment and, as a consequence, their fluorescence spectra.

Similar fluorescence results were obtained with other molecules in literature, for example, methylene blue [18], 9,10-anthraquinone [19], azulene [20], 2-naphthol-6-sulfonato [21] and 5-pyridine-10,15,20-tris-(*p*-chlorophenyl) porphyrin [22]. In these studies, when the guest molecules were entrapped in the CDs cavity, this microenvironment with smaller polarity and stronger rigidity would restrict the freedom of guest molecules and as consequence increase the fluorescence quantum yield. Furthermore, the steric hindrance of cyclodextrins can protect the excited states from nonradioactive and quenching processes that normally readily occur in bulk aqueous solution and enhance the fluorescence efficiencies of guest molecule [23, 24]. Benesi–Hildebrand plot (double reciprocal plot of $1/(F - F_o)$ against 1/[CD]) was used to measure the stoichiometry of complexation and the formation constant of NFOH with different cyclodextrins. The analysis of fluorescent changes to Hildebrand–Benesi equation showed linear plots with correlation coefficient $\geq$0.995, indicating that the stoichiometry was 1:1 (Fig. 2). Grillo et al. [5–7], using other spectroscopic method, also showed that the stoichiometry of the inclusion complex between NFOH with β-cyclodextrins was 1:1.

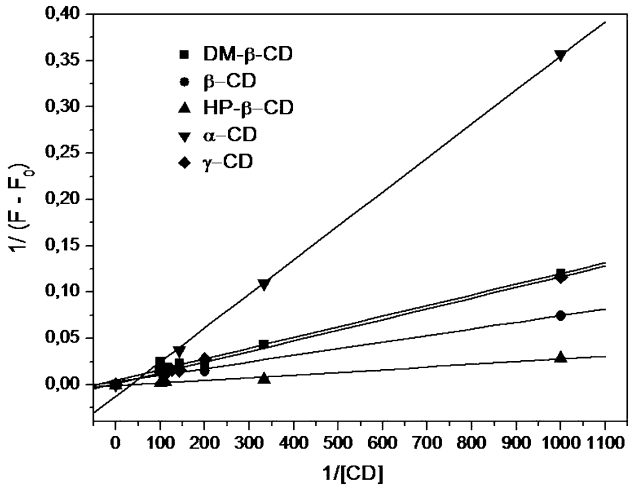

Fig. 2 Benesi–Hildebrand plot of 1:1 complex of NFOH with cyclodextrins (α-CD, γ-CD, DM-β-CD, β-CD and HP-β-CD)

The *K* values of cyclodextrins with NFOH were shown in Table 1 and revealed that the complexing capacity of cyclodextrins presented the following order for NFOH inclusion complex: DM-β-CD > β-CD > α-CD > HP-β-CD > γ-CD. Different values of *K* for cyclodextrins might be due to different characteristics of the inner cavities of cyclodextrins (size and polarity). For β-CD and derivatives, the changes in *K* values reflected the following order: HP-β-CD < β-CD < DM-β-CD and this is probably due to the significant enhancing in the complexation ability caused by methylation, which makes cyclodextrin environment more hydrophobic and benefits the adaptability of the CD–guest interaction [8]. These results indicated that the best complexation of the NFOH occur with DM-β-CD, suggesting that this is a choice for delivery NFOH in oral formulations for Chagas disease treatment.

Regarding α-, β- and γ-CD, the increase on binding constant follows the order β-CD>α-CD $\gg$ γ-CD and this is due to the differences in the cavity sizes of these cyclodextrins, where the volume of β-CD, α-CD and γ-CD cavity were 262 A^3, 174 A^3 and 427 A^3 respectively [2], showing that the size of the β-CD cavity seems to be more important for NFOH binding..

Figure 3 describes the docking of flexible NFOH into the CD cavities aiming to correlate structure to activity and selectivity. The docked binding mode is used to establish a link between the ArgusLab scoring function, structural properties of these compounds and the experimental results in this paper. Thus, the orientations with the best intermolecular energy (van der Waals + electrostatic) were obtained and the accepted ligand (NFOH)/CD models were then subjected to QM calculations. There are two possible orientations of the compounds with the furan ring close to the primary alcohol end of the cavity and with the furan ring near the primary hydroxyl from CD.

The scoring function for the binding energy encoded in the ArgusLab program was applied to evaluate the binding affinities between CDs and NFOH. The inner cavity makes cyclodextrins particularly useful as hosts for inclusion compounds, but the electronic effects in the interaction with the inclusion complexes may be quite important. In order to get more insights about those electronic effects, we performed HF calculation. Table 1 lists the calculated binding energies, at HF level, of NFOH with cyclodextrins. We can note that the relationship between the calculated binding energies and the experimental data for stability constants is satisfactory. Thus, with these results we could also predict the selectivity related to the cyclodextrins with NFOH.

It is important to mention that the evaluation of the docking results was based on CD-ligand complementarity considering steric and electrostatic properties as well as calculated potential interaction energy in the complex and ligand intramolecular energy. At this point, three primary

Table 1 Stability constants (K), (M^{-1}) for NFOH with cyclodextrins inclusion complexes determined by fluorescence Benesi–Hildebrand plot, 25 °C ($n = 3$) and docking energy values (kJ mol^{-1}), at Hartree-Fock level, for NFOH with cyclodextrins

	DM-β-CD	β-CD	α-CD	HP-β-CD	γ-CD
Stability constant (K), (M^{-1})	47.6 ± 0.4	45.5 ± 0.3	32.2 ± 0.1	28.5 ± 0.6	17.5 ± 0.8
Docking energy values (kJ mol^{-1})	−8.74	−8.44	−6.56	−6.04	−4.48

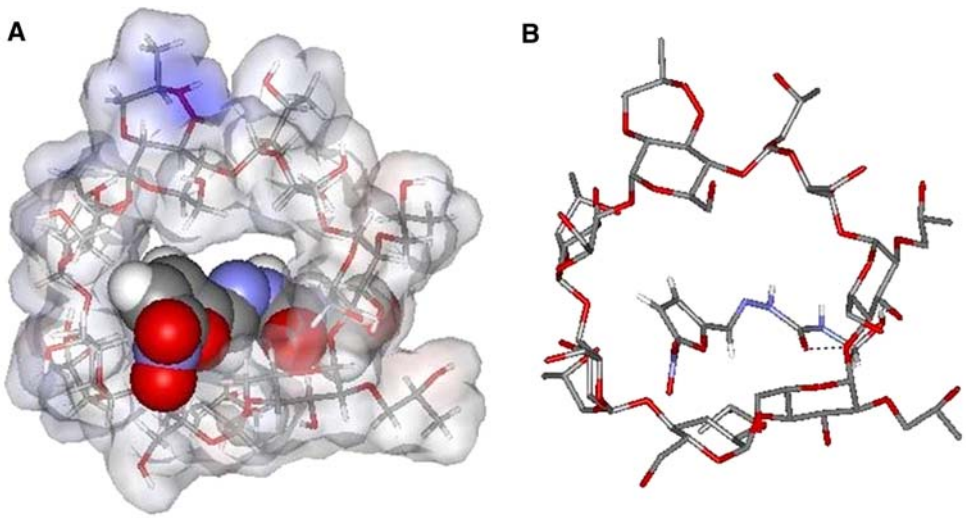

Fig. 3 **a** Van der Waals surface of the HP-β-CD complexed with NFOH. **b** H-bonding interactions of the HP-β-CD with NFOH

factors are known to influence the conformation of a ligand bound to an inclusion process: hydrogen bonding, binding affinity, and total interaction energy [25].

The docking energy values obtained from the QM calculations showed the same relation with the affinity constant values determined by fluorescence spectroscopy. Thus, the inclusion processes involving DM-β-CD and NFOH is preferential. In fact, the inclusion of methyl groups in cyclodextrins leads to a more significant hydrophobic core in the CD cavity, favoring the interaction with NFOH due to a dipole induced mechanism. Interestingly, the hydroxyl groups in HP-β-CD interact through the stronger hydrogen bonds with the terminal hydroxyl group from NFOH. That is certainly a force that avoids the NFOH entrance in the CD cavity, reducing and making hydrophobic interactions with CD difficult, so, our theoretical data reinforce our experimental findings. Regarding α-CD and γ-CD, the interaction with NFOH showed that the size of the cavity determines the insertion of NFOH inside these cyclodextrins, since it is well-known that CD forms inclusion compounds with hydrophobic guest molecules of suitable diameters.

Conclusions

The complexation between cyclodextrins and NFOH was investigated by fluorescence spectroscopy and molecular modeling studies. Cyclodextrins and NFOH could form 1:1 inclusion complexes. The characterization of the inclusion complex of NFOH with cyclodextrins was investigated through the analysis of the stability constants determined by Benesi–Hildebrand. The plots were compared and resulted in the following order of interaction: DM-β-CD > β-CD > α-CD > HP-β-CD > γ-CD. Molecular modeling studies corroborate the experimental data showing that the results are consistent. Our results are in close agreement with predictions from more sophisticated quantum mechanical techniques and give support for the experimental assignments, in favor of the formation of an inclusion complex between cyclodextrins and NFOH, providing additional information on the probable inclusion mode and molecular structure of the complex. The results presented here indicated that the best interaction of cyclodextrin with NFOH occur with DM-β-CD, suggesting that this complex can be promising for oral formulations for Chagas disease treatment. Therefore, this is an important study for the characterization of potential formulations to be used as therapeutic options for the treatment of Chagas disease.

Acknowledgments We are grateful to FAPESP (05/03045-9), FAPEMIG and CNPq, Brazilian agencies for funding this work and CENAPAD-SP for the computational facilities. RG (08/01222-9) and NFSM (06/00121-9) are recipients of fellowships from Fapesp.

References

1. Chung, M.C., Guido, R.V.C., Martinelli, T.F., Gonçalves, M.F., Polli, M.C., Botelho, K.C.A., Varanda, E.A., Colli, W., Miranda,

M.T.M., Ferreira, E.I.F.: Synthesis and in vitro evaluation of potential antichagasic hydroxymethylnitrofurazone (NFOH-121): a new nitrofurazone prodrug. Med. Chem. **11**, 4779–4783 (2003)
2. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
3. Moraes, C.M., Abrami, P., de Araújo, D.R., Braga, A.F.A., Issa, M.G., Ferraz, H.G., de Paula, E., Fraceto, L.F.: Characterization of lidocaine:hydroxypropyl-β-cyclodextrin inclusion complex. J. Incl. Phenom. Macrocycl. Chem. **57**, 313–316 (2007)
4. Atwood, J.L., Davies, J.E.D., Macnicol, D.D., Vögtle, F.: Comprehensive supremolecular chemistry. In: Szejtli, J., Osa, T. (eds.) Cyclodextrins, vol. 3, pp. 652–658. Elsevier Science Ltd., Oxford (1996)
5. Grillo, R., Melo, N.F., Moraes, C.M., Rosa, A.H., Roveda, J.A.F., Menezes, C.M., Ferreira, E.I., Fraceto, L.F.: Hydroxymethylnitrofurazone: dimethyl-B-cyclodextrin inclusion complex: a physical-chemistry characterization. J. Biol. Phys. **33**, 445–453 (2007)
6. Grillo, R., Melo, N.F., Fraceto, L.F., Brito, C.L., Trosssini, G.H.G., Menezes, C.M., Ferreira, E.I., Moraes, C.M.: Physical-chemical characterization of inclusion complex between Hydroxymethylnitrofurazone and Hydroxypropyl beta-cyclodextrin. Química Nova **31**, 290–295 (2008)
7. Grillo, R., Melo, N.F., Moraes, C.M., Lima, R., Menezes, C.M., Ferreira, E.I., Rosa, A.H., Fraceto, L.F.: Study of the interaction between Hydroxymethylnitrofurazone and 2-Hydroxypropyl-beta-cyclodextrin. J. Pharm. Biomed. Anal. **47**, 295–302 (2008)
8. Melo, N.F., Grillo, R., Rosa, A.H., Fraceto, L.F.: Interaction between nitroheterocyclic compounds with β-cyclodextrins: phase solubility and HPLC studies. J. Pharm. Biomed. Anal. **47**, 865–868 (2008)
9. Melo, N.F., Grillo, R., Moraes, C.M., Brito, C.L., Trosssini, G.H.G., Menezes, C.M., Ferreira, E.I., Rosa, A.H., Fraceto, L.F.: Preparation and initial characterization of inclusion complex between nitrofurazone and 2-Hydroxypropyl-beta-cyclodextrin. J. Basic Appl. Pharm. Sci. **28**, 35–44 (2007)
10. Doriguetto, A.C., Silva, C.H.T.P., Ellena, J., Trossini, G.H.G., Chung, M.C., Ferreira, E.I.: 5-Nitro-2-furaldehyde N -(hydroxymethyl)semicarbazone. Acta Crystallogr. E **61**, 2099–2101 (2005)
11. Shuang, S.M., Pan, J.H., Guo, S.Y., Cai, M.Y., Liu, C.S.: Fluorescence study on the inclusion complexes of rutin with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and γ-cyclodextrin. Anal. Lett. **30**, 2261–2270 (1997)
12. Steiner, T., Koellner, G.J.: Crystalline b-cyclodextrin hydrate at various humidities: fast, continuous, and reversible dehydration studied by X-ray diffraction. J. Am. Chem. Soc. **116**, 5122–5128 (1994)
13. Chacko, K.K., Saenger, W.: Topography of cyclodextrin inclusion complexes. 15. Crystal and molecular structure of the cyclohexaamylose-7.57 water complex, form III. Four- and six-membered circular hydrogen bonds. J. Am. Chem. Soc. **103**, 1708–1715 (1981)
14. Saenger, W., Jacob, J., Gessler, K., Steiner, T., Hoffmann, D., Sanbe, H., Koizumi, K., Smith, S.M., Takaha, T.: Structures of the common cyclodextrins and their larger analogues—beyond the doughnut. Chem. Rev. **98**, 1787–1802 (1998)
15. Ramalho, T.C., Figueroa-Villar, J.D.: Thermodynamic evaluation of complexes of zinc and cadmium that mimetize metallic centers in transcription factors. J. Mol. Struct. Theochem. **580**, 217–223 (2002)
16. Viseu, T.M.R., Hungerford, G., Coelho, A.F., Ferreira, M.I.C.: Dye–Host interactions for local effects recognition in homogeneous and nanostructured media. J. Phys. Chem. B **107**, 13300–13312 (2003)
17. Gaussian 98, Revision A.9, M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al. Gaussian, Inc., Wallingford CT (1998)
18. Zhang, G., Shuang, S., Dong, C., Pan, J.: Study on the interaction of methylene blue with cyclodextrin derivatives by absorption and fluorescence spectroscopy. Spectrochim. Acta A Mol. Biomol. Spectrosc. **59**, 2935–2941 (2003)
19. Shamsipur, M., Yari, A., Sharghi, H.: Spectrofluorometric study of complexation of some amino derivatives of 9,10-anthraquinone with β-cyclodextrin. Spectrochim. Acta A Mol. Biomol. Spectrosc. **62**, 372–376 (2005)
20. Abou-Zied, O.K.: A spectroscopic study of the inclusion of azulene by beta-and gamma-cyclodextrins. Spectrochim. Acta A Mol. Biomol. Spectrosc. **62**, 245–251 (2005)
21. Abdel-Shafi, A.A.: Spectroscopic studies on the inclusion complex of 2-naphthol-6-sulfonate with β-cyclodextrin. Spectrochim. Acta A Mol. Biomol. Spectrosc. **66**, 732–738 (2007)
22. Guo, Y.J., Chao, J.H., Pan, J.H.: Study on the interaction of 5-pyridine-10,15,20-tris-(p-chlorophenyl)porphyrin with cyclodextrins and DNA by spectroscopy. Spectrochim. Acta A Mol. Biomol. Spectrosc. **68**, 231–236 (2007)
23. Rajendiran, N., Swaminathan, M.: Luminescence characteristics of 4,4'-diaminodiphenyl methane in different solvents and at various pH. Spectrochim. Acta A Mol. Biomol. Spectrosc. **52**, 1785–1792 (1996)
24. Husain, N., Ndou, T.T., Muñoz de la Pena, A., Warner, I.M.: Complexation of doxorubicin with β- and γ-cyclodextrins. Appl. Spectrosc. **46**, 652–658 (1992)
25. Kuntz, I.D., Blaney, J.M., Oatley, S.J., Langridge, R., Ferrin, T.E.: A geometric approach to macromolecule–ligand interactions. J. Mol. Biol. **161**, 269–275 (1982)