

Synthesis and Characterization of Inclusion Complex of Diminazene Aceturate with β -Cyclodextrin

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Cyclodextrins (CD) are macrocyclic biopolymers with potential applications in the delivery of small and macro-molecular therapeutic agents. Despite the potent host-guest inclusion property, their inherent lack of cellular binding ability has limited applications in drug delivery. Herein, we functionalized β -cyclodextrin (β -CD) with diminazene aceturate (DIMA), which are bioactive molecules, widely distributed some cells, and responsible for antiprotozoal activity. The inclusion complex of DIMA with β -CD was confirmed with textural, thermogravimetric, calorimetric, spectroscopic, and microscopic techniques. Thus, the proposed inclusion complex β -CD-DIMA system could be used as a site-specific drug delivery carrier.

Keywords: diminazene aceturate, β -cyclodextrin, inclusion complex

Diminazene aceturate (DIMA) is an aromatic diamidine compound derived from Surfen C, which has been used as an anti-trypanolytic agent during the past decades [1]. In addition to its parasitocidal activities, a large amount of recent studies have found the potential benefits of diminazene aceturate in animal models with asthma, gastric lesions, ischemic stroke and uveitis [2–5]. Therefore, the therapeutic values of DIMA are far greater than that has been initially expected and it has consequently attracted much research interest [6]. However, the potential effects of DIMA in hepatic disorders remain unknown [7].

The pharmacological mechanisms underlying the beneficial effects of DIMA largely remains unknown, but the anti-inflammatory properties of DIMA were recently suggested to be associated with its pharmacological benefits [8].

Cyclodextrins (CDs) are oligosaccharides consisting of six, seven or eight glucopyranose units connected by 1, 4-glycosidic linkage and have capacity to form inclusion complex with both hydrophilic and hydrophobic guest molecules [9]. The CD-drug host-guest inclusion complexes have capacity to increase the aqueous solubility of poorly water-soluble drugs, stability, and taste masking of the unacceptable taste of drugs [10–12]. In modern drug delivery system, cyclodextrins are the choice of supramolecular host molecules for preparation of drug carriers in different pharmaceutical forms and nanoparticles [13–15]. The resulting noncovalent inclusions or host-guest complexes are of current scientific and technological interest for their peculiar physical, chemical and biological properties. Such noncovalent associations can actually improve the guest water solubility, bioavailability and stability; they can also regulate the release of the guest molecules [16]. Thus, complexation of tolafenamic and flufenamic acids in anionic form with β -cyclodextrin was investigated in solution by 1D and 2D proton NMR spectroscopy. By analyzing the spectroscopic data, it was pointed out that the simultaneous inclusion of both rings of tolafenamic and flufenamic acids gave rise to two isomeric complexes in a 1: 1 ratio. A bimodal bond between these two drugs and β -cyclodextrin was shown in an association order in complexes of 1: 1. [18]

In another paper [19] the molecular mechanisms of formation of inclusion complexes in aqueous ternary systems of caffeine and β -cyclodextrin were studied. Estimation of the equilibrium constant was achieved by mathematical modeling. Information on the driving forces required for the introduction of caffeine into the β -cyclodextrin molecule was obtained from the volume changes. In another paper [20], increasing the solubility of nifedipine in water by β -cyclodextrin complexing and the influence of the preparation method on dissolution were studied. The stoichiometric ratio for the complexation of nifedipine with β -cyclodextrin was determined by solubility analysis and was 1: 1. The binary complex was characterized by various methods (XRD, DSC and FT-IR).

The object of this study was to investigate the molecular structure of the diminazene aceturate/CD complex by BET analysis, thermogravimetric analysis, DSC, FTIR and TEM methods, and find some new correlation between the structure and characteristics.

Experimental part

Chemicals

Diminazene aceturate (purity > 97%) and β -cyclodextrin (purity > 98%) were purchased from Sigma-Aldrich. All other reagents (DMSO, PEG 400 and phosphate buffer) were of analytical grade. The water used was double distilled and deionized.

Complex preparation and characterization

The diminazene aceturate/ β -cyclodextrin (DIMA/ β -CD) complex was synthesized in a blender equipped with a temperature sensor (Heidolph sensor; Heidolph Instruments), and the solvent evaporation was performed by freeze drying method (FD) using a Martin Christ, ALPHA 1-2 LD plus freeze dryer. The proportion between diminazene aceturate and β -cyclodextrin was according to the equimolar ratio. Diminazene aceturate powder was dissolved in DMSO to produce a 0.5% solution, and β -CD was dissolved in distilled water to produce a 0.5% aqueous solution. The complexes were formed in an aqueous medium. To improve the solubility of the active substance, PEG 400 and a phosphate buffer were used.

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The solutions were mixed for 30 min in a blender equipped with a temperature sensor, until the complete homogenization, then the obtained complex underwent solvent evaporation for 3 h, at 45°C, at a constant temperature with steering. After solvent evaporation, the complex was freeze-dried for 12 h [17].

Textural characteristics of the inclusion complex were determined by BET analysis on Autosorb1Quantacrome instrument. Thus the specific surface area (S_{BET}) was determined by BET method in the linear portion of the adsorption-desorption isotherm. The thermogravimetric analysis has been determined by the thermal desorption of diethylamine on a DuPont Instruments Thermal Analyst 2000/2100 coupled with a module 951 Thermogravimetric Analyzer. Differential Scanning Calorimetry Measurements (DSC) was performed using a DSC 823 (Mettler Toledo). The sample was packed in aluminum pans, placed in the DSC cell and then was heated at a rate of 10°C/min from room temperature to 200°C, kept 2 min at 200°C, then cooled to ambient temperature.

FT-IR spectra of the complex was obtained on a FTIR Jasco 610 spectrometer with the ATR method, in the range of 4000 to 550 cm^{-1} , at a scanning rate of 4 $\text{cm}^{-1} \cdot \text{s}^{-1}$, with an average of 128 measurements for each spectrum. FTIR images of the complex were obtained by using a Thermo iN10 MX FT-IR microscope operated in reflection mode. All the spectra were obtained in reflection mode, using a cooled imaging detector (MCT Array) by co-adding 16 spectra at a spectral resolution of 8 cm^{-1} while the time time of acquisition was 3 s/scan. The morphological analysis of the complex has been determined by transmission electron microscopy (TEM) using a Tecnai G2 F20 TWIN Cryo-TEM, 2015 - FEI Company™ system with a field emission 200kV S/TEM and with TWIN lens and high brightness field emission electron gun (FEG).

Results and discussions

The isotherm of adsorption / desorption and the pore-size distribution curves for diminazene aceturate/ β -cyclodextrin complex is shown in figure 1. The adsorbed inert volume is much lower than in the case of porous solids, which highlights the high degree of β -cyclodextrin cavity utilization. The isotherm is specific for capillary condensation process on macroporous solid materials with low content of pores. The hysteresis curve shows a volume

of adsorbed inert greater than the volume of inert desorbed, probably due to a higher affinity of inert for certain areas of the β -cyclodextrin cavities with low polarity.

The distribution of pore sizes highlights the presence of mesopores and especially of macropores (fig. 2). The textural properties (BET surface area, pore volume and average pore diameter) of the sample are summarized in table 1. It can be noted a very small surface area and a very small pore volume. The pore average diameter is specific for the mesopores, which demonstrates that most of the β -cyclodextrin cavities were occupied, because the diameter of the β -cyclodextrin cavities (0.78 nm) is much smaller than the average diameter of the pores determined experimentally (3.901 nm).

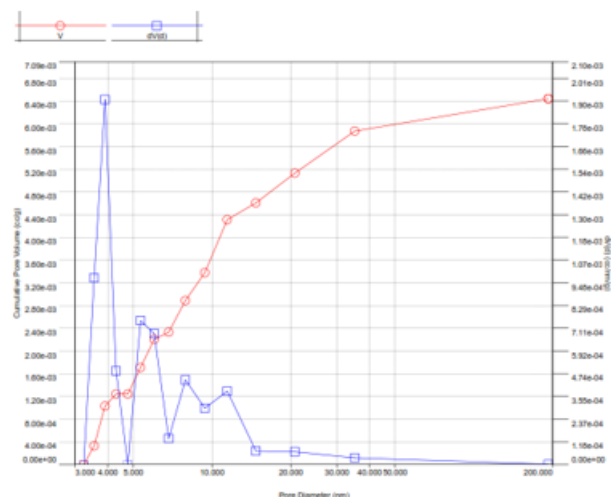


Fig. 2. Pore size distributions of the diminazene aceturate- β -cyclodextrin complex

Table 1
TEXTURAL CHARACTERISTICS OF DIMINAZENE ACETURATE - β -CYCLODEXTRIN COMPLEX

Specific Surface Area, m^2/g	Total pore volume, cm^3/g	Average pore diameter, nm
3.135	0.006	3.901

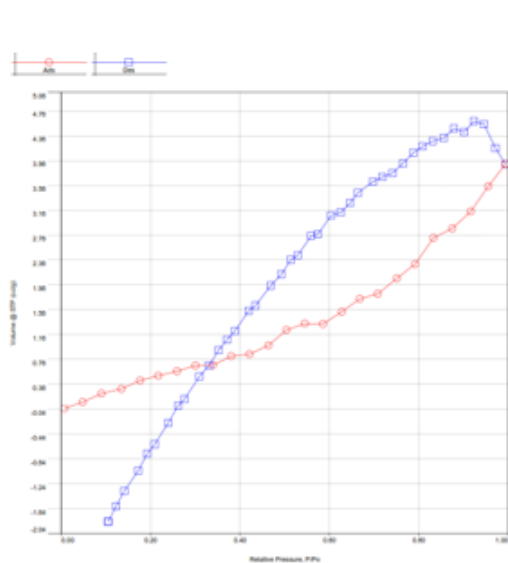


Fig. 1. The adsorption /desorption isotherm and the pore-size distribution curves for diminazene aceturate/ β -cyclodextrin

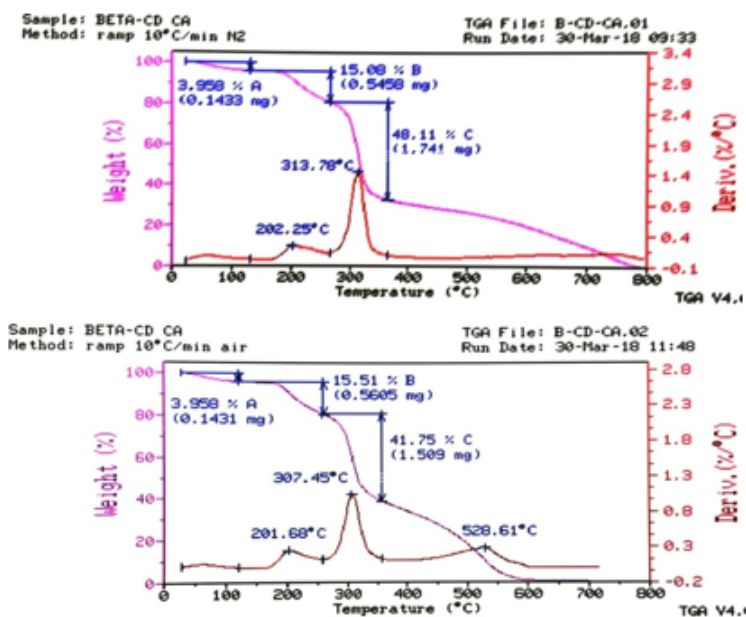


Fig.3. Termogravimetric analysis in inert gas and in synthetic air for diminazene aceturate/ β -cyclodextrincomplex

The stability of the inclusion complex was determined both in the presence of inert as in the presence of air. Figure 3 shows the both thermogravimetric analysis for diminazene aceturate/ β -cyclodextrin complex. The mass loss curves in air and in inert are similar, which demonstrates a high stability to oxidation of the inclusion complex. There is a mass loss of about 4% at temperatures up to 160 °C which corresponds to evaporation of the solvents, a mass loss of about 15% at temperatures up to 250 °C and a mass loss of over 40% at temperatures up to 350 °C. Mass loss occurring in the temperature range of 200-250 °C is probably due to dehydration of cyclodextrin and at temperatures of 250-350 °C, probably to the dextrin degradation.

Thermal curves obtained by DSC provide information about the stability of the complex and, implicitly, of the degradation temperature of one of the components (fig. 4). The presence of exothermic phenomena at temperatures up to 146.4 °C, as well as the occurrence of stronger interactions between the two components of the inclusion compound, and from the temperature of about 160°C, endothermic processes such as phase changes or even degradations.

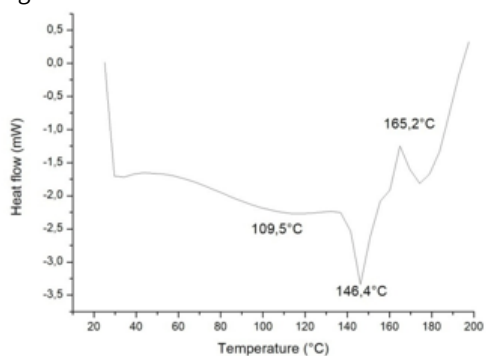


Fig.4. DSC analysis of diminazene aceturate/ β -cyclodextrin complex

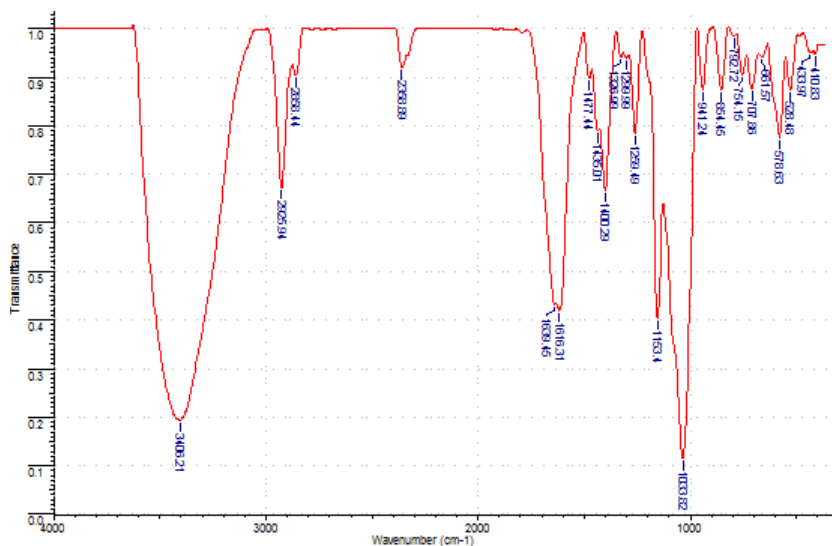


Fig.5. IR spectra for diminazene aceturate / β -cyclodextrin complex

To confirm the existence of the inclusion complex, a Fourier-Transform Infrared Spectroscopy (FT-IR) was performed and the graph shows as in the bottom image (fig.5).

Variation of the shape, passage, and intensity of the IR absorption peaks of guests or hosts may provide sufficient information for the occurrence of inclusion. The graph showed the IR spectra of diminazene, β -CD and their inclusion complex. The IR spectrum of diminazene showed its characteristic bands. There was a very strong absorption band at 1616 cm^{-1} for C=O stretching vibrations. The absorption band at 1400 cm^{-1} was indicated for the stretching vibration of C-C in the diminazene molecule. 941 cm^{-1} was for the C-H absorption band in the C-conjugated system. The IR spectra of the inclusion complex are similar to β -CD because of the reduced amount of diminazene in the system. However, some variations in the spectra were found. The absorption band at 1639 cm^{-1} disappeared or was shifted to the small wave numbers in the diminazene/ β -CD inclusion complex, indicating that the C=O stretch vibration was restricted after the formation of the inclusion complex. 1400 (1477) cm^{-1} was strongly weakened, indicating that a majority of the diminazene molecules were included by β -CD, but perhaps only one part of diminazene was included.

FTIR images of the complex DIMA - β -CD surface (fig. 7) appear that the complex is heterogeneous as shown in the video image (fig. 6), image which identifies green areas that have a slightly different spectrum compared to the rest of the sample. The *green* areas in the video image correspond to an increased intensity of the bands at 1067, 1093, 1213, 1273, 1415, 1450, 1495, 1620, 1650, 1700, etc. Zones surrounded are identical.

By enlarging the spatial resolution, we get the video images, and the FTIR below, the spectrum being unchanged. It can be easily seen that the green area is

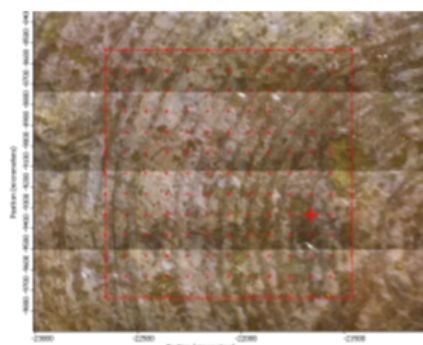


Fig.6.

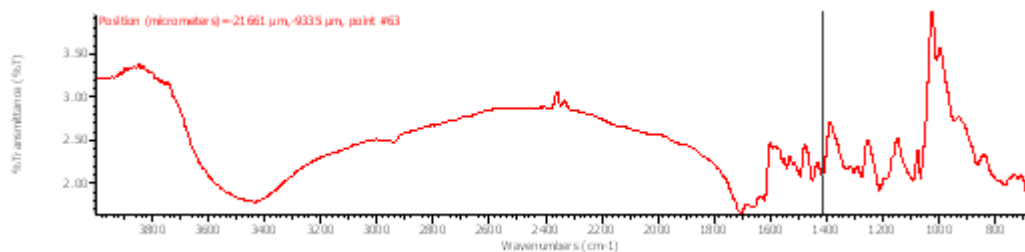


Fig.7. FTIR images of the complex DIMA- α -CD surface

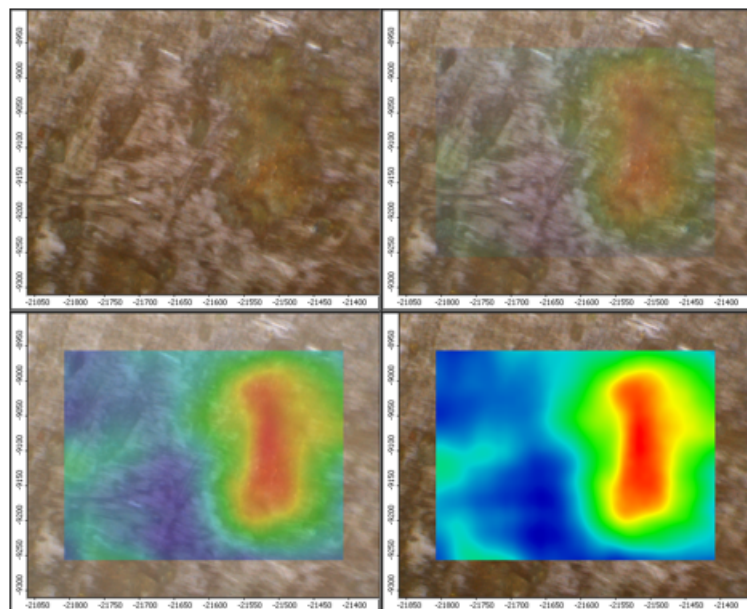


Fig.8. FTIR images of the complex DIMA- β -CD surface with enlarged spatial resolution

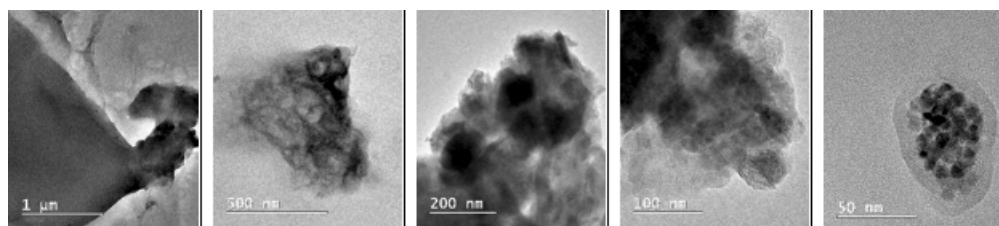


Fig.9. TEM images for the complex DIMA- α -CD

ovaloid, 250 μ m x 100 μ m, the video overlay and FTIR being very good, which confirms that the different color of the video is due to a composite heterogeneity (fig. 8).

In the blue area, based on the FTIR spectrum, the presence of a cyclodextrin can be identified, but the database does not contain information about the red area of the FTIR image.

Figure 9 show the TEM images of the DIMA- β -cyclodextrin complex. From the TEM analyzes it is observed, based on the color difference, the alternation between the two components of the inclusion complex and the shape and size of the inclusion compound.

Conclusions

In the present work, diminazene aceturate/ β -Cyclodextrin inclusion complex was prepared by co-evaporation method and was characterized.

The isotherm of adsorption / desorption curve for diminazene aceturate/ β -cyclodextrin complex highlights a much lower inert volume adsorbed than in the case of porous solids, which demonstrates the high degree of β -cyclodextrin cavity utilization.

The distribution of pore sizes is higher than the diameter of β -cyclodextrin cavities, which demonstrates that the most of the β -cyclodextrin cavities were occupied, because the diameter of the β -cyclodextrin cavities (0.78 nm) is much smaller than the average diameter of the pores determined experimentally (3.901 nm).

The stability of the inclusion complex was determined both in the presence of inert as in the presence of air. The

both thermogravimetric analysis for diminazene aceturate/ β -cyclodextrin complex, in air and in inert are similar, which demonstrates a high stability to oxidation of the inclusion complex.

The existence of the inclusion complex was confirmed by Fourier-Transform Infrared Spectroscopy (FT-IR). Thus, the intensity of the IR absorption peaks showed sufficient information for the presence of inclusion.

FTIR images of the complex DIMA- β -CD surface appear that the complex is heterogeneous. The presence of a cyclodextrin was identified, but the database does not contain information about a part of area of the FTIR image.

From the TEM analyzes it is observed, based on the color difference, the alternation between the two components of the inclusion complex and the shape and size of the inclusion compound.

References

- PEREGRINE, A.S., MAMMAN, M., Pharmacology of diminazene: a review, *Acta Trop.* **54**,1993, p.184.
- DHAWALE,V.S., AMARA,V.R., KARPEPA., MALEK,V., PATEL,D., TIKOO,K., Activation of angiotensin-converting enzyme 2 (ACE2) attenuates allergic airway inflammation in rat asthma model, *Toxicol. Appl. Pharmacol.*,**306**, 2016, p.16.
- SOUZA,L.K., NICOLAU,L.A., SOUSA,N.A., ARAUJO,T.S., SOUSA,F.B., COSTA,D.S., SOUZA,F.M., PACIFICO,D.M., MARTINS,C.S., SILVA, R.O., SOUZAM.H., CERQUEIRA, G.S., MEDEIROS,J.V.,Diminazene aceturate, an angiotensin-converting enzyme II activator, prevents gastric mucosal damage in mice: role of the angiotensin-(1-7)/mas receptor axis, *Biochem. Pharmacol.*, **112**,2016, p.51.

4. BENNION, D.M., HALTIGAN, E.A., IRWIN, A.J., DONNANGELO, L.L., REGENHARDT, R.W., PIOQUINTO, D.J., PURICH, D.L., SUMNERS, C., Activation of the neuroprotective angiotensin converting enzyme 2 in rat ischemic stroke, *Hypertension*, **66**, 2015, p.140.
5. ZHENG, C., LEI, C., CHEN, Z., ZHENG, S., YANG, H., QIU, Y., LEI, B., Topical administration of diminazene aceturate decreases inflammation in endotoxin-induced uveitis, *Mol. Dream.*, **21**, 2015, p.403.
6. KURIAKOSE, S., UZONJ.E., Diminazene aceturate (berenil), and new use for an old compound, *Int. Immunopharmacol.*, **21**, 2014, p. 343.
7. PU, G, XIN, Y., JING, LI, JIANG, R., DAI, J., ZHANG, L., Diminazene aceturate alleviated lipopolysaccharide/D-galactosamine-induced fulminant hepatitis in mice, *Biomedicine & Pharmacotherapy*, **98**, 2018, p. 142.
8. QIU, Y., SHIL, P.K., ZHU, P., YANG, H., VERMA, A., LEI, B., LI, Q., Angiotensin-converting enzyme 2 (ACE2) activator diminazene aceturate ameliorates endotoxin-induced uveitis in mice, *Invest. Ophthalmol. Vis. Sci.*, **55**, 2014, p.3808.
9. SZEJTLI, J., Introduction and General Overview of Cyclodextrin Chemistry, *Chemical Reviews*, **98**, no.5, 1988, p.1742.
10. CRINI, G., A History of Cyclodextrins, *Chemical Reviews*, **114**(21), 2014, p.10940-10975;
11. GUO, Z., WU, F., SINGH, V., GUO, T., REN, X., YIN, X., ZHANG, J., Host guest kinetic interactions between HP- β -cyclodextrin and drugs for prediction of bitter taste masking, *Journal of Pharmaceutical and Biomedical Analysis*, **140**, 2017, p.231.
12. LOFTSSON, T., BREWSTER, M. E., MASSON, M., Role of cyclodextrins in improving oral drug delivery, *American Journal of Drug Delivery*, **2**, no.4, 2004, p.261.
13. HARTLIEB, K. J., HOLCROFT, J. M., MOGHADAM, P. Z., VERMEULEN, N. A., ALGARADAH, M. M., NASSAR, M. S., STODDART, J. F., A Versatile Separation Medium, *Journal of the American Chemical Society*, **138**, no.7, 2016, p.2292.
14. MEJIA-ARIZA, R., GRANA-SUAREZ, L., VERBOOM, W., HUSKENS, J., Cyclodextrin-based supramolecular nanoparticles for biomedical applications, *Journal of Materials Chemistry B*, **5**, no.1, 2017, p.36.
15. WANG, Y., LIU, Y., LIANG, J., ZOU, M., A cyclodextrin-core star copolymer with Y-shaped ABC miktoarms and its unimolecular micelles, *Rsc Advances*, **7**, no.19, 2017, p.11691.
16. LOFTSSON, T., DUCHENE, D., Cyclodextrins and their pharmaceutical applications. *International Journal of Pharmaceutics*, **329**, no.1-2, 2007, p.1.
17. BLANCO, J., VILA-JATO, J.L., OTERO, F., ANGUIANO, S., Influence of method of preparation on inclusion complexes of naproxen with different cyclodextrins, *Drug Dev. Ind. Pharm.*, **17**, 1991, p. 943.
18. FLOARE, C.G., PIRNAU, A., BOGDAN, M., ¹H NMR spectroscopic characterization of inclusion complexes of tolfenamic and flufenamic acids with β -cyclodextrin, *Journal of Molecular Structure*, **1044**, 2013, p.72-78.
19. SANTOS, C.I.A.V., TEIJEIRO, C., RIBEIRO, A. C.F., RODRIGUES, D. F.S.L., ROMERO C. M., ESTESO, M. A., Drug delivery systems: Study of inclusion complex formation for ternary caffeine- β -cyclodextrin-water mixtures from apparent molar volume values at 298.15 K and 310.15 K, *Journal of Molecular Liquids*, **223**, 2016, p/209-216.
20. JAGDALE, S. C., JADHAV, V.N., CHABUKSWAR, A. R., KUCHEKAR, B.S., Solubility enhancement, physicochemical characterization and formulation of fast-dissolving tablet of nifedipine- β -cyclodextrin complexes, *Brazilian Journal of Pharmaceutical Sciences*, **48**, no.1, 2012.

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