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ARTICLE

Study of Inclusion Complex of Baclofen® Analogues with β-Cyclodextrin and Determination of its Stability Constant by UV-Visible Spectroscopy

Assia Keniche[1,2,✉], Si Said Mohammed El-Amine[2] and Joseph Kajima Muengi[3]

Asian Journal of Materials Chemistry

Volume: 3 Year: 2018
Issue: 3 Month: July–September
pp: 42–46
DOI: https://doi.org/10.14233/ajmc.2018.AJMC-P55

Received: 4 April 2018
Accepted: 30 June 2018
Published: 24 September 2018

ABSTRACT

This work reports the interactions of an analogue of baclofen® (A-BF) with β-cyclodextrin and the calculation of stability constant (K) of inclusion complex using UV-visible spectroscopy. 0.1 M solutions of a steady concentration of baclofen® and varying concentrations of (β-cyclodextrin) were prepared in water. The final β-cyclodextrin solutions concentrations ranged between 0.0 and 0.00019 M. Each solution was examined at 202 nm. Absorbances were recorded and plotted against cyclodextrin concentrations. From the plot, the concentrations of both free and bound baclofen® and free β-cyclodextrin were calculated by using the Bensi-Hildebrand method. Then stability constant K was calculated. The magnitude of the stability constant is discussed and the stoichiometry of inclusion complex was determined by means of Job's plot.

KEYWORDS

β-Cyclodextrin, Inclusion complex, Baclofen® analogues, Alcoholic.

INTRODUCTION

Baclofen® is an FDA-approved $GABA_B$ agonist used for the treatment of spasticity since in early seventies [1]. In 2004, Ameisen attempted to cure with baclofen® and found that the high doses of the drug got rid of his frequent cravings for drink. Baclofen® was mediated and presented as a new alternative for the treatment of alcoholic and related addictions [2-6] after the release of his book "Le dernier verre" [7].

Being a racemate, commercial baclofen® is a source of concern as (R)-(-) enantiomer is more active and toxic than its (S)-(+) counterpart. Therefore, it is very important to perform a good resolution of the mixture and use a single useful form in order to achieve optimal therapeutic response [8]. In addition, baclofen® is an usual γ-amino butyric acid antagonist, but it can cross only weakly the blood-brain barrier [9,10]. Despite above-mentioned drawbacks, baclofen® is the subject of several studies, where the main challenge is to optimize both its bioavailability and vectorization through the development of efficient separation of its enantiomers while developing the synthesis of new analogues. In this work, we report the synthesis of baclofen® analogue, along with the study of its complexation with β-cyclodextrin.

Author affiliations:

[1]University Centre of Maghnia, Tlemcen, Algeria
[2]Laboratoire de Chimie Organique, Substances Naturelles et Analyses (COSNA), University of Tlemcen, Tlemcen, Algeria
[3]Faculty of Sciences, University of Tlemcen, Tlemcen, Algeria

✉To whom correspondence to be addressed:

E-mail: keniche_assia@yahoo.fr

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Much of the interest in cyclodextrins (CDs) research has been fuelled by commercial interests and several published reports of complexation papers were focused on pharmaceuticals or potential pharmaceuticals as regards small molecules-cyclodextrins interactions (Fig. 1). This was because drugs formulation with cyclodextrins could be a way to increase their solubility, stability or other relevant properties [11,12]. Therefore, we investigated the encapsulation of this synthetic derivative in commercial β-cyclodextrin and also determined the stability constant (K_a) of the resulting inclusion complex by using UV-visible spectroscopy. This technique proved to be simple to use especially for its availability and enabled us rapid identification of aromatic rings present in the guest compound.

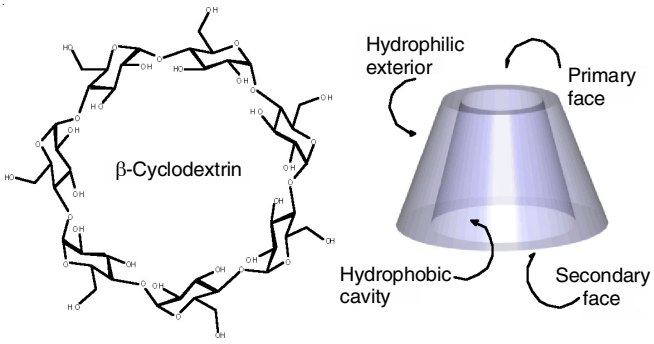


Fig. 1. Chemical structure of β-cyclodextrin

The synthesis of analogues was based on theoretical study of interactions between baclofen® and $GABA_B$ receptor (Fig. 2). The main idea in our synthetic scheme was to preserve integrity of both terminal ends of the target, *i.e.* carboxylic acid and amino group because of their interaction with the residues Asp 471 and Ser 246 of $GABA_B$ receptor [13]. The best method to access those analogues was starting the synthesis with amino acids as compared to procedures described in the literature [14-21].

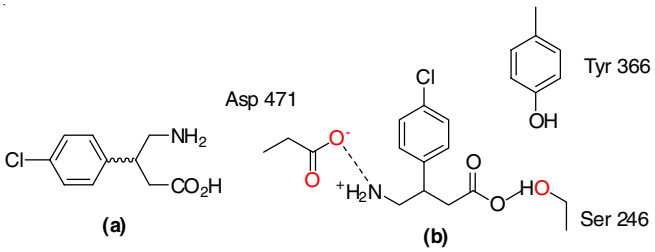


Fig. 2. (a) Baclofen®, (b) Docking of baclofen® into binding pockets of $GABA_B$ receptor [Ref. 13]

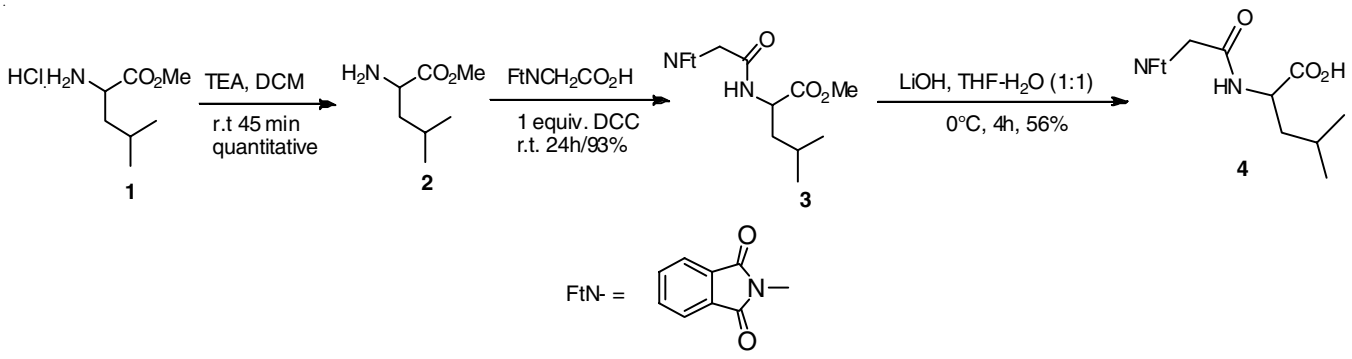
EXPERIMENTAL

Synthesis: In this paper, present discussion focused only on the analogue of baclofen®, *i.e.* A-BF1, which showed best interactions with the β-cyclodextrin among all derivatives synthesized in our laboratory [22-24]. The synthetic scheme leading to **4** was achieved in three steps (**Scheme-I**). The starting material, namely *N*-phthaloyl-L-glycyl-L-leucine (**4**) was obtained after previous neutralization of commercially available *L*-leucine methyl ester hydrochloride (**1**) with triethylamine (TEA) in dichloromethane (DCM). Then coupling its methyl ester with *N*-phthaloyl-L-glycine ($FtNCH_2CO_2H$) using dicyclocyclohexylcarbodiimide (DCC) in the presence of triethylamine in CH_2Cl_2 afforded the corresponding dipeptide **4** (A_BF) in 56 % yield after hydrolysis of **3** in the final step with LiOH solution.

UV spectroscopy: First of all, A-BF and β-cyclodextrin stock solutions were prepared in distilled water, each with 1.9 × 10^{-5} M concentration. Then mixing equimolar amounts of previous solutions afforded the complex solution to be examined along with previous individual stock solutions. Each solution was analyzed at 25 °C within the 190-240 nm range, thus enabling to determine λ_{max} 202 nm.

Determination of stochiometry: The method of continuous variation (Job's method) was used to determine the stoichiometry of the inclusion complexes with β-cyclodextrins. During this work, an attention has been paid to modifications of absorbances of guest *versus* those produced as a result of any complexation. The data were plotted against ratios of β-cyclodextrin and absorbances of the complex. Eleven solutions were prepared from both previously prepared A-BF and β-cyclodextrin stock solutions, in such a way to reach a constant analytical volume of 2 mL. Solutions were left during 18 h for optimal complexation and the results were analyzed at 202 nm.

Binding constant (K_a): To determine the apparent formation constant for the inclusion complex of A-BF and β-cyclodextrin, the concentration of A-BF was held constant whereas those of β-cyclodextrin were varied. The absorbances of solutions were measured at 202 nm against a blank. Then a constant concentration of A-BF1 (2 mL, 5 × 10^{-5} mol L^{-1}) was added to diluted solutions of β-cyclodextrin in 5 mL volumetric flasks and standardized up to 4 mL. The flasks were kept at room temperature and analyzed at 202 nm using a UV-visible spectrophotometer (25 ºC). Every measure was repeated thrice.



Scheme-I: Synthesis of A-BF, analogue of Baclofen®

RESULTS AND DISCUSSION

Complexation: Cyclodextrin inclusion complexes are of interest for scientific community when they achieved in aqueous solutions as they allow investigations on hydrophobic interactions that are so important in biological systems. The most important property of inclusion compounds is that a "host" can accommodate "guest" components into its cavity without establishing any covalent bond, depending mainly on the size of the guest and the compatibility between the host and the guest [25,26]. It has also been suggested that forces inherent in the cyclodextrin contribute to association. To this respect, the water enclosed within the "empty" cyclodextrin cavity could exert such force. Despite the water present in the cavity provides unfavourable hydrophobic environment, its expulsion from the cavity is favoured by both a gain of entropy and potential energy [27-29].

UV spectroscopy: Fig. 3 showed no β-cyclodextrin absorption throughout the wavelengths range used so that its very weak absorbance could be disregarded [30]. The inclusion complex UV-plot showed a decreasing intensity at any point of wavelength, as a result of inclusion between β-cyclodextrin and guest.

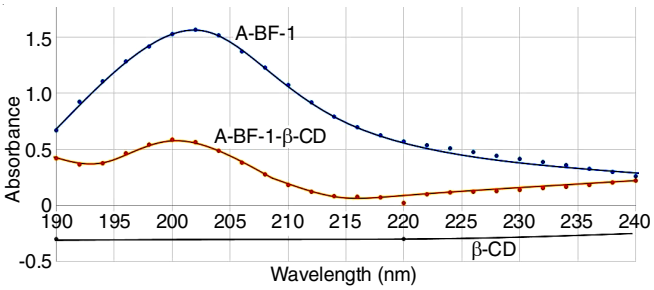


Fig. 3. UV-plots of free A-BF, A-BF-β-CD complex and free β-cyclodextrin

It is obvious that absorptions observed are due to the presence of chromophores such as phthaloyl group, carbonyl moiety of amide and carboxylic acid. As far as the complex was concerned, A-BF plot showed a hypochromic effect, clearly showing the occurrence of both the complexation of guest and its chromophores. The significant reduction of absorbance, ΔA = 0.5, indicated chromophores were encapsulated within the hydrophobic cavity of β-cyclodextrin.

Stochiometry: According to plot (Fig. 4), the slope (r) was found to be 0.5, indicating that the complex was formed with a 1:1 stoichiometry.

Binding constant (K_a): Every absorbance related to a single concentration of β-cyclodextrin was recorded (Table-1) and then plotted *versus* β-cyclodextrin (Fig. 5). This allowed to calculate the stability constant of complex.

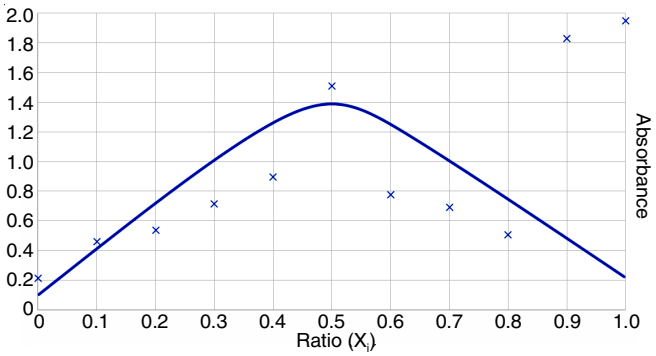


Fig. 4. Job's plot of inclusion complex A-BF-β-CD

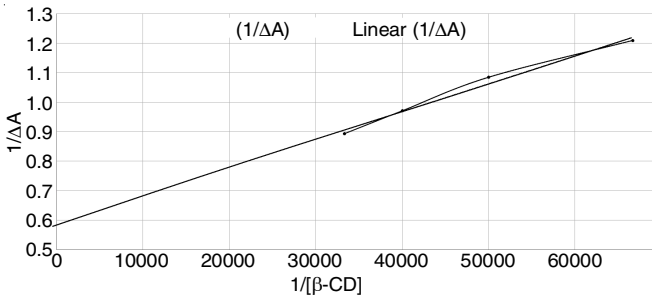


Fig. 5. Plot of K_a of the inclusion complex A-BF-β-CD

To estimate the value of the exchange of complexation, we relied on Benesis-Hildebrand's technique [31]. The authors determined the association constant K_a through the absorbance difference between complexed form and free molecule. The following equation was then used:

$$\frac{1}{\Delta A} = \frac{1}{[PA]\times K\times \Delta\varepsilon\times[CD]} + \frac{1}{[PA]\times\Delta\varepsilon}$$

where, ΔA: absorption difference between the complexed and free molecule; Δε: The extinction coefficient difference between the complexed and free molecule; [PA]: concen-tration of active ingredient (guest molecule); [CD]: β-cyclodextrin concentration.

The association constant may then be determined by plotting 1/ΔA as a function of 1/[CD]. Δε was provided by the intercept and the association constant and K_a was calculated from the slope of the line. From plot, we got the following equation: $y_1 = (9 \times 10^{-6})x + 0.5917$. The value of extinction coefficient difference was $\Delta\varepsilon_1 = 33800.91$ and stability constant was found to be $K_a = 65744.49\ M^{-1}$.

Structure of inclusion complex: It is important to mention that one source of the best structural information might be the individual chemical shift modifications of protons δ of both guest and host. This parameter was referred to as the complexation-induced shift (CIS or Δδ). Therefore, modifications of chemical shifts of β-cyclodextrin H-3 and H-5 protons

TABLE-1
UV ABSORBANCE OF INCLUSION COMPLEX A-BF-β-CD USING DIFFERENT CONCENTRATIONS OF β-CD

A-BF Vol. (mL)	β-CD Vol. (mL)	[A-BF] (mol/L)	[β-CD] (mol/L)	1/[β-CD] (L/mol)	A	ΔA	1/ΔA
2	2	0.00005	0.000030	33333.33330	0.287	1.119	0.89365504
2	2	0.00005	0.000025	40000.00000	0.372	1.030	0.97087378
2	2	0.00005	0.000020	50000.00000	0.480	0.922	1.08459869
2	2	0.00005	0.000015	66666.66667	0.575	0.827	1.20918984
4	0	0.00010	0	∞	1.402	0	∞

provided good evidence for the formation of an internal complex. The geometry of these inclusion complexes could be derived from the evidence of the spatial neighbourhood between the guest molecule and β-cyclodextrin protons. This, was achieved by investigating dipola]r interactions using 2D ROESY NMR experiment [32] according to previous work [33]. This technique proved to be the most sensitive for the structural analysis of inclusion complexes of β-cyclodextrin formed in solutions [34-36].

In this work, we focused on chromophores UV absorbances in order to determine the geometry of the complex. The decrease of absorbance of the complex relative to the absorbance of free A-BF showed that the phthaloyl group linked to major absorbance also showed the greatest differences of absorbance between the free form and the complexed one. This suggested that those modifications were the results of an encapsulation of this moiety inside β-cyclodextrin cavity (Fig. 6).

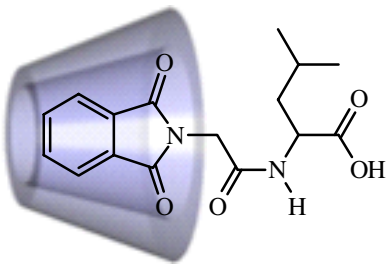


Fig. 6. Suggested structure for the inclusion complex A-BF-β-cyclodextrin

Conclusion

We have carried out the complexation study between a stable A-BF and β-cyclodextrin. The stoichiometry and association constant were determined by Job's and Bensi-Hildbrand's method. Based on the relatively high values of the stability constant (K_a), of the inclusion complex obtained in this work. It could be suggested, that under the experimental conditions used the β-cyclodextrin and A-BF measuring the absorbance, one can quite easily conclude that the interaction between β-cyclodextrin (host) and A-BF (guest) may be very strong. Thus it is suggested that the inclusion complex between A-BF with other modified β-cyclodextrin like those having polar and ionic functional groups attached to the β-cyclodextrin molecule (either at C-3 or C-6) and with the linked cyclodextrins deserve be investigated.

ACKNOWLEDGEMENTS

The authors are grateful to the General Directorate for Scientific Research and Technological Development (Ministry of Higher Education and Scientific Research, Algeria) for financial support.

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