Study of interaction between sugammadex as a modified gamma cyclodextrin and propranolol using different methods

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□ ABSTRACT □

Sugammadex, a new modified gamma cyclodextrin, reverses the neuromuscular blockage induced by rocuronium by forming a strong complex with this muscle relaxant. In order to evaluate possible interactions with potentially co-administered drugs, the interaction between sugammadex and propranolol was investigated using three different methods; the affinity capillary electrophoresis (ACE) method coupled to an ultraviolet (UV) detector, the affinity capillary electrophoresis method coupled to fluorescence detector and Fluorimeter, for the first time. Using ACE, changes in the effective mobility of guest drug were correlated with the increasing concentration of the host molecules in background electrolyte (BGE). Using Fluorimeter, changes in the fluorescence intensity of guest drug were correlated with the increasing host concentration in sample solution. Detected changes were successfully fitted into a nonlinear curve equation; assuming 1:1 stoichiometric interaction. The calculated association constants (K_a) were: 4137 M^{-1}, 4215 $M^{-1}$and 4110 M^{-1} using three methods as mentioned above; respectively. These values confirm the affinity strength of the predicted inclusion complex between sugammadex and propranolol.

Keywords: Sugammadex, Propranolol, Affinity Capillary Electrophoresis, Fluorometry

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دراسة التأثير المتبادل بين سوغاماديكس (الغاما سيكلوديكسترين المعدل) والبروبرانولول باستخدام طرائق مختلفة

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□ ملخّص □

يعمل سوغاماديكس، وهو الغاما سيكلوديكسترين المعدل، على عكس فعل التثبيط العصبي العضلي الناجم عن الروكورونيوم وذلك عن طريق تكوين معقد قوي مع هذا المرخي العضلي. ومن أجل تقييم التأثيرات المتبادلة المتوقعة مع الأدوية التي يُحتمل أن تكون قد أُعطيت بشكل مشترك مع السوغاماديكس، تمت ولأول مرة دراسة التأثير المتبادل بين السوغاماديكس والبروبرانولول باستخدام ثلاث طرائق مختلفة؛ طريقة الرحلان الكهربائي الشعري المقترن بكاشف الأشعة فوق البنفسجية، وطريقة الرحلان الكهربائي الشعري المقترن بكاشف الفلوريسنت ومقياس الفلورة. باستخدام طرائق الرحلان الكهربي الشعري، ارتبطت التغييرات في الحركة الفعالة للضيف بالتركيز المتزايد للجزيئات المضيفة في محلول الوقاء ضمن الأنبوب الشعري. وباستخدام مقياس الفلورة، ارتبطت التغيرات في شدة التألق للضيف بزيادة تركيز المضيف في محلول العينة. تمت ملائمة قيم هذه التغييرات المكتشفة بنجاح في معادلة منحنى غير خطي؛ بافتراض تفاعل متكافئ 1:1. كانت قيم ثابت التفاعل 4137 و 4215 و 4110 مولارتي$^{-1}$ باستخدام الطرائق الثلاث كما هو مذكور أعلاه على التوالي. تؤكد هذه القيم شدة التأثير المتبادل لتفاعل السوغاماديكس والبروبرانولول المتنبأ به.

الكلمات المفتاحية: الرحلان الكهربائي الشعري، مقياس الفلورة، سوغاماديكس ، بروبرانولول

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Introduction

Cyclodextrins as excipients in drugs tend to form inclusion complexes with various molecules due to their chemical structure. Pharmaceutics benefis can be gained from these complexation. Enhancing the bioavailability of many problemaic drugs could be achieved by improving their physical and chemical properties through these complexaion[1, 2, 3, 4].
Sugammadex (Figure 1), as a drug itself, is a modified gamma cyclodextrin substituted with eight thio carboxylated groups. These modified extensions enhance the ability of sugammadx to form strong inclusion complex with neuromuscular blocking agents (NMBs) like roccorunium. This complexation hinders the pharmacological action of the guest molecule, resulting in a reverse of its effect [5, 6]. The potential exists for this complex to occur with other drugs, not only NMBs molecules. The interaction between drugs and sugammadex was investigated and evaluated by calculating the binding constant [7, 8].
The analysis and characterization of cyclodextrin's inclusion complexes, either in solid form or in solution, has been done using various analytical methods; nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), fluorescence and etc. [9, 10, 11, 12, 13]. The use of affinity capillary electrophoresis (ACE) is a flexible analytical method that can determine the binding constant of drug-drug interactions [14] and complexations of CDs and their derivatives [15, 16].
This study considered propranolol as a potential co-administered drug with sugammadex. As a result, it may form a complex with sugammadex, which could have a substantial impact on both the host and guest molecules' pharmacokinetic and pharmacodynamics profiles. To confirm or deny the affinity strength of the predicted complex, three different methods were employed to investigate the complex formation between propranolol and sugammadex.

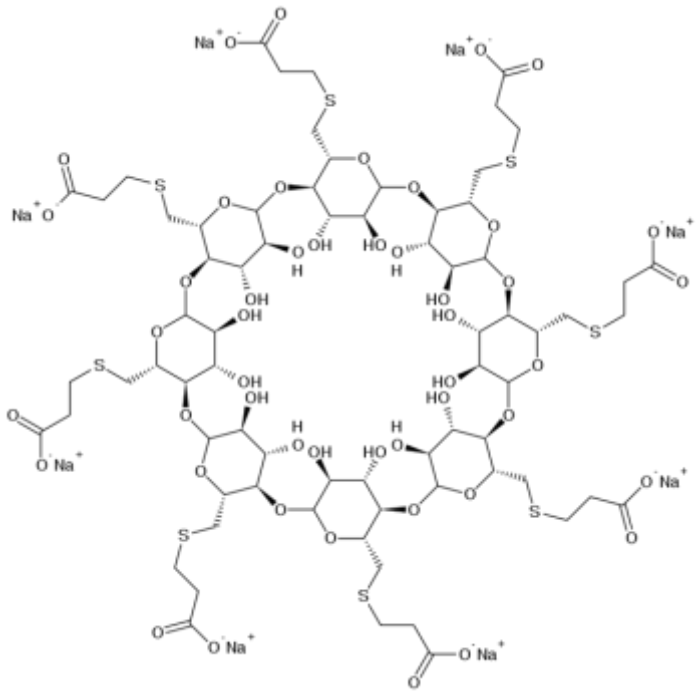

Figure 1 The chemical structure of Sugammadex

Experimental Part

Materials

Propranolol hydrochloride (Figure 2) from sigma Aldrich was used. Sugammadex from N.V. Organon was used. Potassium hydrogen-phosphate and potassium di-hydrogen-phosphate were used for buffer preparation.

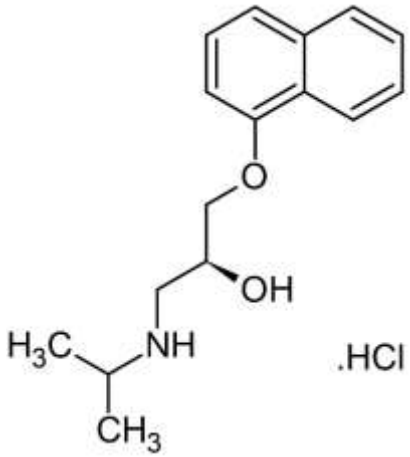

Figure 2 The chemical structure of Propranolol hydrochloride

Methods

CE coupled to ultraviolet detector

An ultraviolet detector coupled to capillary electrophoresis was used to detect propranolol at 220 nm wavelength. Samples were running through fused silica capillary with an internal diameter of 50 µm and a total length (L_T) of 50 cm.

Analysis procedure: for 15 minutes at 40° C, a new capillary was activated with 1 N NaOH as the initial step. Rinsing the capillary with 0.1 N NaOH followed by the buffer solution for 3 minutes and 5 minutes, respectively, was done prior to measuring for each run. The measurements were carried out under the following conditions: 25° C of the temperature, 21 kV of the separation voltage and 50 mbar for 9 s of the injection pressure. The measurement was repeated 3 times for each sample (n=3).

Analysis Principle: the formation of inclusion complexes between the guest molecule and the host molecule affects the migration time (t_{Guest}), and as consequence the effective electrophoretic mobility (μ_{eff}), of the injected drug due to increased receptor concentration. The inclusion complex in solution was investigated by calculating the value of the association constant (K_a) using the origin (7.0) program. The equilibrium between the free and complexed guest molecule was fast and achieved at 1:1 molar ratio according to the following equation:

$$\mathbf{[Guest] + [Host] \rightleftarrows [Complex]} \qquad \textbf{equation (1)}$$

$$K_a = \frac{[Complex]}{[Guest]\,[Host]} \qquad \textbf{equation (2)}$$

$$\mu_{eff} = \frac{\mu_{Guest} + K_a.[Host].\mu_{Comlex}}{1 + K_a.[Host]} \qquad \textbf{equation (3)}$$

Where μ_{eff} is calculated from,

$$\mu_{eff} = \frac{L_D \cdot L_T}{U} \left(\frac{1}{t_{Guest}} - \frac{1}{t_{EOF}} \right) \quad \text{equation (4)}$$

The distance from the capillary inlet to the detector is L_D. EOF is the electro-osmotic flow has a migration time (t_{EOF}) of the neutral substance marker.

CE coupled to fluorescence detector

A fluorescence detector coupled to capillary electrophoresis was used to detect propranolol at 320 nm excitation wavelength and 360 nm emission wavelength.

Analysis procedure and analysis principle were same as mentioned in previous study.

Fluorimeter

A fluorescence molecule, propranolol, was detected at 320 nm excitation wavelength and 360 nm emission wavelength. Analysis principle: the formation of inclusion complexes between the guest molecule and the host molecule affects the fluorescence intensity of the analyzed drug due to increased receptor concentration. The inclusion complex in solution was investigated by calculating the value of the association constant (K_a) using the origin program. The equilibrium between the free and complexed guest molecule was fast and achieved at 1:1 molar ratio according to the following equation:

$$DI = \frac{CI \cdot K_\alpha \cdot [Host]}{1 + CI \cdot [Host]} \quad \text{equation (5)}$$

Where CI is the intensity of the complexed form of drug. DI is the difference in drug intensity that resulted from subtracting the intensity of complexed drug from the one of free drug.

Buffer preparation

10 mM of phosphate buffer at 6.5 pH was used to prepare the buffer samples of increasing concentrations of sugammadex (The host molecule). For CE-ultraviolet study, (0, 0.2, 0.3, 0.5, 0.7 and 1 M) concentrations of sugammadex dissolved in phosphate buffer were used. For CE- fluorescence study, (0, 0.5, 1, 1.5, 2.5, 3.5, and 5) × 10^{-4} M concentrations of sugammadex dissolved in phosphate buffer were used. All the samples were filtered and degassed.

Sample Preparation

Propranolol (The guest molecule) samples were prepared in the concentrations of 10^{-5} M and 10^{-4} M for CE-ultraviolet and CE- fluorescence studies, respectively. The drug samples were spiked with dimethylsulfoxide (DMSO) as a neutral marker.

In contrast to CE studies, the flourometry study required the preparation of samples from both the guest and host molecules in the same solution. A fixed amount of propranolol was dissolved into phosphate buffer (pH = 6.5) samples that were spiked with increasing amount of sugammadex.

Results and discussion

The inclusion complex of propranolol with sugammadex was successfully characterized using three different methods. For CE coupled to ultraviolet detector, Propranolol running in blank buffer has a positive electrophoretic mobility. As sugammadex concentration increased in the background electrolyte solution, the effective mobility of propranolol decreased. At 0.3 M concentration of sugammadex, the effective mobility of propranolol inverted to negative one as shown in figure (3). This change could be resulted from

complex formation and depend basically on the increasing of both the total negative charge and the total mass of the complexed drug in comparison to the free one.

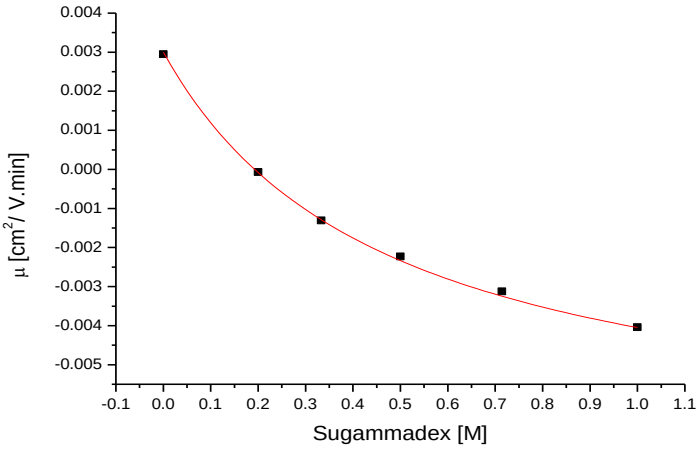

Figure 3 Correlation of the effective mobility of propranolol as a function of the increasing concentration of sugammadex in background electrolyte solution (non linear fitting equation) using CE coupled to ultraviolet detector

Fluorescence detector coupled to CE was successfully confirmed the positive effective mobility of the drug (propranolol). The decreasing values of the effective mobility versus the increasing concentration of sugammadex was shown clearly in figure 4. And that could be attributed to the same circumstances of the previously discussed method.

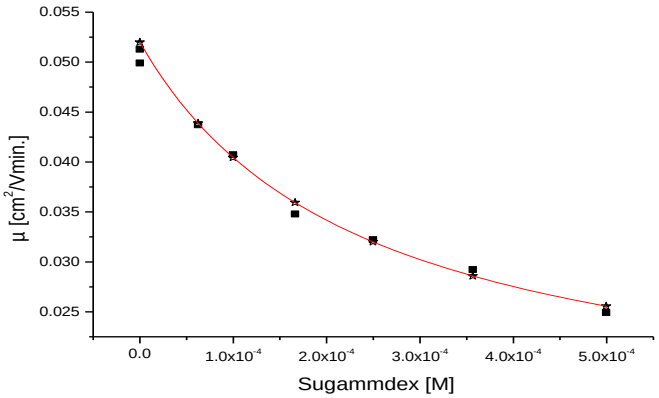

Figure 4 Correlation of the effective mobility of propranolol as a function of the increasing concentration of sugammadex in background electrolyte solution (non linear fitting equation) using CE coupled to fluorescence detector

The affinity strength of complex formation was detected by calculating the association constant K_a using non linear curve fitting method. For both studies, the electrophoretic mobility of guest drug as a function of the concentration of the host drug was correlated and fitted to equation (3). The values of K_a were plotted in table (1).

Table 1 values of the association constant K_a detected using different methods

Method	K_a (M^{-1})
CE coupled to ultraviolet detector	4137 ± 80
CE coupled to fluorescence detector	4215 ± 82
Fluorimeter	4110 ± 85

For fluorimeter study, descending change in the fluorescence intensity of propranolol was observed upon the increasing concentration of sugammadex in the sample mixture as shown in figure (5). This change could be caused by the formation of inclusion complex between the drug and modified cyclodextrin. The difference in fluorescence intensity of propranolol as a function of the increasing concentration of sugammadex in sample mixture was successfully correlated and fitted to equation (5). The association constant K_a was concluded in value plotted in table (1).

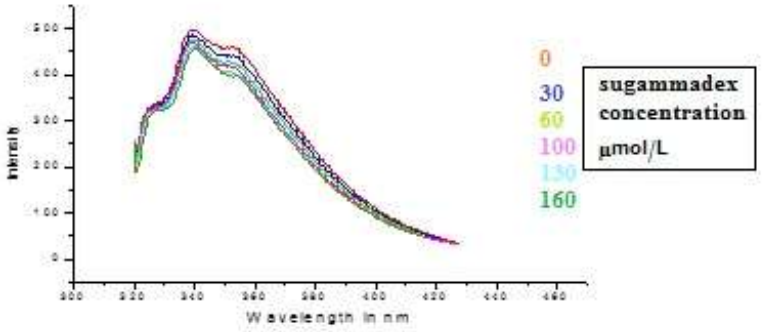

Figure 5 descending fluorescence intensity of propranolol versus ascending concentration of sugammadex

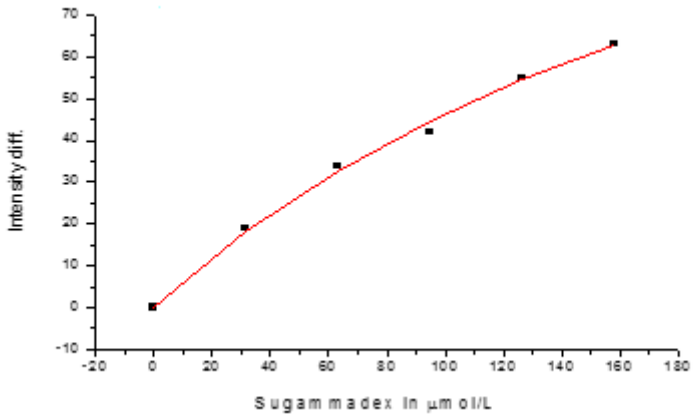

Figure 6 Correlation of the difference in propranolol intensity as a function of the increasing concentration of sugammadex in sample solution (non linear fitting equation) using fluorimeter

According to three different methods, The K_a values (table 1) confirm the presence and strength of the affinity between propranolol and sugammadex. Hydrophobic interaction and hydrogen bonds have been suggested as the reason for the inclusion complex between sugammadex and propranolol. The hydrophobic interaction could result from an entrapment of an organic amine into the extended hydrophobic cavity of modified cyclodextrin. The hydroxyl group attached to propranolol has the potential to form a hydrogen bond.

Conclusion

The inclusion complex between sugammadex and propranolol was successfully characterized using three different methods. This study confirms the affinity strength of sugammadex and potentially co-administrated drug (Propranolol).

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