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Investigation of Haemolytic and Complexation Properties of γ-Cyclodextrin Carbonate Derivatives

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Abstract

New alkyl carbonates of γ-cyclodextrin have been obtained. These derivatives show lower haemolytic effect than the parent γ-cyclodextrin. The complexation behaviour was tested with three important drugs, namely: progesterone, diazepam and flurbiprofen. DSC analyses were consistent with the inclusion complex formation.

Introduction

Cyclodextrin inclusion compounds [1, 2] have been widely used to improve solubility, dissolution rate and bioavailability of hydrophobic drugs. Moreover, cyclodextrins enhance the stability, decrease the volatility, and modify local irritation of drugs. However, the amount of cyclodextrins that can be used in pharmaceutical dosage forms is limited and only few cyclodextrins are suitable for parenteral formulations.

Cyclodextrins have a haemolytic effect at high concentrations, because they extract cholesterol and phospholipids from erythrocyte membrane [3]. γ-ciclodextrin is less haemolytic than β- and α-cyclodextrins, because it has less lipid extraction capacity.

The present work deals with the preparation and characterisation of a series of alkyl carbonates (ethyl, butyl and hexyl) of γ-cyclodextrin; the haemolytic activity of the native and modified γ-CD was measured. The inclusion complexes of the series of alkyl carbonates with lipophylic drugs was examined with Differential Scanning Calorimetry (DSC).

Three drugs, namely progesterone, flurbiprofen and diazepam, have been chosen as model to investigate the complexation capacities of the reported alkyl carbonates. Progesterone and diazepam are two hydrophobic drugs without any dissociable groups. Flurbiprofen is an acidic drug with a pK value of 4.12 and low solubility in water.

Experimental

γ-CD was a gift from Wacker Chemie (Germany). All the reagents (ACS grade) were bought from Aldrich (USA) and used without further purification. Alcohol-free chloroform (Merck-(Germany) was produced extracting twice the chloroform with distilled water and drying the organic phase with anhydrous sodium sulphate. Pyridine was distilled over sodium hydroxide just before use.

TLC analyses were performed on Merck silica plate (art. 5764) by using $CH_3OH : H_2O$ 70 : 30 v : v as eluent and anisaldehyde spray detector.

A series of alkyl carbonates derivatives (ethyl, butyl and hexyl) were prepared following the previously reported procedure with minor modifications [4]. Briefly, the selected alcohol was activated by reaction with excess of carbonyldiimidazole in alcohol free chloroform. In the second step the imidazoil derivative was allowed to react with anhydrous γ-CD in anhydrous pyridine at 80 °C for 4 hours. Once the reaction was over, the residual precipitate was filtered off and distilled water was added to the organic solution. The solid was recovered by filtration, washed many times with water and then liophylized.

The solubility in water of the ethyl, butyl, hexyl carbonates γ-cyclodextrins (γ-CD) were determined by weighing 10 mg of dry powder of each derivative and then adding water until complete dissolution at 25 °C .

Haemolytic activity determination

Human blood was collected from healthy donors. 250 μL of blood were diluted with a isotonic phosphate buffer (pH 7.40) to 1 mL. The three alkyl carbonates γ-cyclodextrins were then added at a concentration corresponding to the saturation solubility. The mixtures were incubated for 90 minutes at 37 °C and then centrifugated at 2000 rpm for 5 minutes. The superrnatants were analysed spectrophotometrically. The percent of haemolysis was determined from the absorbance at 543 nm. γ-CD and β-CD at 15 mM concentration were used as control. 100% haemolysis corresponds to a solution haemolysed completely. All the experiments were performed in triplicate.

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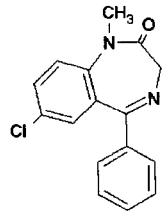

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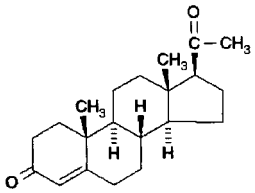

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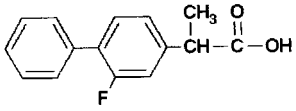

FLURBIPROFEN

Figure 1. Molecular structure of the selected drugs.

Preparation of the drug inclusion complexes

The CD inclusion complexes were prepared by adding the selected drug to a γ-cyclodextrin derivatives water : ethanol solution (70 : 30, v : v) at 1 : 2 molar ratio. The mixtures were stirred at 25 °C for 48 h. A precipitate was obtained which is collected after centrifugation and dried.

Differential scanning calorimetry

Thermal analyses of the inclusion complexes were performed using a DSC 7 instrument (Perkin Elmer) equipped with an instrument controller Perkin-Elmer TAC/DX weighting 3–5 mg samples in aluminium pans at a heating rate of 10 °C/min in the 25–200 °C temperature range under a nitrogen purge.

Results and discussion

Figure 1 shows the structure of the selected drug molecules. All these drugs are poorly soluble in water.

Table 1 reports the physicochemical properties of the series of alkyl carbonate γ-CD. The γ-cyclodextrin carbonates were not charecterised for chemical structure or isomeric composition. The solubility in water decrease with increasing the length of the alkyl chain. Thus, ethyl-γ-CD shows a solubility of about 15 mM (i.e., 2.03 wt%) but the corresponding hexyl derivative is soluble to a lower extent and no more than 0.34 mM (i.e., 0.05 wt%) at room temperature. Table 1 reports also the solubility of the parent γ-CD. The alkyl carbonates of γ-CD show a surface activity, but critical micellar concentration were not determined. Despite their surface properties, the alkyl carbonates derivatives of γ-CD have a lower haemolytic activity than that of the parent of γ-CD. In particular saturated water solution of ethyl-γ-CD shows only 5.5% of haemolysis that is about 4 times less haemolytic than the parent γ-CD. Beside, both butyl and hexyl carbonates of γ-CD have not haemolytic effect. This is a relevant point because γ-CD is the less haemolytic among the native cyclodextrins.

Table 1. Physicochemical properties of alkyl carbonates of γ-CD

	DS	Solubility (mM)	Surface activity	% haemolysis
β-CD	–	16.3	No	44*
γ-CD	–	200.0	No	19.0*
Ethyl γ-CD	3	15.0	Yes	5.5
Butyl γ-CD	3	2.6	Yes	0
Hexyl γ-CD	3	0.34	Yes	0

* % of haemolysis caused by a 15 mM concentration at 37 °C.

The alkyl carbonates of γ-CD are able to form stable inclusion compounds with the selected drugs. The DSC thermograms of progesterone inclusion complexes with ethyl, butyl and hexyl-γ-CD did not show the melting peaks corresponding to the melting temperature of progesterone (literature 127-131 °C). The disappearance of the peaks at the melting temperature of the drug confirm the interaction of progesterone, with the alkyl carbonates γ-CD. Thus, the DSC thermograms are consistent with the formation of the progesterone inclusion complexes.

Similar results were obtained with the other two drugs, diazepam and flurbiprofen. The DSC thermograms for diazepam and flurbiprofen inclusion complexes did not show the melting peaks of the two drugs. The absence of the melting peaks of the drugs in the thermograms could be assumed as evidence for the formation of the inclusion compounds.

Conclusions

Ethyl, butyl and hexyl carbonates of γ-CD have a lower haemolytic activity than the parent CD. Beside, DSC analyses show that they are able to form inclusion complexes with ionic and non ionic drugs such as progesterone, diazepam and flurbiprofen..

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