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Determination of the molecular complexation constant between alprostadil and alpha-cyclodextrin by conductometry Implications for a freeze-dried formulation

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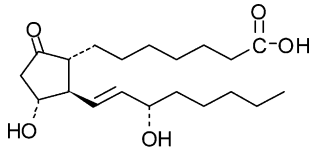
Abstract

The binding constant between alprostadil (PGE_1) and α-cyclodextrin (α-CD) was determined at four temperatures using conductance measurements. Alpha-cyclodextrin is an excipient material in Caverject dual chamber syringe (DCS) that was added to enhance stability. The binding constant was used to calculate the amount of PGE_1 free upon reconstitution and injection, since only the free drug is clinically active. The conductivity measurement is based on a decrease in specific conductance as alprostadil is titrated with α-CD. The change in conductivity was plotted versus free ligand concentration (α-CD) to generate a binding curve. As the value of the binding constant proved to be dependent on substrate concentration, it is really a pseudo binding constant. A value of $742 \pm 60\,M^{-1}$ was obtained for a 0.5 mM solution of alprostadil at 27 °C and a value of $550 \pm 52\,M^{-1}$ at 37 °C. These results compare favorably to values previously obtained by NMR and capillary electrophoresis. Calculation of the fraction PGE_1 free upon reconstitution and injection show it to approach the desired outcome of one. Hence, the amount of drug delivered by Caverject DCS is nominally equivalent to that delivered by Caverject S. Po., a predecessor product that contains no alpha-cyclodextrin.

Keywords: Complexation; Binding constant; Complexation constant; Freeze-dried formulation; Conductometry

1. Introduction

Caverject sterile powder dual chamber syringe (Caverject DCS) is a product that was developed by Pharmacia Corp. for treatment of erectile dysfunction. The active ingredient is the prostaglandin alprostadil (PGE_1). It is a lyophilized product offered in two strengths (10 and 20 μg). Each strength is reconstituted with 0.60 ml bacteriostatic water for injection (BWFI) in a dual chamber syringe, yielding concentrations of 20 and 40 μg/ml. The desired dose is administered by delivering the appropriate volume. The lyophilized powder resides in the forward chamber and the BWFI in the rear. The syringe is packaged together with a disposable administration device that is used to reconstitute and inject the resulting solution.



alprostadil; PGE_1

Caverject DCS differs from an earlier product, Caverject S. Po., principally in the inclusion of α-cyclodextrin (α-CD) in the lyophilate. α-CD is added to enhance stability in the solid state, notably, to inhibit decomposition of PGE_1 to PGA_1, a hydrolysis product [1]. Addition of α-cyclodxtrin has resulted in at least a two-year shelf life at room temperature.

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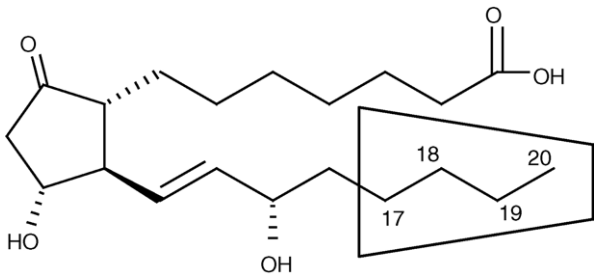

Fig. 1. Schematic representation of the molecular complex between PGE_1 and α-CD [2].

When in solution with alprostadil, α-CD is believed to associate with alprostadil in the manner depicted in Fig. 1 [2]. The degree to which PGE_1 and α-CD associate is reflected in the binding constant for the complex. As the bulk solution containing PGE_1 and α-CD becomes more concentrated during freeze-drying, the fraction of PGE_1 that combines with α-CD increases to the point where, if a sufficient excess of α-CD is present, virtually all of the PGE_1 substrate will be complexed in the solid state. This contrasts with the situation for the reconstituted solution, where the fraction bound is dictated by the strength of the interaction between PGE_1 and α-CD as defined by the binding constant. The degradation kinetics of alprostadil in the solid state are second order [1]. This mechanism requires that two PGE_1 molecules collide and interact with one another. α-CD is presumed to enhance stability by impeding mobility and thereby reducing the frequency of collision and hence inhibiting the decomposition process. Interestingly, the reactive parts of the alprostadil molecule, the five-member ring and the carboxylic group, are not contained within the cyclodextrin cavity (Fig. 1). Including α-CD in the formulation is essential to achieving a two-year room temperature shelf life for Caverject DCS. In addition to alprostadil and α-CD, the formulation contains lactose, sodium citrate, and benzyl alcohol (the latter a constituent of the BWFI).

In devising the formulation a sufficient amount of α-CD had to be included to impart the desired stability in the solid state, yet not be so high that upon reconstitution PGE_1 remains substantially bound. If PGE_1 remains significantly bound after injection, efficacy may be reduced, hence the need for determining the binding constant for the molecular inclusion complex between PGE_1 and α-CD.

Many techniques have been reported in the literature for determination of binding constants. They include optical absorption spectroscopy, infrared spectroscopy, nuclear magnetic and electron spin resonance spectroscopy, potentiometry, reaction kinetics, solubility, liquid–liquid partitioning, dialysis, gas and liquid chromatography, fluorometry, refractometry, polarimetry, conductometry, polarography, dielectrometry, capillary electrophoresis, thermal methods, and others [3]. The only requirement for a technique is that the parameter being measured differ between the free and complexed substrate, i.e., that the parameter changes with the fraction bound. As to cyclodextrins, binding studies have been conducted with a wide assortment of compounds. Techniques utilized include kinetics [4–6], spectrophotometry [6,7], potentiometry [8], dialysis [9], circular dichroism [7], thermal analysis [10–12], and NMR [7,13–18]. NMR is the most generally informative of these various approaches, as it affords high specificity and can yield structural information on the nature of the complex. Optical absorbance is an attractive technique when applicable because of its simplicity and accessibility. However, in order to use optical absorbance, there must either be a shift in the wavelength of maximum absorbance or a change in A_{max} as a function of ligand concentration. Unfortunately, since PGE_1 possesses only end absorption, absorption spectroscopy is not applicable. Capillary electrophoresis (CE) has gained popularity in recent years as a technique for the determination of binding constants. Conductometry has been used less than CE, although it has long been used to study binding in inorganic metallic complexes. Because the intrinsic aqueous solubility of alprostadil is low (60–80 μg/ml at room temperature), either a high pH ($pK_a = 5.1$) or a salt of PGE_1 is needed in order to utilize conductometry. In this report, we present our work on the determination of the binding constant for the inclusion complex PGE_1–α-CD using conductometry. We compare the results obtained with those previously obtained using NMR and CE.

1.1. Background

We first present some general background, then develop the relevant equations for conductivity. Molecular complexation for a 1:1 stoichiometry may be represented by

$$S + L \rightarrow \mathrm{SL} \tag{1}$$

where S refers to the substrate (PGE_1), L to the ligand (α-CD), and SL to the 1:1 complex. In turn, the binding or equilibrium constant is written as

$$K_{11} = \frac{[\mathrm{SL}]}{[\mathrm{S}][\mathrm{L}]} \tag{2}$$

where the $_{11}$ subscript signifies binding for a 1:1 stoichiometry.

Only three loci in alprostadil are potential sites for inclusion inside the torus of α-CD (see Fig. 1): the terminal alkyl chain, the hydroxycyclopentanoyl ring, and/or the carboxylic moiety. Molecular modeling and NMR measurements utilizing the nuclear Overhauser effect (NOE) provide support for the structure shown in Fig. 1 [13,19]. Most studies on complexes between carboxylic acids and cyclodextrins have concluded that carboxylic groups, regardless of ionization state, are repelled from the apolar interior of cyclodextrins [7]. Other work conducted by us utilizing NMR [20] and CE [21] similarly supports a 1:1 stoichiometry for the complex between PGE_1 and α-CD. Also, results of a prior NMR study on the PGE_1/α-CD system argue against interaction at the carboxylic site [13]. Hence, we worked from the assumption that a 1:1 stoichiometry exists between PGE_1 and α-CD.