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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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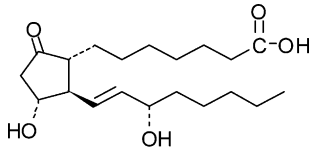
Abstract

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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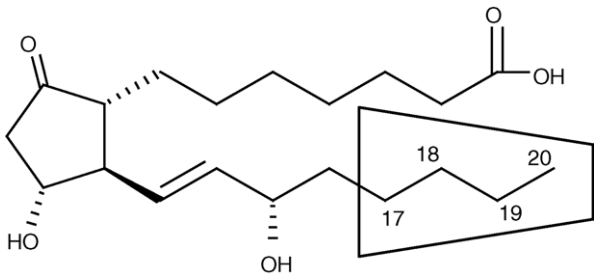

Fig. 1. Schematic representation of the molecular complex between PGE_1 and α-CD [2].

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.

In Eq. (2) [S] is the concentration of *free* (unbound) substrate and [L] the concentration of free ligand. We followed the nearly universal approach of determining the molecular complexation constant by generating a binding curve, and from the curve extracting K via regression analysis.

Consider the expression

$$f_b = \frac{[SL]}{S_t} \quad (3)$$

where S_t is the total substrate concentration and f_b is the fraction of S bound. Combining Eqs. (2) and (3), we arrive at the *binding isotherm*

$$f_b = \frac{K_{11}[L]}{1 + K_{11}[L]} \quad (4)$$

To utilize this expression, f_b has to be described in terms of a measurable system parameter, i.e., a parameter whose magnitude varies as a function of the amount of substrate bound.

1.2. Literature on use of conductivity for determination of binding constants

Conductivity has been extensively used to determine formation constants of inorganic metallic complexes [22,23]. Its application to the determination of molecular complexation constants involving organic species is a more recent development. In order to utilize conductometry for measurement of formation or binding constants, the *specific* conductivity of the reactant must differ from the specific conductivity of the product. Application of conductometry to the determination of binding constants includes various studies on cyclodextrins in both aqueous and nonaqueous systems [24–26].

Sataki et al. determined association constants between α-cyclodextrin and several ionic surfactants [27]. They argue that for dilute electrolyte solutions (2.5 mM), the activity coefficients may be assumed to be unity, and hence Debye–Hückel type corrections accounting for ion-pair formation are unnecessary. The alkanesulfonates and sulfates have been popular substrates in the study of binding to cyclodextrins by conductometry. Lavandier et al. investigated the binding of sodium alkane-1-sulfonates (C_5–C_{10}, C_{12}) to four modified, neutral β-cyclodextrins [28]. Association between alkyl sulfonates (C_6–C_{12}) and fluorocarbon surfactants (C_3–C_6) with α- and β-cyclodextrin was studied by Aman and Serve [29]. Funasaki noted a concentration dependence to the binding constants for the sodium dodecylsulfate (SDS)–β-CD system, with the binding constant decreasing with increasing surfactant concentration [30]. Palepu and Reinsborough studied the stoichiometries of the surfactants SDS and tetradecyltrimethyl ammonium bromide with α-, β-, and γ-CD [31].

A clear theoretical treatment on conductance that resulted in the commonly cited square root expression was given by Tawarah and Wazwaz for the binding of methyl orange, *o*-methyl red, and *p*-methyl red anions with α-CD in water [32]. They meticulously addressed the question of whether viscosity corrections were necessary when calculating a binding constant. If the solution viscosity increases during a titration, then not all of the measured change in conductivity will be due to binding, but some will be due simply to the change in viscosity. If this viscosity-induced change is not subtracted from the overall change, then an error will be introduced into the binding constant calculation. Tawarah and Wazwaz concluded that, except for highest accuracy measurements, no correction for viscosity was necessary. In contrast, Wojoik and Rohrbach included a viscosity correction of up to 3% when studying the binding of various inorganic anions to α- and β-CD over essentially the same concentration range [33].

Several authors have compared values for formation constants obtained by conductivity to values obtained by other techniques. Jobe et al. compared conductivity with fluorescence [34], Junquera and Aicart conductivity to potentiometry and fluorometry [35] and to the speed of sound [26], while Gelb et al. compared conductivity to ^{13}C NMR [36,37]. Although agreement between techniques is not always good, ranking of a series of compounds using the same technique (such as conductometry) is ordinarily reliable.

1.3. Theoretical treatment for conductivity

Conductance, G, is directly proportional to (electrophoretic) mobility μ [38]. The mobility of the complex is lower than that of the substrate alone due to its greater bulk at the same charge. It follows then that the conductivity decreases with the fraction bound.

The measured parameter in conductivity is *specific conductivity*, κ, given by

$$\kappa = \frac{1}{R}\frac{d}{A} \quad (5)$$

where R is the solution resistance in S^{-1} (=1/G), d the distance between electrodes in cm, and A is the area of the electrodes in cm^2; the units of κ are $S\,cm^{-1}$. The greater the difference in specific conductivity between the free and bound species, the greater will be the accuracy of the binding constant determination. Additionally, the greater the fraction (transport number) of the total solution conductivity carried by the target analyte (PGE_1 here), the more accurate will be the determination. Because the intrinsic solubility of PGE_1 in aqueous solution is low (60–80 μg/ml in unbuffered solution), either the pH of the solution needs to be raised to ionize the carboxylic acid group, or a salt of the analyte needs to be prepared. The preferred approach, clearly, is to prepare a salt, as the former tack will result in a higher background conductance. Accordingly, we prepared the lithium salt of PGE_1. $Li^+PGE_1^-$ is a strong electrolyte, and hence dissociates completely into Li^+ and PGE_1^-. Li^+ was chosen because its equivalent ionic conductance is lower than that of either Na^+ or K^+, a consequence of its larger hydration shell [39].The

measured specific conductivity may be expressed as

$$\kappa_{\Sigma} = \kappa_{Li^+} + \kappa_{E^-} + \kappa_{ECD^-} \quad (6)$$

where κ_{Σ} is the observed (measured) conductivity, and κ_{Li^+}, κ_{E^-}, and κ_{ECD^-} are the specific conductivities due to Li^+, free PGE_1, and bound PGE_1, respectively. We do not include a scavenger term to account for any conductive impurities. Rather, this may be lumped into the κ_{Li^+} term. Considering the generic equation for f_b (Eq. (3)) and noting that κ for an individual ionic species is defined as

$$\kappa = \lambda C \quad (7)$$

where λ is the molar ionic conductivity of the species and C is its concentration, we arrive at the following general equation for the conductivity measurement:

$$\kappa = (\kappa_{Li^+} + \lambda_{E^-} E_t) + f_b(\lambda_{ECD^-} - \lambda_{E^-})E_t \quad (8)$$

E_t in Eq. (8) refers to the total concentration of PGE_1. Rearranging the terms, and using the equation for a binding isotherm (Eq. (4)), we arrive at

$$\Delta\Lambda = \frac{(\Delta\lambda)K_{11}[CD]}{1 + K_{11}[CD]} \quad (9)$$

where CD was substituted for L and where Λ is the molar conductivity, defined as

$$\Lambda = \frac{\kappa_{\Sigma}}{C} \quad (10)$$

k_{Σ} in Eq. (10) refers to the sum of the individual specific conductivities, defined by Eq. (6). The Δ terms in Eq. (9) are defined as

$$\Delta\Lambda = \Lambda_i - \Lambda \quad (11)$$

where Λ_i is the initial molar conductivity and

$$\Delta\lambda = \lambda_{E^-} - \lambda_{ECD^-} \quad (12)$$

The molar conductivity of the substrate is calculated after each addition of titrant. An exact expression for [CD] is defined by

$$[CD] = CD_t - E_t\left(\frac{\Delta\Lambda}{\Delta\lambda}\right) \quad (13)$$

where CD_t and E_t refer to the total concentrations of α-CD and PGE_1, respectively, which are always known.

N.B.: Although we did not see this exact treatment in the literature, equations derived by different authors are equivalent. For example, we were able to readily interconvert our expressions to the derivation of Satacki et al. [27,40].

2. Materials and methods

The binding isotherm represents a change in the fraction bound as a function of free ligand concentration (Eq. (4)). This equation is ordinarily utilized by maintaining the concentration of either the substrate or ligand constant (usually the former) and then varying the concentration of the other over a suitable range. This was accomplished by charging the conductivity cell with a fixed volume of the lithium salt of PGE_1, then titrating with α-CD while monitoring the change in conductivity. The underlying principle is that as the amount of PGE_1 bound increases with increasing [α-CD], the conductivity decreases due to the larger bulk of the complex relative to free PGE_1. A rule of thumb in binding experiments is that the amount and concentration of ligand (α-CD) should be sufficient to sweep out ≥75% of the full binding range [3] to allow adequate comparison of the curve fit to the experimental data, and thereby evaluate the suitability of the assumed stoichiometric model (1:1 here). Accordingly, in each experiment the concentration and amount of α-CD was adjusted to allow the requisite range to be swept out. The cell was ordinarily charged with 20 ml of ∼0.5 mM $Li^+PGE_1^-$ and titrated with 15 ml of 23 mM α-CD.

2.1. Chemicals

Lithium carbonate and benzyl alcohol were purchased from Aldrich, and ethanol from Quantum Chemical Company. Purified water (Milli Q) was obtained from a Millipore water purification unit, prepared as needed. The alprostadil used was P&U lot 143AW (purity, 98.6%). The alpha-cyclodextrin was lot 8156E from Wacker. A potency of 100.5% was measured and KF coulometry found 9.8% water. Hence, a purity of 100.5 − 9.8 = 90.7% was assigned.

The α-CD as received contained trace ionic impurities. Ionic impurities in the titrant complicate the experiment due to a higher background, but more importantly, will lead to errors in the binding constant because the measured change in conductivity will be due not only to binding but also to the introduced impurities. We therefore passed aqueous α-CD through a short (2 cm × 8 cm) column packed with a mixed ion-exchange resin, AG-501 ×8. For a final desired concentration of about 20 mM, a solution ca. 40 mM in α-CD was passed through the resin, then diluted to 20 mM.

2.2. Preparation of the lithium salt of PGE_1

Our initial attempt to produce the lithium salt of PGE_1 was to titrate alprostadil in methanol with lithium methoxide, also in methanol. Unfortunately, although only a slight excess of base was present during the freeze-dry process, it was enough to severely degrade the alprostadil. We subsequently prepared the lithium salt according to the following reaction:

$$2PGE_1 + Li_2CO_3 \rightarrow 2Li^+PGE_1^- + H_2O + CO_2\uparrow$$

762.4 mg of PGE_1 was dissolved in 750 ml of 42% EtOH in water (∼1 mg/ml) and placed in a three-neck flask chilled by immersion in an ice bath. A solution of 77.9 mg Li_2CO_3 in 100 ml water (10 mM) was then added dropwise to the solution over a 20 min period while stirring vigorously. The

solution was allowed to stand for an additional 10 min, again with vigorous stirring. The ethanolic solution of the lithium salt of alprostadil was then frozen with a FTS Systems shell freezer. This frozen solution was lyophilized over a 48 h period with the FTS freeze drier to yield a dry, solid product. The yield was ∼75%. The isolated salt was analyzed by HPLC, for percent water (KF), for residual solvents (by GC) and for lithium (by AA). The results were: purity, 0.7% impurity formation (PGA_1); water, 4.1%; residual solvents (EtOH), <0.02%; Li, 1.8% (w/w). The theoretical amount of lithium was 1.9%. The overall purity was then calculated as $\{100 - 0.7 - 4.1 - 0\}\% = 95.2\%$.

2.3. Preparation of benzyl alcohol solution in purified water

Because Caverject DCS is reconstituted in BWFI, which contains 0.945% benzyl alcohol, determination of the binding constant in the presence of benzyl alcohol is also of interest. A 1 l volumetric flask containing a small magnetic stirrer was filled about 2/3 full of Milli Q water. 9.45 g of benzyl alcohol weighed into a small beaker was transferred to the 1 l flask with several water rinsings. The solution was mixed for 30 min, then brought to volume with Milli Q water. Finally, the flask was inverted several times, then allowed to stand for another 10 min. A desired amount of α-CD was added to the solution, and the solution then passed through a mixed ion-exchange bed, AG-501 × 8, to remove ionic impurities. N.B.: This manner of preparing the solution had the effect of stripping out some of the benzyl alcohol. The desired concentration of 0.945% was reduced to 0.83%. (A preferred way of preparing this solution would have been to dissolve the benzyl alcohol in an aqueous α-CD solution already stripped of ionic impurities.)

2.4. Instrumentation/equipment

A Brinkmann Metrohm research grade conductivity meter, the 712 Conductometer, was used as part of a fully automated titration system, the Brinkmann Titrino Model 751-1. Due to the dependence of conductivity on temperature, all titrations were carried out in a jacketed, 50 ml cell (cat. no. 20-29-300-4). Temperature was regulated using a Model 1166 Polyscience Circulator with Digital Controller capable of controlling the temperature to ±0.01 °C. Precise temperature control is imperative in order to make accurate and reproducible conductivity measurements [27]. The electrode was a double platinized (to increase surface area) electrode from Metrohm (cat. no. 20-49-017-9). The cell constant (d/A, where d is the distance between electrodes in cm and A is the area of the electrode in cm^2) was determined to be 0.900 cm^{-1} using the calibration solution available from Brinkmann (KCl, cat. no. 020-10-040-1). A nitrogen blanket was used to prevent carbon dioxide from entering the cell by passing a constant flow of gas at a rate of 2.5 l min^{-1}. Dissolved CO_2 leads to drift as it reacts with water to form trace amounts of bicarbonate and carbonate. Ascarite®-filled fittings were also used to exclude CO_2 from the cell. Raw data in the form of specific conductivity, κ, was collected for each addition of titrant (0.5 ml) after a user-set delay (25 s, time required to achieve a stable reading).

In a typical experiment, the cell was charged with 20 ml of PGE_1 (from $Li^+PGE_1{}^-$) solution, equilibrated for several minutes (∼5 min, longer for temperature investigations) with stirring, then titrated with α-CD under the control of the Titrino 751-1. The titrations were performed using 15 ml of a fixed concentration of α-CD (23 mM) in 0.5 ml steps against varying concentrations of the lithium salt of PGE_1 (0.25–2.5 mM), with the 200 μg/ml (0.50 mM) concentration the standard for temperature investigations. As noted, the amount and concentration of titrant was selected in order to sweep out a sufficient expanse of the binding curve (approximately ≥85%).

2.5. Data analysis

The data were analyzed according to Eq. (9) using nonlinear regression analysis from which both ($\Delta\lambda$) and K_{11} were extracted. The change in molar conductivity, $\Lambda_i - \Lambda$, was plotted against free α-CD, [α-CD], to obtain the curve fit. The curve fitting and nonlinear regression were performed with SigmaPlot.

3. Results

In order to accurately calculate a binding constant, it is essential that the change in conductivity be due only to binding and not to a change in solution viscosity. An increase in viscosity could result from α-CD as its concentration increases in the course of a titration. To test for this, we titrated a 5 mM KCl solution with α-CD over the applicable α-CD concentration range. If the viscosity is unchanged, the solution conductance should decrease only in accordance with dilution. Note than an assumption here was that neither K^+ nor Cl^- binds to α-CD. We conducted the viscosity check at four temperatures, 25, 27, 30, and 37 °C. The result was that at each temperature the conductivity decreased slightly (after correction for dilution), but measurably (slightly more than 1% at 25 °C). The literature is somewhat confusing on this point, as some authors state that a small viscosity correction is appropriate [29,33,37] while others argue that the change is so slight that, except for measurements of highest accuracy, no correction is necessary [29,32]. Due to this ambiguity, and also because there is some suggestion in the literature that even at low millimolar concentrations Cl^- can bind [29], the measurements were repeated at a lower concentration, 1 mM KCl. At this lower concentration the change in conductivity was negligible (all under 1%). This change was deemed too small to warrant making corrections.

Preliminary experiments revealed a concentration dependence on the inclusion complex between alprostadil and

α-CD, similar to what has been reported in the literature for other systems, notably for binding between α-cyclodextrin and C_6, C_8, and C_{10} sulfonates [27,29]. The concentration dependence of the alprostadil–α-CD system was assessed over the range ~0.25–2.5 mM (0.24, 0.48, 0.95, and 2.4 mM). The mean binding constants obtained ranged from $1224 \pm 369\ M^{-1}$ at 0.24 mM to $514 \pm 24\ M^{-1}$ at 2.4 mM (at 25 °C). The fit to the binding curve was relatively poor at the lowest concentration, exhibiting a fair amount of scatter. This was a consequence of small Δk values, thereby making for noisy measurements.

While 0.25 mM proved too low a concentration for reliable measurement by conductivity, 0.50 mM was satisfactory. When Caverject DCS is reconstituted with diluent from the rear chamber, the resultant concentration of PGE_1 is either 20 μg/ml (for the 10 μg strength) or 40 μg/ml (for the 20 μg strength), which correspond to 0.06 mM and 0.11 mM, respectively. Clearly, this low concentration cannot be duplicated in a conductivity experiment. Therefore, 0.5 mM was selected for subsequent conductivity measurements.

At 0.5 mM formation of ion-pairs may be disregarded [27]; therefore all ions can be assumed to migrate independently [38]. Hence, the activity coefficients may be taken as unity and Debye–Hückel type corrections are therefore unnecessary [27,40]. Also, at this low concentration, hydrolysis, as represented by

$$E^- + H_2O \rightarrow HE + OH^-$$

is negligible. For PGE_1 at 200 ppm $LiPGE_1$ (0.5 mM) with $K_a = 1.1 \times 10^{-5}$, less than 1% is hydrolyzed. Furthermore, this percentage does not change as a function of the fraction bound since the charged carboxylic group is not contained within the cyclodextrin cavity. Hence, neither of these potential complications needed to be taken into account in the analysis.

In NMR and CE experiments conducted earlier, the binding measurements were made at 27 °C (300 °K) [20,21]. Hence, 27 °C was chosen for conductometry as well, but additionally three other temperatures were selected in order to study the temperature dependence of the binding. The values of the binding constant obtained by conductometry at 25, 27, 30, and 37 °C (all at 0.50 mM) are given in Table 1. As expected, the higher the temperature, the smaller the binding constant. Corresponding representative plots are shown in Fig. 2. Fig. 3 shows the residuals at each temperature. The excellent fit exhibited by the binding curves of Fig. 2 offers strong support for the assumed 1:1 stoichiometry.

Although binding constants are most commonly reported for aqueous systems, of greater relevance for Caverject DCS is the reconstituted solution in BWFI, which contains 0.945% benzyl alcohol. For a solution 0.83% in benzyl alcohol (see Section 2) a mean value of $559 \pm 120\ M^{-1}$ (557 ± 86 and $561 \pm 84\ M^{-1}$) at 25 °C was obtained. This compares with a value of $393\ M^{-1}$ obtained by NMR at 27 °C [20].

Table 1
Regression results by conductance at 25, 27, 30, and 37 °C

Replicate	Temperature (°C)	$\Delta\lambda$ (S cm^2 mol^{-1})	K_{11} (dm^3 mol^{-1})
1	25	5.98	890 ± 88 (2σ)
2		5.75	986 ± 74
3		5.86	889 ± 76
4		5.23	926 ± 133
5		5.23	986 ± 126
Average		5.61	935 ± 187
1	27	7.17	699 ± 38
2		6.70	784 ± 46
Average		6.94	742 ± 60
1	30	7.98	588 ± 20
2		6.97	617 ± 54
3		7.50	664 ± 30
Average		7.48	623 ± 64
1	37	9.26	523 ± 30
2		9.35	565 ± 24
3		8.62	562 ± 36
Average		9.08	550 ± 52

4. Discussion

4.1. Comparison of techniques

In Table 2, the results obtained here are compared with results previously obtained by us using NMR [20] and CE [21]. Because of the concentration dependence of the PGE_1–α-CD binding constant (vide supra), comparison of results by the three techniques must be done with caution. The agreement between CE and conductivity is excellent, albeit with the recognition that they correspond to different PGE_1 concentrations, 0.07 mM for CE, 0.50 mM for conductivity. The explanation for the concentration dependence observed by conductivity may be that surface-active alprostadil seeks out the air–water interface in an attempt to escape the aqueous milieu in which alprostadil is only very slightly soluble. This behavior is exacerbated at higher concentrations due to alprostadil's propensity to self-aggregate,

Table 2
Comparison of results by NMR, CE, and conductometry

Temperature (°C)	K_{11} (M^{-1})		
	NMR[a]	CE[b]	Conductivity[c]
25			$935 \pm 114_{n=5}$ (2s)
27	$966 \pm 130_{n=1}$	$708 \pm 64_{n=3}$	$742 \pm 60_{n=2}$
30		$642 \pm 51_{n=3}$	$623 \pm 46_{n=3}$
37		$537 \pm 27_{n=3}$	$550 \pm 38_{n=3}$

[a] A fixed ratio in accordance with the composition of Caverject DCS was maintained between PGE_1 and α-CD: [α-CD]/[PGE_1] = 11.8; absolute concentration of PGE_1 varied from 0.56 to 0.03 mM.
[b] [PGE_1] = 0.07 mM.
[c] [PGE_1] = 0.50 mM.

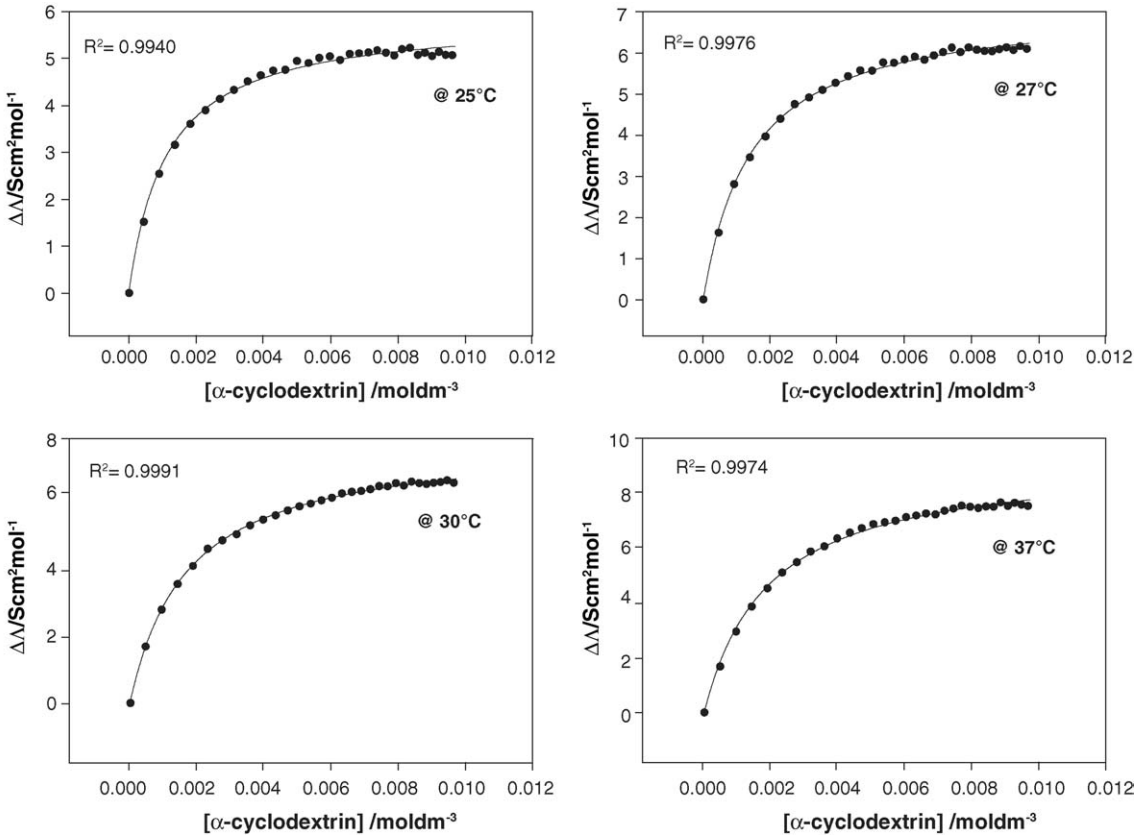

Fig. 2. Representative binding curves at four temperatures for the lithium salt of alprostadil; $[LiPGE_1] = 0.50 \times 10^{-3}$ mol dm^{-3}. The filled circles represent the data points, the curves the best-fit regression.

hence the decrease in the binding constant with increasing concentration. If the same behavior is presumed in the CE experiment, one would expect the CE results to be higher than those obtained by conductivity since the PGE_1 concentration in the CE experiment was eight-fold lower than in the conductivity experiment (0.07 mM versus 0.56 mM). On the other hand, the same behavior may not apply to CE. In CE, the same escape route is not available to PGE_1, as the separation capillary is completely filled with fluid (buffer); hence, there is no air–water interface. Furthermore, the fact that the 0.07 mM sample injected in CE is diluted in the course of the separation makes it difficult to make a direct analogy to the conductivity experiment. Interestingly, a concentration dependence was noted when using microcalorimetry (unpublished results). The air–water interface is also present in the calorimetry experiment. In the case of NMR, it has been reported that NMR can give different results depending on which protons are measured [20]. Hence, the manner in which the experiment is carried out may affect the results. Another difference vis-à-vis CE and conductometry is that in the NMR experiment there was no buffering, although, in principle, one would not expect dissociation to play a role since the carboxylic acid group does not enter the CD cavity (vide supra). In reality, it is likely that every technique introduces its own bias.

4.2. Calculation of percent PGE_1 bound from K_{11}

Once K_{11} is known, the free PGE_1 can be calculated for any combination of $(PGE_1)_t$ and α-CD. A goal of this work was to be able to predict how much drug is available to the patient upon injection and subsequent dilution. An equation can be derived using an analysis similar to that used to arrive at the binding isotherm above. Combining Eqs. (1) and (2) with the expressions $E_t = [E] + [ECD]$ and $CD_t = [CD] + [ECD]$, one readily obtains the quadratic equation

$$K_{11}E^2 + (K_{11}CD_t - K_{11}E_t + 1)E - E_t = 0 \tag{14}$$

which, when solved for E, gives

$$E = \frac{-(K_{11}CD_t - K_{11}E_t + 1)}{2K_{11}} + \frac{\sqrt{(K_{11}CD_t - K_{11}E_t + 1)^2 + 4K_{11}E_t}}{2K_{11}} \tag{15}$$

where E is the free PGE_1 concentration and E_t is the total PGE_1 concentration.The fraction free, f_f, may then be calculated from

$$f_f = \frac{E}{E_t} \tag{16}$$

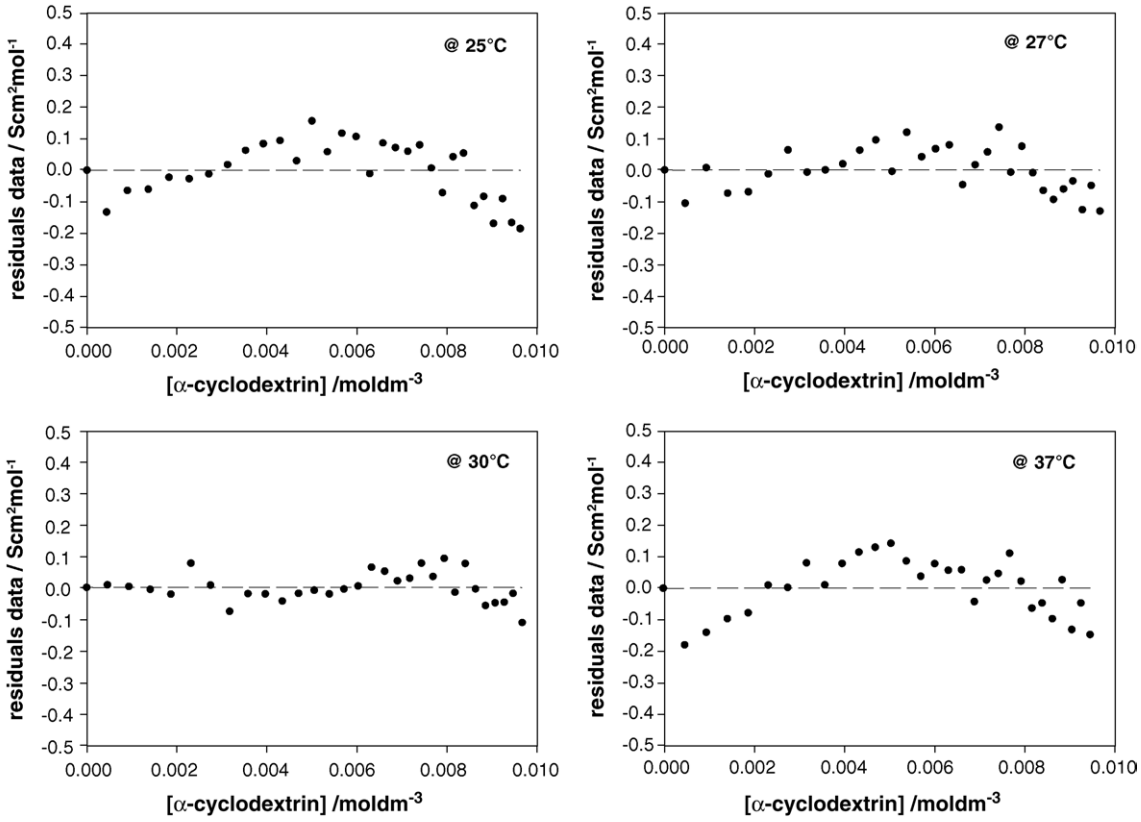

Fig. 3. Residuals plots for the binding isotherms in Fig. 2.

The fraction PGE_1 free may be calculated for any concentration of PGE_1 and α-CD and for any value of the binding constant through application of Eqs. (15) and (16). The fraction PGE_1 free for both strengths of Caverject DCS upon reconstitution[2] and after dilution (injection) for select values of K_{11} over the range 300–800 M^{-1} is shown in Table 3. The column labeled $\{0.5 \rightarrow 10\,ml\}$ assumes an intracavernosal blood volume of 10 ml in the flaccid state, and the column labeled $\{0.5 \rightarrow 50\,ml\}$ assumes a blood volume of 50 ml after tumescence [41]. The table reveals that for $K_{11} = 742\,M^{-1}$ (27 °C) more than 97% of the PGE_1 is free after injection for the 10 μg strength (20 μg/ml) and more than 95% for the 20 μg strength (40 μg/ml). For $K_{11} = 550\,M^{-1}$ (value obtained at physiological temperature, 37 °C), more than 98% is free after injection for the 10 μg strength and more than 96% for the 20 μg strength. From the last column, it is seen that, regardless of the value of K_{11}, as erection proceeds, the alprostadil becomes almost entirely free. The value of K_{11} obtained in the presence of BWFI was 559 M^{-1} (27 °C), and hence would result in a higher percentage of free PGE_1 upon reconstitution. After injection (and dilution by blood) the additional benefit gained from the BWFI would be only slight because of the dilution effect. Applied to the real (biological) system, the calculated values of Table 3 constitute a lower bound. Competitive displacement by endogenous lipophilic constituents that are more strongly bound than alprostadil, binding to plasma and tissue proteins, and preferential uptake of the drug in tissue may all act to promote release of drug from the drug–CD complex [42]. These competing influences become increasingly important the stronger the complexation between the drug and cyclodextrin. Where complexation is relatively weak, i.e., for binding constants less than about 1000 M^{-1}, as here, dilution is thought to play the dominant

Table 3
Effect of magnitude of K_{11} on percent PGE_1 free after reconstitution and injection[a]

K_{11} (M^{-1})	1× (reconstituted)	0.5 → 10 ml	0.5 → 50 ml
20 μg/ml PGE_1 (10 μg Caverject DCS)			
300	83.5	99.0	99.8
400	79.2	98.7	99.7
500	75.4	98.4	99.7
600	71.9	98.0	99.6
700	68.7	97.7	99.5
800	65.9	97.4	99.5
40 μg/ml PGE_1 (20 μg Caverject DCS)			
300	71.9	98.0	99.6
400	65.9	97.4	99.5
500	60.8	96.8	99.3
600	56.5	96.2	99.2
700	52.7	95.6	99.1
800	49.5	95.0	98.9

[a] In a strictly aqueous system (i.e., no benzyl alcohol).

[2] For a strictly aqueous system, i.e., no benzyl alcohol present.

role in release of the drug [42]. Interestingly, over the range shown in Table 3, the magnitude of the binding constant has only a moderate effect on the amount free immediately after injection, and virtually no effect after tumescence is achieved.

5. Conclusion

The binding constant between alprostadil and α-cyclodextrin (α-CD) was determined at four temperatures by conductometry. The values were calculated via nonlinear regression analysis applied to binding isotherms. The resultant curve fits were excellent, thereby lending support to the assumed 1:1 complexation stoichiometry. A value of $742\,M^{-1}$ was obtained by conductometry at 27 °C, compared with $966\,M^{-1}$ obtained by NMR and $708\,M^{-1}$ by CE. Because the values obtained were dependent on the concentration of PGE_1, they are actually pseudo-binding constants. The calculated binding constants translate to nearly all of the PGE_1 being released, and hence delivered to the patient after injection and subsequent dilution. For $K_{11} = 559\,M^{-1}$ at 27 °C in the presence of benzyl alcohol (not measured at 37 °C) greater than 98% is free after injection and nearly 100% upon tumescence for the 10 μg strength. The corresponding values for the 20 μg strength are 96 and 99%. Although some differences were noted between techniques, these differences translate to only minor differences in the calculated amount of PGE_1 free after injection.

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