European Journal of Pharmaceutical Sciences 87 (2016) 22–29

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Thermodynamic investigation of the interaction between cyclodextrins and preservatives — Application and verification in a mathematical model to determine the needed preservative surplus in aqueous cyclodextrin formulations

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ARTICLE INFO

Article history:
Received 11 April 2015
Received in revised form 17 September 2015
Accepted 17 September 2015
Available online 21 September 2015

Keywords:
Cyclodextrins
Preservation
Equilibrium
Stability constant
Benzoic acid
Parabens

ABSTRACT

Preservatives are inactivated when added to conserve aqueous cyclodextrin (CD) formulations due to complex formation between CDs and the preservative. To maintain the desired conservation effect the preservative needs to be added in apparent surplus to account for this inactivation. The purpose of the present work was to establish a mathematical model, which defines this surplus based upon knowledge of stability constants and the minimal concentration of preservation to inhibit bacterial growth. The stability constants of benzoic acid, methyl- and propyl-paraben with different frequently used βCDs were determined by isothermal titration calorimetry. Based upon this knowledge mathematical models were constructed to account for the equilibrium systems and to calculate the required concentration of the preservations, which was evaluated experimentally based upon the USP/Ph. Eur./JP monograph. The mathematical calculations were able to predict the needed concentration of preservation in the presence of CDs; it clearly demonstrated the usefulness of including all underlying chemical equilibria in a mathematical model, such that the formulation design can be based on quantitative arguments.

1. Introduction

Within the pharmaceutical field cyclodextrins (CDs) are primarily applied as solubilisers in order to enhance the apparent aqueous solubility of poorly soluble drugs through formation of inclusion complexes (Brewster and Loftsson, 2007). CDs are cyclic macromolecules consisting of glucose units linked together with α-1,4-glycosidic bonds, which creates a shape of a truncated cone with cavities of different sizes depending on the number of glycopyranose units. The most frequently investigated CDs consist of six, seven, or eight units of α-D-(+)-glucopyranose, denoted α, β, or γ-CDs, respectively (Szejtli, 1988), where the most frequently used in pharmaceutics is βCDs (Brewster and Loftsson, 2007; Davis and Brewster, 2007). CDs can be chemically modified by e.g. hydroxylation, alkylation or sulfoalkylation and two uncharged and one charged βCD derivatives, 2-hydroxypropyl β-cyclodextrin (HPβCD), 2-O-methyl βCD (mβCD) and sulfubutylether βCD (SBEβCD), have been widely investigated in the pharmaceutical literature on the account of their fast dissolution rate, high solubility in water and low toxicity (Järvinen et al., 1995; Stella and Rajewski, 1997; Savolainen et al., 1998). Further, these modified CDs have been used in a number of marketed pharmaceutical products (Brewster and Loftsson, 2007; Davis and Brewster, 2007).

Multi-dose pharmaceutical products, e.g. oral solutions and eye droplets etc., may require addition of preservatives, if the compound in the formulation does not in itself have an antimicrobial effect, to prevent microbial spoilage upon storage and during use. Loftsson et al. (1992) studied the interaction between several commonly used preservative compounds and HPβCD and reported two problematic effects; i) the examined preservatives interacted with CDs and competitively displaced the drug and thereby reduced the desirable solubilising of the drug; and ii) the activity of the preservative was similarly reduced since the efficacy of the antimicrobial agents was due to its free and unbound fraction. Miyajima et al. (1987) and Simpson (1992) reported results on neutralisation of antimicrobial activity of quaternary ammonium compounds due to complexation with natural α-, β- and γCD and Lehner et al (1993) noted that higher concentrations of parabens was needed in the presence of HPβCD. Further, a few patents were

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http://dx.doi.org/10.1016/j.ejps.2015.09.011
0928-0987/

published on compositions containing a pharmaceutical compound and CDs, which could be preserved (e.g. Castillo and Espino, 1998; Tetsuro et al., 1985; Shin-Ichiro et al., 1984). From the regulatory agencies it is required that the concentration of preservative is justified in term of efficacy and safety such that the preservative is used in the minimum concentration, which provides the required level of efficacy. Lehner et al. (1993) developed a semi-quantitative model to correlate for the preservation inactivation due to the inclusion by the CD. However, no other systematic work has to our knowledge been published to provide a generalised method on how to obtain the preservative effect in CD solutions taking all parameters in the formulation into account.

A number of equilibriums can be formed in a pharmaceutical product, the acid/base equilibrium, the interaction between CD and the drug, the interaction between the preservative system and the CD etc., depending on the individual components, concentrations and pH. These may in a pharmaceutical formulation be approached as competitive equilibriums defined by complexation constants and acid/base constant, pK_a. Given that these parameters are determined experimentally and the minimum inhibitory concentration (MIC) for the unbound preservative is known, a mathematical evaluation of the needed amount of preservative surplus in a CD formulation should be possible. The purpose of the present work was, hence, to i) determine the complexation constant of orally used preservatives (benzoic acid and methyl- and propylparaben) to frequently used CDs; ii) develop mathematical models to predict the amount of preservative needed in CD systems; and iii) verify experimentally if the efficacy of antimicrobial preservation was sufficient according to the requirements defined in USP, JP and the European Pharmacopoeia.

2. Theoretical considerations

In a system containing drug, preservatives, and CD several competitive chemical equilibria are present, given that both drug and preservative interact with the CD. The preservative will displace the drug in the drug-CD complex, thereby reducing the solubilising effect of the CD. Furthermore, preservative bound in the CD cavity loses its antimicrobial effect as discussed above (Loftsson et al., 1992; Miyajima et al., 1987; Simpson, 1992; Lehner et al., 1993). To compensate for the complexed preservative a surplus may, hence, be necessary to obtain the desired level of preservation. Knowing the different equilibriums in the formulation is therefore of crucial importance in order to design a formulation where both the drug is solubilised and the formulation preserved sufficiently. The estimation of preservative surplus necessary in aqueous CD formulations can be defined according to a number of equilibrium models, dependent upon the given situation. In the simplest situation the system consists of a neutral CD and both a nonionisable drug and nonionisable preservative (at least at the pH range relevant for the given formulation), whereby the following equilibriums will be present:

$$D + CD \leftrightarrows D:CD \tag{1}$$

$$K_{D:CD} = \frac{[D:CD]}{[D]\cdot[CD]} \tag{2}$$

$$P + CD \leftrightarrows P:CD \tag{3}$$

$$K_{P:CD} = \frac{[P:CD]}{[P]\cdot[CD]} \tag{4}$$

where [D], [P] and [CD] are the concentrations of free components of drug, preservative and cyclodextrins, [D:CD] and [P:CD] are the concentration of CD complexes at equilibrium and $K_{P:CD}$ and $K_{D:CD}$ the stability constants for the two reactions. The equations for conservation of mass are given below:

$$[D]_t = [D] + [D:CD], \quad \text{where } [D] \le [D]_{sol} \tag{5}$$

$$[P]_t = [P] + [P:CD] \tag{6}$$

$$[CD]_t = [CD] + [D:CD] + [P:CD] \tag{7}$$

where $[D]_t$, $[P]_t$ and $[CD]_t$ are the total concentration of drug, preservative, and CD, respectively. As drug molecules of low aqueous solubility are normally considered for CD solutions, a requirement was introduced in Eq. (5), that the equation is only valid when the free drug concentration is below its intrinsic solubility, i.e. $[D] \le [D]_{sol}$.

To design this type of formulation, the formulation scientist will have to meet two requirements: 1) the drug should be fully solubilised by the CD such that no drug will precipitate, and 2) the solution should be properly preserved according to the MIC. To optimise the formulation, the dosing should be done in such a way that the minimal amount of excipients (CD and preservative) is used.

In the supporting information it is shown that the concentration, which achieves these two requirements and simultaneously uses the minimal amount of excipients is given by:

$$[CD]_t = \frac{1 + K_{D:CD}[D]_{sol}}{K_{D:CD}[D]_{sol}}\left([D]_t - [D]_{sol}\right) + \mathrm{MIC}\cdot\frac{K_{P:CD}}{K_{D:CD}}\frac{[D]_t - [D]_{sol}}{[D]_{sol}} \tag{8}$$

$$[P]_t = \mathrm{MIC}\cdot\left(1 + \frac{K_{P:CD}}{K_{D:CD}}\frac{[D]_t - [D]_{sol}}{[D]_{sol}}\right) \tag{9}$$

where MIC is the minimum inhibitory concentration in molarity.

2.1. Theoretical background for the microbiological tests conducted on placebo solutions

The experiments conducted in the present work were conducted on placebo solutions. When no drug molecule with solubility limitations is present the only requirement for the formulation is that the free preservative concentration should be equal to the MIC. In that case it was straight-forward to obtain the concentrations of the free species by numerical solutions of the equations for conservation of mass and the complexation constants. Numerical solutions can easily be derived even for very complicated systems with both ionisable drugs and ionisable preservatives forming complexes with CD. Alternatively, algebraic formulas for the required amount of preservative can be derived to gain insight into, which quantities that are dominant for the concentration of the free species. This is done in the following for the systems considered experimentally in this work.

For a nonionisable preservative (e.g. methylparaben), the total preservative concentration to achieve MIC is given by:

$$[P]_t = MIC + K_{P:CD}\cdot MIC\cdot\frac{[CD]_t}{1 + K_{P:CD}\cdot MIC}. \tag{10}$$

If the preservative is an acid (e.g. benzoic acid), the total preservative concentration to achieve MIC is given by:

$$[P]_t = MIC + \frac{K_{H^+:P}\cdot MIC}{[H^+]} + \frac{K_{PH:CD}\cdot MIC + K_{P^-:CD}\dfrac{K_{H^+:P}\cdot MIC}{[H^+]}}{1 + K_{PH:CD}\cdot MIC + K_{P^-:CD}\dfrac{K_{H^+:P}\cdot MIC}{[H^+]}}[CD]_t \tag{11}$$

where $[H^+]$ is the concentration of protons, $[P^-]$ and $[P^-{:}CD]$ are the free ionised preservative and the complex between ionised preservative, [PH] and [PH:CD] are the free neutral preservative and the complex between neutral preservative and CD and $K_{PH:CD} = \frac{[PH:CD]}{[PH]\cdot[CD]}$, $K_{P^-:CD} = \frac{[P^-:CD]}{[P^-][CD]}$, $K_{H^+:P} = \frac{[PH]}{[H^+][P^-]}$ are the stability constants for the neutral preservative, the ionised preservative and the acid association constant.