International Journal of Pharmaceutics 531 (2017) 504–511

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

# Soluble 1:1 complexes and insoluble 3:2 complexes – Understanding the phase-solubility diagram of hydrocortisone and γ-cyclodextrin

Christian Schönbeck[a,*], Tobias L. Madsen[b], Günther H. Peters[b], René Holm[c], Thorsteinn Loftsson[d]

[a] *Department of Science and Environment, Roskilde University, Universitetsvej 1, DK-4000 Roskilde, Denmark*
[b] *Department of Chemistry, Technical University of Denmark, Building 207, DK-2800 Kongens Lyngby, Denmark*
[c] *Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium*
[d] *Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata 53, IS-107 Reykjavik, Iceland*

ARTICLE INFO

*Article history:*
Received 24 February 2017
Received in revised form 5 May 2017
Accepted 10 May 2017
Available online 11 May 2017

*Keywords:*
Cyclodextrin
Phase-solubility
Calorimetry
Hydrocortisone
Inclusion complex
Binding constant

ABSTRACT

The molecular mechanisms underlying the drug-solubilizing properties of γ-cyclodextrin were explored using hydrocortisone as a model drug. The $B_S$-type phase-solubility diagram of hydrocortisone with γ-cyclodextrin was thoroughly characterized by measuring the concentrations of hydrocortisone and γ-cyclodextrin in solution and the solid phase. The drug-solubilizer interaction was also studied by isothermal titration calorimetry from which a precise value of the 1:1 binding constant ($K_{11} = 4.01\ \text{mM}^{-1}$ at 20 °C) was obtained. The formation of water-soluble 1:1 complexes is responsible for the initial increase in hydrocortisone solubility while the precipitation of entities with a 3:2 ratio of γ-cyclodextrin: hydrocortisone is responsible for the plateau and the ensuing strong decrease in solubility once all solid hydrocortisone is used up. The complete phase-solubility diagram is well accounted for by a model employing the 1:1 binding constant and the solubility product of the precipitating 3:2 entity ($K_{32}^{S} = 5.51\ \text{mM}^5$). For such systems, a small surplus of γ-cyclodextrin above the optimum concentration may result in a significant decrease in drug solubility, and the implications for drug formulations are briefly discussed.

## 1. Introduction

Addition of cyclodextrins (CDs) is an often used formulation strategy to increase the apparent aqueous solubility of poorly soluble drugs (Williams et al., 2013). Formation of water-soluble inclusion complexes between drug and cyclodextrin increases the amount of drug in solution but other mechanisms may also contribute to the increased drug solubility. Both CDs and their complexes may form aggregates in which drug molecules are included, similar to solubilization of drugs by incorporation into micelles. The exact contribution of CD aggregates to the increased drug solubility is difficult to quantify but it is nevertheless anticipated that aggregate formation can be exploited as a new type of drug delivery system (Ryzhakov et al., 2016). It is clear, however, that a more thorough understanding of the aggregation phenomenon is required.

Hydrocortisone (HC) solubilized by CDs is an often used model system for the study of CD aggregates and their impact on drug solubility (Jansook et al., 2010; Jansook and Loftsson, 2008; Messner et al., 2011a,b; Saokham et al., 2016). In the case of modified cyclodextrins (e.g. hydroxypropylated β- and γCDs) the equilibrium concentration of hydrocortisone increases linearly with the CD concentration resulting in a so-called $A_L$-type phase solubility (PS) diagram, using the classification system of Higuchi and Connors (Higuchi and Connors, 1965). For the natural β- and γCDs, $B_S$-type PS diagrams have been reported which are characterized by an initial linear increase in the amount of dissolved drug followed by a plateau where the concentration of dissolved drug is independent of the CD concentration. After the plateau, the drug concentration decreases with increasing amounts of CD (Fig. 1). From a formulation point of view, $B_S$-type systems are problematic as just the right amount of CD is required to obtain maximum drug solubility and addition of too much CD (relative to the amount of drug) may significantly lower the amount of dissolved drug. According to the traditional interpretation (Higuchi and Connors, 1965) $B_S$-type PS diagrams

---

[*] Corresponding author.
*E-mail address:* jechsc@ruc.dk (C. Schönbeck).

http://dx.doi.org/10.1016/j.ijpharm.2017.05.024
0378-5173/

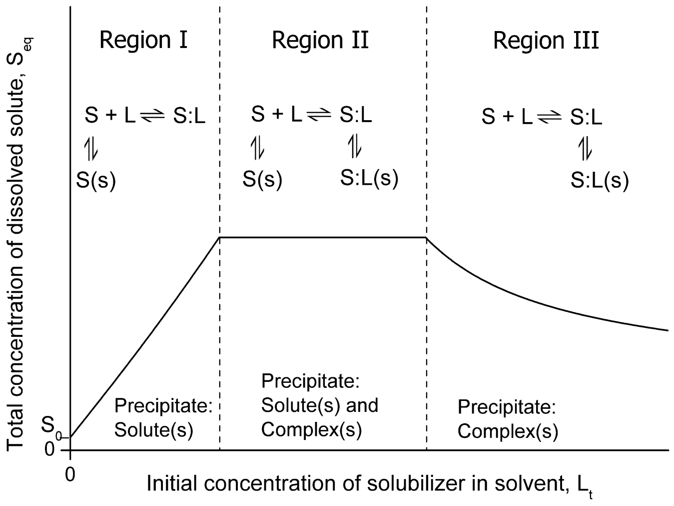

**Fig. 1.** Illustration of a $B_S$-type PS diagram. $S_0$ is the intrinsic solubility of the drug. For each of the three regions the relevant equilibria and the composition of the precipitate are shown. *S*, *L* and *S:L* denotes drug, ligand and complex in solution.

occur due to formation of complexes with a limited aqueous solubility but more recent studies suggest that formation of nano- and micrometre-sized aggregates also play an important role (Messner et al., 2011a; Saokham and Loftsson, 2017). However, a detailed mechanistic description involving aggregates has not been provided. The present work aims to fill in this gap via a thorough characterization of the system and subsequent modelling of the experimental data.

In order to understand the mechanisms that determine the shape of $B_S$-type PS diagrams we have used a novel multifaceted experimental approach combining traditional PS analysis and Isothermal Titration Calorimetry (ITC). As shown in 1965 by Higuchi and Connors, $B_S$-type PS diagrams can be analyzed to provide the stoichiometry and binding constant of the formed complexes. More modern techniques are also available to determine the binding constants, e.g. ITC which can provide precise estimates of binding constants and stoichiometries of drug: CD complexes. ITC can normally only be used in the lower concentration range as the technology requires both of the complexing species to be in solution, i.e. the drug solubility in water can define the limitation. So while ITC is very precise for the initial part of the $B_S$-type PS diagram it fails in characterizing the following parts where the species are present at higher concentrations. This underlines the importance of multiple characterization approaches for complicated PS diagrams.

Traditionally, PS diagrams are constructed for only one of the components in solution, the drug, and the total concentration of drug in solution is plotted as a function of added CD. Measuring the concentration of the complexing agent is in principle not necessary and is rarely done. However, quantifying the amount of CD in solution allows for a more detailed mechanistic picture in terms of the composition of the precipitate and the identity of the molecular species in solution. Unfortunately, CDs are not easy to quantify as they contain no chromophores or fluorophores, and researchers have resorted to other analytical techniques, e.g. measuring the refractive index (Jansook et al., 2010). Recently, a method have been developed where the concentration of CDs is determined accurately by HPLC coupled to a Charged Aerosol Detector (Saokham and Loftsson, 2015), thereby assisting the interpretation of the PS diagram. In addition to quantifying the amount of drug and γCD in solution, we also characterize the overall composition of the precipitate as well as the amount of uncomplexed drug in the precipitate. By combining the information from these complementary analytical techniques we intend to obtain a more complete picture of the molecular mechanisms that determine the features of $B_S$-type PS diagrams. Once a mechanistic model is established, it can provide a full map of the formulation space and thereby define the optimal formulation space for a compound with a $B_S$-type PS diagram, in the present case HC.

## 2. Theoretical background

The analysis of $B_S$-type phase diagrams was pioneered by Higuchi and Connors in 1965 (Higuchi and Connors, 1965), but a more exhaustive and formal mathematical treatment has later been developed which also takes various higher-order complexes into account (Zughul and Badwan, 1998, 1997; Zughul, 2007).

Complex usually refers to molecular assemblies formed by combination of substrates (*S*) and ligands (*L*), and in most cases complex formation is a reversible process. PS diagrams are typically constructed by mixing a constant amount of solid substrate (in this work HC) with a medium containing varying concentrations of solubilizer or ligand, $L_t$, (in this work γCD) and then measuring the equilibrium concentration of dissolved solute, $S_{eq}$. If one of the formed complexes has a limited solubility and starts precipitating from the solution above a given concentration of solubilizer $B_S$-type diagrams are observed. These are divided into 3 regions as sketched in Fig. 1.

**Region I**: Total concentration of solute in solution, $S_{eq}$, increases with increasing concentration of solubilizer. Precipitate consists of solid solute.

**Region II:** The solubility limit of one of the formed complexes is exceeded, and the concentrations of all species in solution are constant. Precipitate consists of solid solute and precipitated complexes.

**Region III:** All solid *S* is depleted and exists as free and complexed *S* in the solution and as complexed *S* in the precipitate. $S_{eq}$ decreases with increasing $L_t$ due to the gradual conversion of free *S* and soluble complexes to precipitated complex.

In each of the 3 regions the following mass balances apply for the molecular species in solution:

$$S_{eq} = [S] + [LS] + \cdots \tag{1}$$

$$L_{eq} = [L] + [LS] + \cdots \tag{2}$$

where the concentration of 1:1 complexes, [*LS*], is related to the concentrations of free solutes, [*S*], and hosts, [*L*], via the equilibrium constant:

$$[LS] = K_{11} \cdot [L] \cdot [S] \tag{3}$$

The ( . . . )'s in Eqs. (1) and (2) represent possible higher-order complexes, $L_xS_y$. If these are present, their concentrations are given as:

$$[L_xS_y] = K_{xy} \cdot [L]^x \cdot [S]^y \tag{4}$$

These equations pertain to each of the 3 regions but the mathematical description of the PS diagram requires additional restrictions which are specific to each region. In a standard PS diagram $S_{eq}$ is plotted versus the known initial concentration of ligand, $L_t$, in the liquid phase. In order to express $S_{eq}$ as a function of $L_t$ it is necessary to restrict some of the variables in Eqs. (1)–(4). These restrictions are specific to each of the 3 regions:

**Region I**: Solid solute is present, and the concentration of free solute, [*S*], is therefore equal to its intrinsic solubility $S_0$ (its solubility in the aqueous medium when no ligand (i.e. cyclodextrin) is present). As no host has precipitated, $L_{eq}$ is equal to the