International Journal of Pharmaceutics 469 (2014) 1–9

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Do cyclodextrins bound to dextran microspheres act as sustained delivery systems of drugs?

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 1 April 2014
Accepted 5 April 2014
Available online 12 April 2014

Paper dedicated to the 65th anniversary of "Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy, Iasi, Romania.

Keywords:
Cyclodextrin
Inclusion complex
Association constant
Liquid chromatography
Drug release

ABSTRACT

The use of cyclodextrins (CDs) for controlled delivery of drugs is largely presented in the literature. However, the question of whether CDs themselves linked to a polymeric network are able to sustain the release of drugs still persists. Here, CD immobilization within dextran microspheres is reported, and CD-dextran complexes were packed in a glass column and then, the retention time of different drugs and drug model compounds was determined by liquid chromatography. The release profiles of drugs and of drug model compounds (indole, 3-nitrophenol, *p*-hydroxybenzoic acid, diclofenac), characterized by different values of the retention time (high, moderate or low), were investigated. The release rates were quite high even for drugs that exhibit very high retention time (high association equilibrium constant). Moreover, the volume of the release fluid strongly influences the rate of drug release. As a whole, "the sink conditions" must be continuously maintained, since at each drug concentration in the release medium, equilibrium occurs between the free and the CD-bound drug.

1. Introduction

Cyclodextrins (CDs) are widely used in the pharmaceutical industry due to their specific properties to form inclusion complexes with a large variety of drugs (Misiuk and Zalewska, 2009; Xu et al., 2010; Zingone and Rubessa, 2005). Inclusion complexes improve chemical stability of drugs (Fundueanu et al., 2004), mask the drug unpleasant smell (Szejtli and Szente, 2005), and affect the release rate of drugs in physiological fluids (Bibby et al., 2000). In most cases, CDs are not used as such, but grouped with polymeric materials such as hydrogels. These hydrophilic three-dimensional networks enhance the biocompatibility of CDs and prevent dilution in the physiological medium, increasing the stability of the inclusion complex (Concheiro and Alvarez-Lorenzo, 2013).

CD linking to a polymeric network could be obtained in a single step by covalent cross-linking of the two components (Fundueanu et al., 2003) or in two steps by coupling functionalized CDs to a polymeric backbone (Yuan et al., 2013). If the access of drugs to the CD cavity is not blocked, CDs in hydrogels can still form inclusion complexes. However, values of the association equilibrium constants for drug binding to CD-hydrogels may be lower (Sreenivasan, 1997) or higher (Crini et al., 1998) than those reported for free CDs. In CD-rich hydrogels, CDs can form complexes with higher association equilibrium constants than those observed for parent CDs dispersed in an aqueous medium. In fact, the guest molecule released from one CD may interact with another empty CD that it may meet during diffusion along the hydrogel network (Concheiro and Alvarez-Lorenzo, 2013).

The formation of CD-guest molecule complexes can be monitored in aqueous solution by several methods including NMR spectroscopy (Floare et al., 2013), UV–vis spectrophotometry (Borodi et al., 2008), spectrofluorimetry (Martínez et al., 2011), polarography (Taraszewska and Piasecki, 1987), and potentiometry (Diard et al., 1985). Values of the association equilibrium constants for binding of guest molecules to α-, β- and γ-CDs are well known (Connors, 1995; Rekharsky and Inoue, 1998). In contrast, the determination of values of the association equilibrium constants

Abbreviations: CAB, cellulose acetate butyrate; CD, cyclodextrin; Dex, dextran; Dex ms, dextran microspheres; DT, dextran with standard molecular weights; ECH, epichlorohydrin; ESEM, environmental scanning electron microscopy; ISEC, inverse size exclusion chromatography; ms, microspheres; PB, phosphate buffer solution; T_R, retention time.

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http://dx.doi.org/10.1016/j.ijpharm.2014.04.016
0378-5173/

for drug binding to CDs linked to polymeric hydrogels is a difficult task, due to the insolubility of the matrix and the occurrence of non-specific interactions determined by the characteristics of three-dimensional network (*e.g.*, hydrohylic/hydrophobic balance, cross-linking degree, and cross-linker characteristics). However, values of the cumulative interactions between the guest molecules and the microgels containing covalent-linked CD were determined by inverse size exclusion chromatography (ISEC) (Fundueanu et al., 2003). The microgels in the swollen state were packed in a chromatographic column, and the elution profile of the guest molecules was evaluated. Compounds that form strong inclusion complexes with CDs display high values of the retention time while chemicals that form weak inclusion complexes display low values. The release rate of drugs from these hydrogels is mainly controlled by the dissociation of the guest molecule from CDs, followed by diffusion through the network of the hydrogel. In general, drug association to CDs and drug dissociation from CD-drug complexes are dynamic processes. For both strong and weak CD-drug complexes, these reactions are very fast with average lifetimes in the milli- to micro-second time range or shorter (Turro et al., 1982). Also, these processes occur at equilibrium. After oral or parenteral administration the main driving force for the dissociation of CD-bound drugs seems to be a simple dilution. Since the association and dissociation processes occur at fast rates and when the organism realizes permanently "sink" conditions (*i.e.*, the released drug is taken by tissues components) (Rajewski and Stella, 1996), the role of CDs on the intrinsic pharmacokinetics of drugs is openly debated. However, hydrogel platforms that release drugs rapidly or in a sustained way are well known. For example, the hydrogels with pendant β-CDs are able to sustain the delivery of diclofenac in artificial lacrimal fluid for several days, even up to 2 weeks (Rosa dos Santos et al., 2009). The high affinity of the drug for the hydrogels with pendant β-CDs impairs the drug release when a certain drug concentration in the medium is reached. In fact, as the volume of the release fluid is low, the equilibrium between the drug release and the drug re-absorption by the hydrogel occurs. However, if the content in water of the hydrogel is high and the drug molecules have no affinity for the polymer chains, the time that the network can sustain the release is quite low. For example, the release of gabexate mesylate from starch hydrogels with linked α-CDs occurs in no more than 60 min, even if the affinity is quite high. Kinetics behavior of drug release suggests that the release of gabexate mesylate appears to be controlled by the swelling and diffusion processes instead of the dissociation of the CD-drug complex (Fundueanu et al., 2004). Here, the preparation of dextran/cyclodextrin (Dex/CD) microgels by a single step procedure, based on the covalent cross-linking with epichlorohydrin, is reported. The microgels were packed in a glass column and the elution time of different drugs and drug models was determined under pseudo-physiological conditions (*i.e.*, pH 7.4). Compounds with high retention time were considered to form strong inclusion complexes with CDs while those with low retention time form weak inclusion complexes. The release of drugs or drug models with different retention properties from microgels was investigated comparatively.

2. Materials and methods

2.1. Materials

Dextran (Dex) (M_w = 150,000 g mol^{-1}) was provided from Sigma–Aldrich (St. Louis, USA). α-, β-, and γ-cyclodextrin (α-, β-, γ-CD) were provided from Roquette Frères (Lestrem, France). Cellulose acetate butyrate (CAB) was purchased from Eastman Inc. (Kingsport, Tennesse, USA). Epichlorohydrin (ECH) was supplied from Fluka AG (Buchs, Switzerland). Drugs and drug models used for chromatographic and release studies were provided from different suppliers; the chemical structures are shown in Fig. 1. As molecular weight standards, deuterated water (D_2O) and glucose (from Fluka AG, Buchs, Switzerland) were used as small probe molecules whilst dextran (DT) with different molecular weights, namely 1000, 4000, 5000, 12,000, 25,000, 50,000, 80,000, 150,000, 2,000,000 g mol^{-1} (from Pharmacia, Uppsala, Sweden) were used as high molecular weight standards. Phosphate buffer solution at pH 7.4 (PB) (50 mM NaH_2PO_4 + 39 mM NaOH) were prepared in our laboratory. All chemicals were of analytical grade.

2.2. Preparation of microspheres

Dex/CD microspheres were prepared in a glass reactor equipped with an anchor type glass stirrer, and a reflux condenser, by the suspension cross-linking method as previously reported (Fundueanu et al., 2004). Briefly, 4 g of Dex and 2 g of CD were dissolved in 20 mL of a NaOH aqueous solution (10%, w/v), in the presence of 50 mg $NaBH_4$, as the reducing agent. The solution was sonicated for deaeration and then it was emulsified in 100 mL of dispersion phase (1,2-dichloroethane) containing 2.4 g of CAB (dispersion agent). The water-in-oil emulsion was stirred for 1 h at 55 °C, thereafter 8 mL of the cross-linking agent (ECH) were added and the reaction continued for 24 h at 55 °C. The hardened microspheres were filtered through a sintered glass filter. The residuals were removed by washing the microspheres with solvents in the fixed order: 1,2-dichloroethane, acetone, aqueous acid acetic solution (30%, v/v), water, acetone. Finally, the microspheres were dried under vacuum at 55 °C. Blank sample of Dex microspheres were obtained under the same conditions.

2.3. Morphological and dimensional analysis

The size and morphology of the microspheres were evaluated by observation on optical and environmental scanning electron microscopy (ESEM). Size-based separation of microspheres was performed using a system of sieves with well determined exclusion diameter. Particularly, the fraction characterized by dimensions comprised between 60 and 125 μm was further used for the experiments.

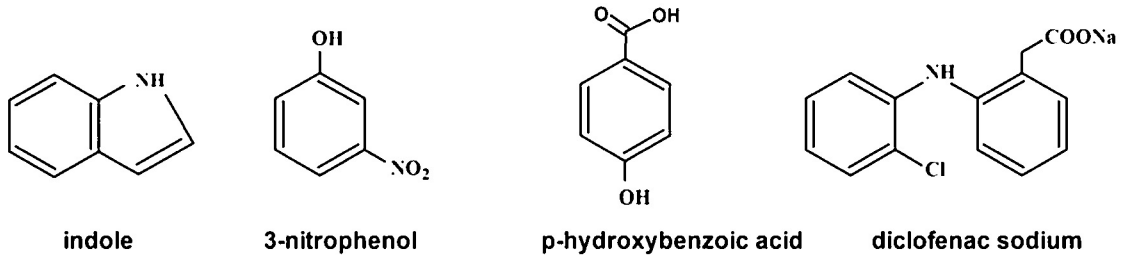

Fig. 1. Chemical structure of drugs and drug model compounds.