International Journal of Pharmaceutics 522 (2017) 98–109

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Characterisation of aggregates of cyclodextrin-drug complexes using Taylor Dispersion Analysis

Hadar Zaman[a], Andrew G. Bright[a], Kevin Adams[a,b], David M. Goodall[c,**], Robert T. Forbes[a,*]

[a] Faculty of Life Sciences, University of Bradford, Bradford BD7 1DP, UK
[b] Incanthera Ltd, 76 King Street, Manchester, M2 4NH, UK
[c] Paraytec Ltd, York House, Outgang Lane, Osbaldwick York, YO19 5UP, UK

ARTICLE INFO

Article history:
Received 9 October 2016
Received in revised form 3 February 2017
Accepted 4 February 2017
Available online 6 February 2017

Keywords:
Taylor Dispersion Analysis
Peptide prodrug
Aggregation
Cyclodextrin
Solubility enhancement
Formulation

ABSTRACT

There is a need to understand the nature of aggregation of cyclodextrins (CDs) with guest molecules in increasingly complex formulation systems. To this end an innovative application of Taylor dispersion analysis (TDA) and comparison with dynamic light scattering (DLS) have been carried out to probe the nature of ICT01-2588 (ICT-2588), a novel tumor-targeted vascular disrupting agent, in solvents including a potential buffered formulation containing 10% hydroxypropyl-β-cyclodextrin. The two hydrodynamic sizing techniques give measurement responses are that fundamentally different for aggregated solutions containing the target molecule, and the benefits of using TDA in conjunction with DLS are that systems are characterised through measurement of both mass- and z-average hydrodynamic radii. Whereas DLS measurements primarily resolve the large aggregates of ICT01-2588 in its formulation medium, methodology for TDA is described to determine the size and notably to quantify the proportion of monomers in the presence of large aggregates, and at the same time measure the formulation viscosity. Interestingly TDA and DLS have also distinguished between aggregate profiles formed using HP-β-CD samples from different suppliers. The approach is expected to be widely applicable to this important class of drug formulations where drug solubility is enhanced by cyclodextrin and other excipients.

1. Introduction

An understanding of the self-association behaviour and conformation change of a candidate drug in different environments is desirable towards its pharmaceutical development. Insight into drug-excipient interaction behaviour is also required. Rapid, cost-effective techniques using small amounts or volumes of sample that can analyse a drug in a label-free and immobiliser-free solution are of high interest. It is also desirable to be able to rapidly screen a range of formulation buffers and characterise the increasingly complex formulations that are required to enhance solubility without the need for dilution. Size-based analysis and viscosity measurements are key parameters required in the early assessment of parenteral drugs with potential for aggregate formation. Taylor dispersion analysis (TDA) is a technique for measurement of diffusion coefficient and hydrodynamic radius, named after Sir Geoffrey Taylor who developed and provided the first practical test of the theory (Taylor, 1953). There is a considerable body of literature on methodology and applications of TDA, as exemplified for single component systems (Wakeham et al., 1976; Bello et al., 1994; Sharma et al., 2005; Cottet et al., 2007a; d'Orlyé et al., 2008; Ribeiro et al., 2008) and mixtures (Kelly and Leaist, 2004; Cottet et al., 2007b, 2010). An instrument utilizing UV imaging detection at two windows for samples flowing through a capillary has been developed specifically for TDA and complementary solution viscosity measurements, and applied for characterising proteins and their formulations (Hawe et al., 2011; Hulse and Forbes, 2011a, 2011b). Very high precision (RSD <1%) is obtained in protein sizing (Paraytec, 2010; Hulse and Forbes, 2011b; Hulse et al., 2013). Use of methodology with two windows has the benefit that contributions to variance other than Taylor dispersion, e.g. those from injection, are automatically removed (Chamieh and Cottet, 2012; Ye et al., 2012; Hulse et al., 2013). The instrument has recently been used as an early developability screen of therapeutic antibody candidates

* Corresponding author. Present address: School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston, PR1 2HE, UK.
** Corresponding author.
E-mail addresses: david.goodall@paraytec.com (D.M. Goodall), rtforbes@uclan.ac.uk (R.T. Forbes).

http://dx.doi.org/10.1016/j.ijpharm.2017.02.012
0378-5173/

(Lavoisier and Schlaeppi, 2015). The authors noted that the instrumental combination of hydrodynamic radius measurements from nanolitre quantities with solution viscosity measurements allowed screening of candidates with a view to the early identification of candidates with development issues.

A number of solubilisation strategies can be used to enhance the solubility of poorly soluble drugs (Douroumis and Fahr, 2013). For anti-cancer candidate drugs a range of technologies have been trialled for targetting and/or solubilisation purposes. These include the use of liposomes (Deshpande et al., 2013), polymer micelles (Richter et al., 2010), dendrimers (Svenson and Chauhan, 2008), and cyclodextrins (Gidwani and Vyas, 2015). Cyclodextrins are often used to increase drug solubility through complex formation (Uekama et al., 1998). The ability of cyclodextrins to self-associate and the role of cyclodextrin-drug complexes and aggregates in solubilisation and delivery of drugs have been well documented in a series of papers by Loftsson and coworkers amongst others (Loftsson and Brewster, 1996, 2012; Loftsson et al., 2002, 2004; Messner et al., 2010, 2011; Jansook et al., 2010; Loftsson, 2014). The body of work showed that solubilisation and stabilisation of drugs in aqueous CD formulations are strongly influenced by other commonly used excipients. Jansook et al. (2010) were able to conclude that CD solubilisation is also affected in combination formulations containing more than one drug and that CD formulation studies should always be performed in a medium that closely resembles the final drug formulation. Given that subtle changes in the formulation buffer composition can affect solubilisation and aggregate formation, and that inclusion complexes as well as a range of sizes of aggregates can be present in aqueous complexation media, and that such compositions are concentration dependent (Messner et al., 2010), there is a need for analytical tools to characterise such systems and provide formulation fingerprints. Valente and Soderman (2014) affirm this view by pointing out that CD self-assembly is a fundamental issue that remains veiled or not completely clear in this promising field with plenty of challenges.

Physico-chemical methods used to characterise binding and aggregation in such systems include dynamic light scattering (DLS) and NMR. The use of microscopy in this field has been reviewed (He et al., 2008). Messner et al. (2010) highlighted some of the deficiencies of the various instruments including that NMR results can be difficult to interpret, especially when numerous atomic interactions are being investigated. When recently applied to the study of whether native cyclodextrins aggregate in water, three ^{1}H NMR techniques (NMR diffusometry, relaxometry, and proton peak intensity) showed that levels of large aggregates (>100 CDs) were below the detection limit of 1% (Valente et al., 2015). Regarding DLS measurements, it has been commented that R_h is obtained but not shape, it can be difficult to find acceptable laser intensity that produces reliable results, and concentrated solutions produce signal noise (Messner et al., 2010). From the above it is clear that a range of techniques and new tools are helpful in characterising such complexity.

TDA has been used to measure diffusion coefficients and hydrodynamic radii of cyclodextrins (Ribeiro et al., 2007, 2008). TDA methodology can also provide information on binding of guest molecules. With UV absorbing guest species and spectrophotometric detection, the diffusion constants of guest molecules have been investigated as a function of cyclodextrin concentration and binding curves obtained (De Azevedo et al., 2000) from which binding constants can be determined (Bielejewska et al., 2010; Jensen and Østergaard, 2010). Another approach has been to use a differential refractometer to measure mutual diffusion coefficients over a range of guest and host concentrations and fit these data (Barros et al., 2015; Filho et al., 2016).

Whilst binding can be studied in favourable cases, there have been no approaches using TDA to look at aggregation of cyclodextrin complexes. Addressing this challenge, the objective of the present work is to explore the use of a different methodological approach for TDA, alongside DLS, to provide mechanistic insight towards the nature of the solubilisation strategy for a poorly soluble drug candidate, and the aggregation behaviour of drug-CD complexes.

ICT01-2588 (Fig. 1), originally referred to as ICT2588 (Gill et al., 2008), is a novel tumor-targeted vascular disrupting agent activated by membrane-type matrix metalloproteinases (Atkinson et al., 2010; Ansari et al., 2014) which has demonstrated preclinical therapeutic activity against solid tumors and reduced potential for cardiovascular toxicity (Gill et al., 2014). In this paper we report results of TDA experiments on ICT01-2588 in the solvents dimethylsulfoxide (DMSO), methanol, and TDA alongside complementary DLS studies in a potential formulation medium Tris buffer containing 10% hydroxypropyl-β-cyclodextrin.

2. Materials and methods

2.1. Materials

ICT01-2588 was provided by Incanthera Ltd (Bradford, UK). Methanol and dimethyl sulfoxide were purchased from Fisher Scientific UK Ltd (Loughborough, UK). Tris (Tris(hydroxymethyl) aminomethane) and its hydrochloride salt (Trizma-base, Trizma-hydrochloride), fluorescein disodium salt, Hellmanex III and a first sample of (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD), product code H107, were purchased from Sigma-Aldrich Ltd (Poole, UK). This first sample of HP-β-CD was specified to have degree of substitution of 2-hydroxypropyl units 0.5-1.3 per glucose unit and in this paper is referred to as HP-β-CD-s1. A second sample of

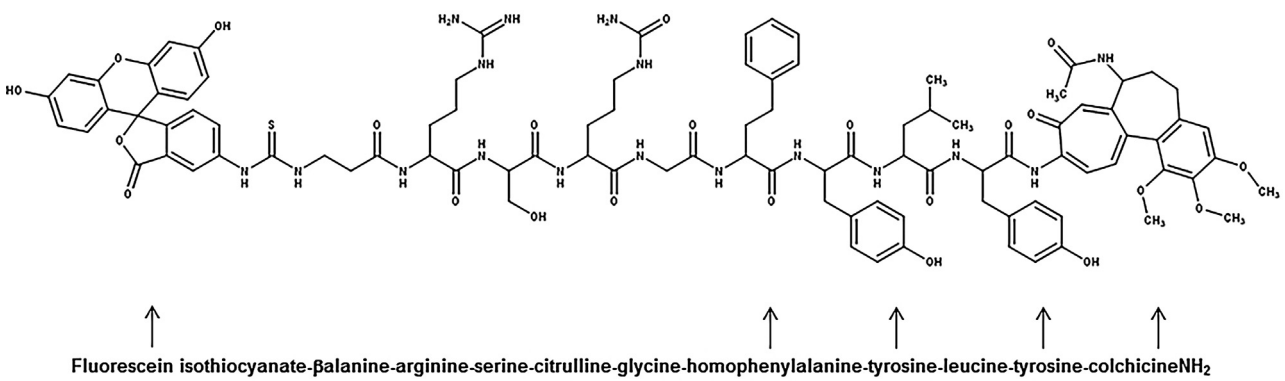

Fig. 1. Structure of ICT01-2588: in the sequence of functional groups, those with arrows are potential binding sites for cyclodextrins.