Ibuprofen-in-cyclodextrin-in-W/O/W emulsion – improving initial and long-term encapsulation efficiency of a model active ingredient

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Abstract

A challenge with formulating water-in-oil-in-water (W/O/W) emulsions is the uncontrolled release of encapsulated compound prior to application. Pharmaceuticals and nutraceuticals usually have amphipathic nature, which may contribute to leakage of the active ingredient. In the present study, cyclodextrins (CyDs) were used to impart a change in the relative polarity and size of a model compound (ibuprofen) by the formation of inclusion complexes. Various inclusion complexes (2-hydroxypropyl (HP)-β-CyD-, α-CyD- and γ-CyD-ibuprofen) were prepared and presented within W/O/W emulsions and the initial and long-term encapsulation efficiency was investigated. HP-β-CyD-ibuprofen provided the highest retention of ibuprofen in comparison to a W/O/W emulsion with unassociated ibuprofen confined within the inner water phase, with a 4 fold increase in the encapsulation efficiency. An improved, although lower encapsulation efficiency was obtained for the inclusion complex γ-CyD-ibuprofen in comparison to HP-β-CyD-ibuprofen, while α-CyD-ibuprofen had similar encapsulation efficiency as unassociated ibuprofen. The lower encapsulation efficiency of ibuprofen in combination with α-CyD and γ-CyD was attributed to a lower association constant for the γ-CyD-ibuprofen inclusion complex and the ability of α-CyD to form inclusion complexes with fatty acids. For the W/O/W emulsion prepared with HP-β-CyD-ibuprofen, the highest retention of ibuprofen was obtained at hyper- and iso-osmotic conditions and by using an excess molar ratio of CyD in comparison to ibuprofen. In the last part of the study it was suggested that the chemical modification of the HP-β-CyD molecule did not influence the encapsulation of ibuprofen, as similar encapsulation efficiency was obtained for an inclusion complex prepared with mono-1-glucose-β-CyD.

1. Introduction

Water-in-oil-in-water (W/O/W) emulsions are a type of double emulsion, consisting of water droplets in oil droplets mixed into an external water phase. The inner water phase of a W/O/W emulsion may be used as a reservoir to encapsulate active ingredients, providing a large range of potential applications. Perhaps the area of most scientific interest, which is highlighted by the number of articles, is W/O/W emulsions designed for controlled and prolonged release of pharmaceuticals and nutraceuticals (Dams & Walker, 1987; Vasiljevic, Parojcic, Primorac, & Vuleta, 2006). Double emulsion delivery systems have been tested both clinically and pre-clinically with different types of pharmaceuticals, including targeting of anti-cancer drug or delivery of vaccine adjuvants (Takahashi, Ueda, Kono, & Majima, 1976; Taylor, Miller, Pollock, Perkins, & Westwood, 1969). Another area of interest is the oral delivery of gastric-labile biopharmaceuticals, e.g. as a delivery system for insulin (Cunha, Grossiord, Puisieux, & Seiller, 1997a, 1997b). In general, it is preferred that a W/O/W emulsion based delivery system is stable during storage, with no release of the active ingredient prior to application. This has been a large scientific challenge, since W/O/W emulsions by nature are thermodynamically unstable. Different types of physical destabilization may occur, such as coalescence, creaming and flocculation of the droplets. However, the use of polymeric emulsifiers, gelling agents and Pickering stabilized emulsions have provided W/O/W emulsion-based delivery systems with enhanced macromolecular stability (Benichou, Aserin, & Garti, 2004; Frasch-Melnik, Spyropoulos, & Norton, 2010; Hattrem, Dille, Seternes, & Draget, 2014; Surh, Vladisavljevic, Mun, & McClements, 2007).

A second major challenge in regards to stability, is the uncontrolled release of active ingredient during storage, in which different mechanisms of release have been suggested: 1) coalescence (rupturing) between the inner and outer water phase, 2) formation of reverse micelles (Matsumoto, Kita, & Yonezawa, 1976), 3) thermal fluctuations causing the formation of transient small pores (Paula, Volkov, VanHoek, Haines, & Deamer, 1996; Pays, Giermanska-Kahn, Pouligny, Bibette, & Leal-Calderon, 2002) and 4) solubilisation of the active ingredient in the oil phase followed by diffusion (Matsumoto, et al., 1976). Several studies have shown that improved encapsulation may be obtained by using different sets of stabilizing agents, e.g. polymeric emulsifiers (Garti & Aserin, 1996; Su, Flanagan, & Singh, 2008). Even though high initial and long-term encapsulation efficiency is reported, an important consideration is that a highly hydrophilic model compound (e.g. simple salts or sugar alcohols) is often used as encapsulated marker. However, pharmaceuticals and nutraceuticals usually have a more complex molecular structure with both polar and non-polar moieties. The articles describing encapsulation of pharmaceuticals and nutraceuticals usually reports significantly lower initial and long term encapsulation efficiency compared to small model compounds with non-amphiphilic nature (Fukushima, Nishida, & Nakano, 1987; Hino, Shimabayashi, Tanaka, Nakano, & Okochi, 2001; O'Regan & Mulvihill, 2010; Paul, Kumar, Yedurkar, & Sawant, 2013). Preliminary studies in our lab have also shown that highly polar/ionic model compounds without hydrophobic domains/moieties are easily entrapped, while drugs with small non-polar moieties are released at a much higher rate, including ionic substances with lipophilic moieties.

Cyclodextrins (CyDs) are oligosaccharides consisting of a minimum of six α(1→4) D-(+) glucopyranose units forming a molecular structure resembling a hollow truncated cone (Szejtli, 1998). The unique molecular structure of CyDs gives the molecule a hydrophilic outer surface and a small non-polar inner cavity, which has an affinity for lipophilic entities. This molecular arrangement facilitates the formation of inclusion complexes between the CyDs and various non-polar molecular structures. The most common CyDs: α, β and γ-CyD consist of 6,7 and 8 glucopyranose units, respectively. The size of the inner cavity differs between the CyDs, with increasing size obtained as a function of the number of glucopyranose-units in the ring. This variation in the size of the inner cavity enables CyDs to form inclusion-complexes with a large range of pharmaceuticals and nutraceuticals (Szejtli, 1998). The formation of inclusion-complexes may improve the aqueous solubility of the respective guest molecule. This characteristic feature is utilized within drug formulation to improve drug dissolution in the gastrointestinal tract. Besides improving aqueous solubility, CyDs may also provide enhanced chemical stability of solubilized drugs (Loftsson & Brewster, 1996)

As described above, the presence of non-polar moieties seem to influence the encapsulation efficiency of active compounds confined within W/O/W emulsions. Thus, a viable approach to increasing the initial and long-term encapsulation would be to shield the non-polar groups of the active compound by forming inclusion complexes with CyDs. These inclusion complexes would be relatively more hydrophilic and larger in size compared to the unassociated active compound, which should assist in an increased retention of the encapsulated substance (Vasiljevic, et al., 2006). Besides potentially improving encapsulation, CyDs can be used to enhance aqueous solubility and improve chemical stability of pharmaceuticals and nutraceuticals. As both high aqueous solubility and chemical stability of the incorporated compound are highly advantageous for a successful W/O/W emulsion, CyDs should have a large potential to improve existing double emulsion delivery systems. Hence, the scope of the present paper is to investigate the potential of encapsulating an active compound as an inclusion complex and compare it to an unassociated pharmaceutical confined within a W/O/W emulsion. The sodium salt of ibuprofen was chosen as model drug, as it is well known, contains both hydrophilic and hydrophobic domains and forms inclusion complexes with CyDs.

2. Materials and methods

2.1 Materials

Alpha (α)-cyclodextrin (CyD), 2-hydroxypropyl (HP)-beta (β)-CyD and gamma (γ)-CyD produced by Wacker Fine Chemicals (Burghausen, Germany) and mono-1-glucose-β-cyclodextrin (Glu-β-CyD) obtained from Ensuiko sugar refining Co. Ltd, Japan were used in this study - information about these CyDs is listed in table 1. Ibuprofen sodium salt, chloroform (anhydrous, containing amylene as stabilizer), methyl chloroformate (MCF, 99%), methanol, sodium hydroxide, sodium sulphate, pyridine, sodium chloride (NaCl), corn oil, sodium bicarbonate and polysorbate 80 were purchased from Sigma Aldrich (Seelze, Germany). Sodium azide was supplied from BDH Laboratory Supplies Poole (BH15 1TD, England). The lipophilic emulsifier Polyglycerol Polyricinoleate (PGPR, Grindsted PGPR 90) was supplied by Danisco (Copenhagen, Denmark). All experiments were performed using deionised water (MQ-water).

Table 1. Information about the cyclodextrins used throughout this study

Scientific name	Product name	Mw (g/mol)
Alpha (α)-CyD	CAVAMAX®W6	972
(2-Hydroxypropyl)-beta (β)-CyD	CAVATEX®W7	~1400
Gamma (γ)-CyD	CAVATEX®W8	1297
Mono-1-glucose-beta (β)-CyD	Dexy Pearl	1297

2.2 Preparation of ibuprofen-cyclodextrin inclusion complex with encapsulated ibuprofen

Cyclodextrin (CyD)-ibuprofen inclusion complexes were prepared by dissolving ibuprofen sodium salt (12 mg/mL – 0.0526 mol/liter) and either α-, HP-β-, γ- or glu-β-CyD in an aqueous phase (0.02 wt.% sodium azide), at different molar ratios of CyD:ibuprofen (2:1, 1:1 or 0.5:1). All of the inclusion complexes prepared in the present study is listed in table 1. The CyD-ibuprofen complex solutions were stored for 2 days at ambient conditions prior to usage.

Table 2. Information about the ibuprofen (12 mg/ml) – cyclodextrins (with varying molar ratio) inclusion complexes prepared in the present study

Nr.	Type of CyD	Molar ratio CyD:IBU
1	HP-β-CyD	2:1
2	α-CyD	2:1
3	γ-CyD	2:1
4	HP-β-CyD	1:1
5	HP-β-CyD	0.5:1
6	Glu-β-CyD	1:1

2.3 Preparation of W/O/W emulsions and investigation of ibuprofen release during 15 days of storage

The oil phase was prepared by dissolving PGPR (10 wt.% of finalized W/O-emulsion) into corn oil (70 wt.% of finalized W/O-emulsion). A W/O-emulsion (20 wt.% aqueous phase, 80 wt.% lipid phase) was manufactured by dispersing the CyD-ibuprofen (listed in table 1) or ibuprofen solution (12 mg/mL) throughout the oil phase using a VDI 12 homogeniser (VWR International, Darmstadt, Germany) equipped with a dispersing element (type S12N-12S, VWR International, Darmstadt, Germany) at a mixing speed of 30 000 RPM at room temperature. The water phase was added continuously during mixing, at a rate of 7.5 mL/min for ~45 seconds. After completing addition of the aqueous solution, the homogenisation procedure was continued for 2 minutes. After the homogenisation, the W/O-emulsion was equilibrated at room temperature for 1 hour. The external aqueous phase was prepared by dissolving polysorbate 80 (2 wt.%) into an aqueous solution containing sodium azide (0.02 wt.%). As the encapsulated CyD-ibuprofen of the W/O/W emulsions would give rise to an osmotic pressure, the external water phases were prepared with varying amounts of sodium chloride (0.15 M and 0.075 M) or MQ-water. The W/O/W emulsions were prepared by mixing the W/O-emulsion (20 wt.%) throughout the external water phase (80 wt.%) at a mixing speed of 12 500 RPM for 3 minutes. Information about the different W/O/W emulsions prepared in this paper is highlighted in table 3. The W/O/W emulsions were stored at ambient conditions during the time span of the study.

Table 3. Different W/O/W emulsions (20 wt.% W/O-emulsion (20 wt.% inner water phase, 70 wt.% corn oil and 10 wt.% PGPR), 80 wt.% external water phase) prepared in this paper, describing type of encapsulated compound (ibuprofen or CyD-ibuprofen inclusion complex) with the corresponding amount of sodium chloride dissolved in the external water phase.

Nr	Type of CyD	Molar ratio CyD:IBU	NaCl in E.W.P.
1	HP-β-CyD	2:1	0.15 M
2	α-CyD	2:1	0.15 M
3	γ-CyD	2:1	0.15 M
4	None		0.15 M
5	None		0.075 M
6	None		MQ-water
7	HP-β-CyD	2:1	0.075 M
8	HP-β-CyD	2:1	MQ-water
10	HP-β-CyD	1:1	0.15 M
11	HP-β-CyD	0.5:1	0.15 M
12	Glu-β-CyD	1:1	0.15 M

*E.W.P. – External Water phase

All of the W/O/W emulsions listed in table 3 were hyper-osmotic, except sample nr. 7 (iso-osmotic) and sample nr. 6 and 8 (hypo-osmotic). The amount of released ibuprofen from the W/O/W emulsions described in table 3 was measured over a period of 15 days. Released ibuprofen was determined by diluting 1.50 gram of the prepared W/O/W emulsion into 10.00 gram aqueous

phase with equal sodium chloride concentration as the external water phase of the sample. The diluted W/O/W emulsion was thereafter filtrated using a 0.1 µm PALL Acrodisc 32 mm syringe filter with 0.1 µm Supor membrane® (Pall life sciences, NY, U.S.A) and the filtrated samples were derivatized and analysed using a Gas Chromatography–Mass Spectrometer (GC-MS), as further described in section 2.4.

2.4 Quantification of ibuprofen by the use of gas chromatography–mass spectrometry (GC-MS)

A modified protocol previously described by Villas-Boas and co-workers was used to prepare a derivative of ibuprofen suitable for analysis using gas chromatography–mass spectrometry (GC-MS) (Villas-Boas, Delicado, Akesson, & Nielsen, 2003). In a test tube, filtrated sample (40 µL) obtained from the encapsulation studies (see section 2.3) was mixed with sodium hydroxide (360 µL of 1 M), methanol (333 µL), and pyridine (67 µL). While mixing the content of the test tube by vortex mixing, derivatization was started by the addition of MCF (40 µL). The sample was thereafter mixed for 60 seconds, followed by the addition of chloroform (400 µL) to the test tube, and vortex mixing for an additional 10 seconds. The reaction was subsequently stopped by the addition of sodium bicarbonate (400 µL of 50 mM) to the test tube. The chloroform phase was extracted, and subsequently dried by the addition of a small amount of anhydrous sodium sulphate. Analytical standards were also prepared by performing a similar derivatization process on samples with known concentration of ibuprofen and CyD-ibuprofen.

The instrumental setup has earlier been described, however, some small modification to the existing procedure was implemented (Wentzel, Sletta, Consortium, Ellingsen, & Bruheim, 2012). The GC/MS QqQ system was an Agilent 7890A series GC coupled with an Agilent 7000B QqQ MS (EI source operated at 70 eV). The GC was equipped with an Agilent VF-5ms 30x0.25 + 5m EZ-guard (CP9012) capillary column. The data acquisition method was run at a constant pressure, and 1 µL of sample was injected in a pulsed split-less mode with an inlet temperature of 290 °C. Ibuprofen derivatives were separated by using the following temperature gradient: start at 45 °C and held for 1 min, followed by a linear gradient of 60 °C/min until 300 °C was reached, with an additional hold time of 1 min at 300 °C, giving a total run time of 6.25 min. The MS transfer line temperature was set to 300 °C. In the present study, the MS was operated in MRM mode (start after 4.5 min) with the following transitions monitored: 161.1 m/z → 91 m/z (quantifier) and 220.4 m/z → 161.1 m/z (qualifier) with collision energy of 25 eV and 5 eV, respectively. The dwell time was set to 10 ms giving a cycle time of 22 ms/cycle. Nitrogen (N2) was used as the collision gas and data was analysed using MassHunter workstation software, quantitative analysis version B.05.01 build 5.1.333.0.

From the GC-measurements, the amount of ibuprofen released to the external water phase of the W/O/W emulsions was calculated. The encapsulation efficiency of the different samples was further determined by the use of equation 1.

$$\text{Encapsulation efficiency (\%)} = \left(1 - \frac{amount\ of\ released\ ibuprofen}{amount\ of\ encapsulate\ ibuprofen}\right) * 100 \quad (1)$$

2.5 Morphological analyses of W/O/W emulsions

Morphological analyses of the W/O/W emulsions were performed using a conventional light microscope (Nikon eclipse TS 100 (Tokyo, Japan)) equipped with a Nikon ELWD 0.3/OD75 camera (Tokyo, Japan) connected to the respective software (NIS-Element F 3.0). One single droplet of diluted W/O/W emulsion (1.50 gram of sample diluted in 10.00 gram aqueous phase) was applied to a microscope slide (VWR International, Leuven, Germany) and covered with a cover glass (VWR International, Leuven, Germany). The images were recorded at a magnification of 400× (40× objective combined with a 10× magnifier lens).

2.6 Droplet size measurements of W/O/W emulsions

The droplet size distributions of the W/O/W emulsions were determined by particle size measurements using a Malvern Mastersizer 3000 (Worcestershire, UK) connected to a Hydro MV, wet dispersion unit (Malvern, Worcestershire, UK) and the data was analysed using the respective software (Mastersizer 3000, v1.0.1). The Mastersizer determines the droplet size distribution by laser diffraction. This is achieved by transforming angular intensity of the scattered light into a droplet size distribution by the use of the Mie theory. The refractive index of continuous and dispersed phase was set to 1.33 (solvent) and 1.47 (corn oil, datasheet from supplier) respectively. The absorption index of the DE was set to 0.01. Diluted W/O/W emulsion (1.50 gram of sample diluted in 10.00 gram aqueous phase) was added to the dispersion unit (containing ~125 mL water), until an obscuration of approximately 10 % was obtained.

3. Results and discussion

Sodium ibuprofen (IBU) was chosen as a model compound to investigate the potential of using cyclodextrins (CyDs) to improve encapsulation of active pharmaceutical ingredients (APIs) in a W/O/W emulsion delivery unit by presenting the active compound as an inclusion complex compared to unassociated ibuprofen. Ibuprofen is known to form inclusion complexes with α-, γ- and β-CyD, in which β-CyD-IBU is regarded as being the most stable, reflected by the high association constant reported for this system (Xing, et al., 2009). The stoichiometry of CyD-IBU inclusion complexes have been reported as 1:1 (Xing, et al., 2009), which restricts the use of traditional β-CyD in W/O/W emulsions, as the requisite of equimolar concentration and the corresponding low solubility of β-CyD (18.5 gram/liter) in water would provide a low payload (amount of IBU/gram delivery unit) of the finalized delivery unit. However, chemically modified β-CyD, e.g. 2-hydroxyropyl (HP)-β-CyD, is known to have a significantly improved aqueous solubility compared to its unmodified parent molecule. Thus, inclusion complexes were prepared between HP-β-CyD, α-CyD or γ-CyD and ibuprofen using molar excess (molar ratio of CyD:IBU of 2:1) concentration of CyD compared to ibuprofen to assure a low concentration of unassociated ibuprofen. These inclusion complexes were further encapsulated within W/O/W emulsions (section 2.3). As a control sample, a W/O/W emulsion with only ibuprofen encapsulated was prepared. For all of the prepared samples, sodium chloride (0.15 M) was used as a counter-solute, providing a hyper-osmotic W/O/W emulsion, which has been previously reported as having

improved encapsulation efficiency compared to samples prepared at iso-osmotic or hypo-osmotic conditions. The amount of released ibuprofen was measured for a period of 15 days and the resulting encapsulation efficiencies are shown in figure 1.

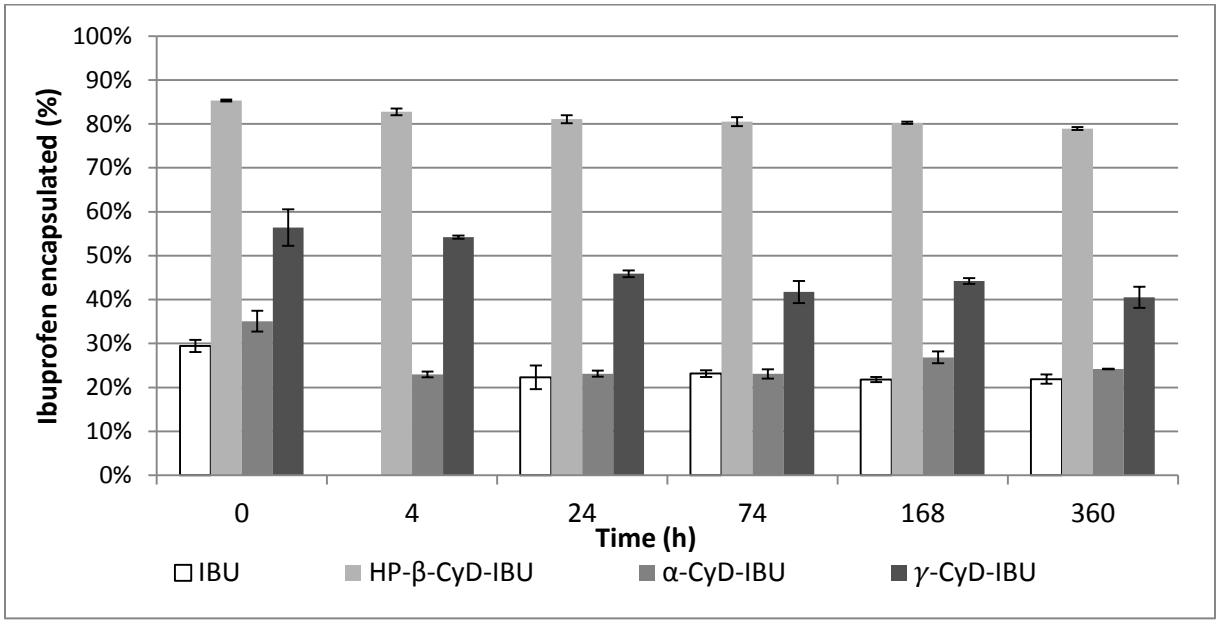

Figure 1. Measured initial and long-term encapsulation efficiencies of ibuprofen (IBU) confined within W/O/W emulsions (20 wt.% W/O-emulsion (20 wt.% inner water phase, 70 wt.% corn oil and 10 wt.% PGPR), 80 wt.% external water phase). The ibuprofen (12 mg/mL) was either presented as an inclusion complex in combination with either HP-β-, α- or γ-CyD (molar ratio of 2:1 of CyD:IBU) or as unassociated ibuprofen (IBU) within the inner water phase. For all of the prepared W/O/W emulsions, 0.15 M sodium chloride was used as a counter solute in the external water phase. The control IBU, 4h, is not reported due to experimental error. The data represent the average value of two independent replicates.

As displayed in figure 1, a significantly higher encapsulation efficiency, initially and long-term, was obtained for the W/O/W emulsions with HP-β-CyD-IBU confined within the inner water phase compared to only ibuprofen, supporting the earlier proposed hypothesis that improved retention of active ingredient can be obtained by presenting it as an inclusion complex. The increased encapsulation may be explained by two sets of mechanisms. The first possible explanation is that a reduced partition of ibuprofen in the lipid phase would be expected for the inclusion complex due to an increased overall polarity and size compared to unassociated ibuprofen. A reduced partition would further be expected to reduce diffusion of the active compound from the inner to the outer water phase, thereby improving long-term encapsulation (Vasiljevic, et al., 2006). Secondly, due to the presence of both hydrophilic and hydrophobic moieties, ibuprofen can be considered as having surface-active properties. This may provide an interaction of the ibuprofen-molecule at the lipid interface, potentially promoting droplet rupturing or mediateing in the release through the mechanisms discussed previously (reverse micelles, transient pores etc.). A decreased affinity for a

lipid bilayer has earlier been reported for an inclusion complex of CyD and nifedipine (lipophilic API) compared to the unassociated API, which highlights CyDs potential to reduce API-lipid interaction (Skalko, Brandl, BecirevicLacan, FilipovicGrcic, & Jalsenjak, 1996).

An increased retention of ibuprofen was also observed for the W/O/W emulsion with encapsulated inclusion complex of γ–CyD and ibuprofen, although to a lesser extent compared to samples prepared with HP-β-CyD. The association constant for the γ-CyD-IBU is reported to be two orders of magnitude lower compared to inclusion complexes prepared with HP-β-CyD (Castronuovo & Niccoli, 2013; Xing, et al., 2009). The lower association constant reflects a reduced stability of the inclusion complex and a higher concentration of unassociated ibuprofen. As highlighted in figure 1, unassociated ibuprofen is released rapidly, potentially explaining the lower retention of ibuprofen in combination with γ-CyD. However, for the W/O/W emulsions with encapsulated inclusion complexes of α-CyD and ibuprofen, no improvement in the encapsulation efficiency was observed compared to the control sample. The α-CyD suffers from the potential of forming inclusion complexes with fatty acids, potentially promoting association with triglycerides, PGPR and polysorbate 80. Hence, although a more stable ibuprofen-inclusion complex is reported compared to γ-CyD (Xing, et al., 2009), a competitive binding between different guest molecules may reduce the degree of association between α-CyD and ibuprofen, causing leakage of the active ingredient during preparation. As W/O/W emulsions with encapsulated HP-β-CyD-IBU provided the highest retention of active ingredient, additional studies were performed on this system to investigate the potential of further improving ibuprofen encapsulation.

In the previous section, W/O/W emulsions were prepared at hyper-osmotic conditions (0.15 M NaCl in the external water phase), in which a migration of water from the inner to outer water phase was expected. It would be of interest to investigate the W/O/W emulsion stability at varying osmotic pressure gradients, as this property is known to influence the encapsulation efficiency (Benichou, et al., 2004). The osmotic regulation of W/O/W emulsions is complicated by both the Laplace pressure of the inner droplets and non-ideal solute behavior (Hattrem, et al., 2014; Mezzenga, Folmer, & Hughes, 2004). In addition, the association of inclusion complexes would affect the osmolarity of the inner water phase. Hence, a crude estimate of the osmolarity of the inner water phase/droplets was performed, and close to iso-osmotically balanced W/O/W emulsions (HP-β-CyD-IBU encapsulated (molar ratio of 2:1 of CyD:IBU)) was prepared by the addition of sodium chloride (0.075 M NaCl) as a counter solute. These W/O/W emulsions were compared to samples prepared at hypo-osmotic (MQ-water in the external water phase) and hyper-osmotic conditions (0.15 M NaCl). The amount of released ibuprofen was measured for a period of 15 days and the resulting encapsulation efficiency are shown in figure 2. A similar osmotically adjusted study was also performed on W/O/W emulsions with unassociated ibuprofen confined within the inner water phase, however these data are not further presented as it was confirmed that the osmotic pressure gradient did not influence the initial and long-term encapsulation efficiency for these samples. In addition to the measured encapsulation yields, the corresponding droplet size (reported as D[4;3]) of both W/O/W emulsions with encapsulated ibuprofen and HP-β-CyD-IBU are shown in table 4.

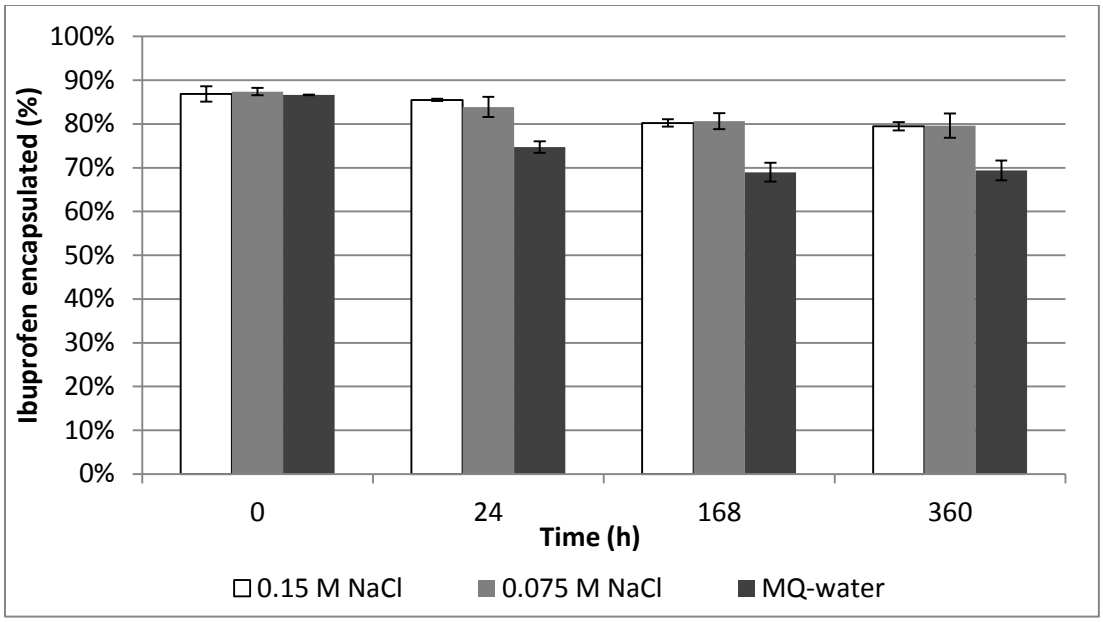

Figure 2. Measured initial and long-term encapsulation efficiencies of ibuprofen (IBU) confined within W/O/W emulsions (20 wt.% W/O-emulsion (20 wt.% inner water phase, 70 wt.% corn oil and 10 wt.% PGPR), 80 wt.% external water phase) prepared using varying amounts of sodium chloride (0.075 or 0.15 M) or MQ-water in the external water phase. The ibuprofen (12 mg/mL) was presented as an HP-β-CyD-IBU inclusion complex (molar ratio of 2:1 of CyD:IBU) within the inner water phase. The data represents the average value of two independent replicates

Table 4. Measured volume moment mean diameter (D[4,3]) for W/O/W emulsions (20 wt.% W/O-emulsion (20 wt.% inner water phase, 70 wt.% corn oil and 10 wt.% PGPR), 80 wt.% external water phase) prepared using varying amounts of NaCl as a counter solute. The ibuprofen (12 mg/mL) was presented as either an ibuprofen-HP-β-CyD inclusion complex (molar ratio of 2:1 of CyD:IBU) or unassociated ibuprofen within the inner water phase

		D[4;3] in µm - hours after preparation		
CyD used	**NaCl in E.W.P.***	0	24	360
HP-β-CyD	0.15 M	51	50	49
HP-β-CyD	0.075 M	57	56	56
HP-β-CyD	MQ-water	58	65	76
None	0.15 M	46	41	38
None	0.075 M	48	42	38
None	MQ-water	57	52	47

*External water phase

As presented in figure 2, a lower retention of ibuprofen is observed for the W/O/W emulsions without any counter solute dissolved in the external water phase (hypo-osmotic conditions). In addition to a lower encapsulation, an increase in droplet size was reported for the hypo-osmotic W/O/W emulsions with encapsulated HP-β-CyD-IBU during the time span of the study (table 4). This swelling of the droplets was expected, due to the unbalanced osmotic pressure gradient,

promoting a migration of water from the outer to the inner water phase. Similar studies performed on hypo-osmotic W/O/W emulsions have earlier reported a swelling-mediated breakdown/rupturing of the samples, contributing to release of the encapsulated substance (Hattrem, et al., 2014; JagerLezer, et al., 1997). For the present system, a similar explanation seems reasonable, as a significant decrease in the encapsulation efficiency for the hypo-osmotic samples are observed during the first 24 hours, with a corresponding increase in droplet size. An interesting observation is that regardless of osmotic pressure, the W/O/W emulsion with encapsulated unassociated ibuprofen showed a distinct decrease in droplet size during the time span of the study. This suggests that unassociated ibuprofen promotes either rupturing of the inner water droplets or migration of water by the earlier discussed mechanisms. An examination of the morphological structure was also performed (data not included), confirming the reduction in the volume of the inner water phase (fewer droplets or reduced size) for the W/O/W emulsion with only encapsulated ibuprofen. The droplet size of W/O/W emulsions with confined HP-β-CyD-IBU was however found to be stable at both iso-osmotic and hyper-osmotic conditions. This suggests that the ibuprofen promotes destabilisation of the W/O/W emulsions, while shielding of the hydrophobic moieties by the formation of inclusion complexes increases the encapsulation efficiency of ibuprofen and improves structural integrity. It should be noted, that the reduction in droplet size is larger than the expected volume of the inner water phase. Contrary to earlier reports, this may suggest that the non-uniformity of the W/O-droplets may influence the refractive index causing a potential overestimation of the initial average droplet size.

To our knowledge, W/O/W emulsions with encapsulated API-inclusion complexes have not previously been reported. However, other compartmentalized drug delivery systems, such as liposomes, have been used to encapsulate API-inclusion complexes compared to unassociated APIs. Liposomes and W/O/W emulsions have many of the same characteristics, including an inner and outer water phase and a lipophilic phase acting as a semipermeable membrane. A large range of APIs have been presented as inclusion complexes confined within liposomes in order to improve the functional properties of the delivery unit, including: ketoprofen, dehydroepiandrosterone (DHEA), retinol, indomethacin and betamethasone (H. L. Chen, Gao, Wang, & Liang, 2007; Maestrelli, Gonzalez-Rodriguez, Rabasco, & Mura, 2005; Mccormack & Gregoriadis, 1994; Piel, et al., 2006). As summarized in a recently published review article (J. Chen, et al., 2014), a slower release of the active ingredient is generally observed for liposomes with encapsulated API-inclusion complexes compared to unassociated API. However, a challenge with these systems is the competitive association between lipophilic excipients (e.g. cholesterol) and the API to interact with the inner cavity of the CyD, often resulting in depletion of API-CyD interaction, providing a fast release of the active ingredient in vivo (J. Chen, et al., 2014). This highlights an important consideration in the formulation of W/O/W emulsions with inclusion complexes, as certain excipients may reduce the applicability of the CyDs.

For the previously discussed W/O/W emulsions, a molar ratio of 2:1 of CyDs:IBU was used to prepare the inclusion complexes. This surplus of CyD was used to assure a minimal concentration of unassociated ibuprofen. However, it would be of interest to investigate the influence of the molar ratio on the encapsulation efficiency further. In addition, chemical modification of the β-CyD is known to influence its association with the guest molecule – e.g. a small reduction in the association

constant is reported between ibuprofen and HP-β-CyD compared to β-CyD (Castronuovo, et al., 2013). The presence of the propyl group at the outer surface of the HP-β-CyD molecule may also enable interaction between the CyD and the lipophilic phase potentially influencing the stability of the W/O/W emulsion. Hence, to elucidate the influence of molar ratio and type of β-CyD used, inclusion complexes of HP-β-CyD-IBU (molar ratios CyD:IBU of 2:1, 1:1 and 0.5:1) and mono-1-glucose-β-CyD-IBU (molar ratio of CyD:IBU of 1:1) were prepared. The described inclusion complexes were further encapsulated into W/O/W emulsions, and the amount of ibuprofen released was measured during a period of 15 days (figure 3).

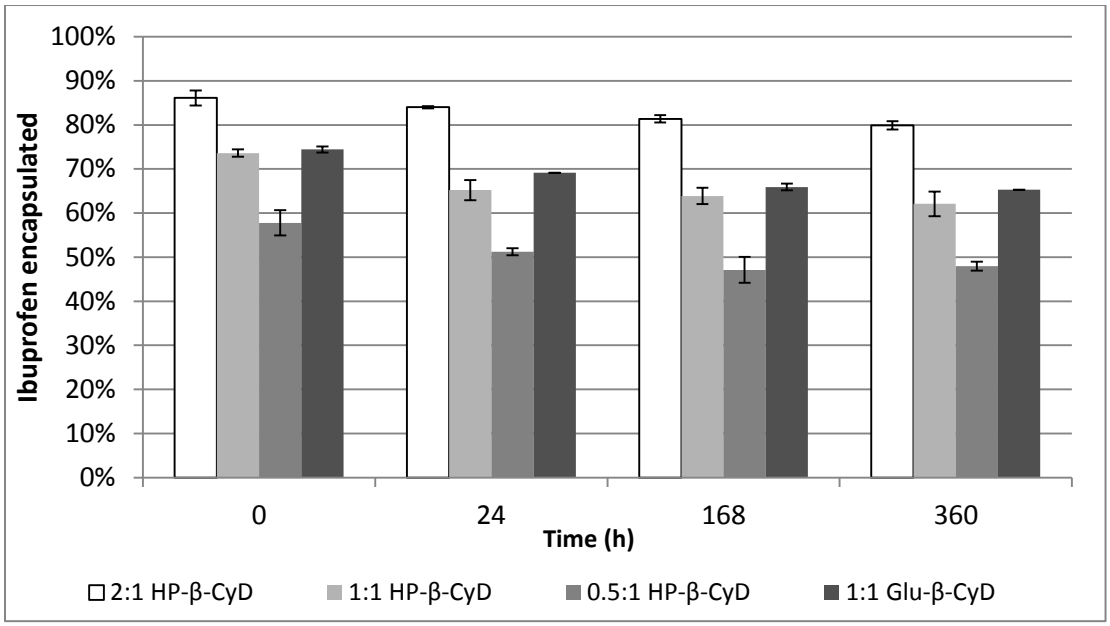

Figure 3. Measured initial and long-term encapsulation efficiencies of ibuprofen (IBU) confined within W/O/W emulsions (20 wt.% W/O-emulsion (20 wt.% inner water phase, 70 wt.% corn oil and 10 wt.% PGPR), 80 wt.% external water phase). The ibuprofen (12 mg/mL) was presented as CyD-IBU inclusion complex using either HP-β-CyD (molar ratios of 2:1, 1:1 or 0.5:1 of CyD:IBU) or mono-1-glucose-β-CyD (molar ratio of 2:1 of CyD:IBU) as host molecule. For all of the prepared W/O/W emulsions, 0.15 M NaCl was used as a counter solute in the external water phase. The data represent the average value of two independent replicates

As observed in figure 3, the molar ratio between CyD and ibuprofen had a significant impact on the encapsulation efficiency. An almost similar reduction in the initial encapsulation efficiency was observed by reducing the molar ratio from 2:1 to 1:1, as from 1:1 to 0.5:1. This highlights the potential of improving encapsulation by using molar excess concentration of CyD. As further shown in figure 3, similar encapsulation efficiency was obtained for both inclusion complex formed by HP-β-CyD and mono-1-glucose-β-CyD, indicating that type of chemical modification had minimal influence on the encapsulation properties of CyD within W/O/W emulsions. Hence even though the propyl groups may interact with the lipophilic phase, this does not seem to influence the stability of the W/O/W emulsions.

4. Conclusion

In this paper, the potential of using cyclodextrins (CyDs) to improve encapsulation of ibuprofen (IBU) within W/O/W emulsion delivery unit have been investigated by presenting the active ingredient as an inclusion complex. By encapsulation of a HP-β-CyD-IBU inclusion complex, a 4 fold increase in the encapsulation efficiency of the ibuprofen was obtained compared to unassociated ibuprofen. The increased retention of ibuprofen was attributed to the polar nature of the inclusion complex and larger size compared to the unassociated API, either providing a reduced interaction between the API and the lipophilic phase or limiting diffusion from the inner to the external water phase. This study suggest that CyDs has a large potential to improve encapsulation for a large range of nutraceuticals and pharmaceuticals encapsulated within W/O/W emulsions, as ibuprofen has similar molecular characteristics as other active ingredients – e.g. small molecular size with both hydrophilic and hydrophobic moieties. Besides improving encapsulation, CyDs can be used to improve the chemical stability of the encapsulated compound or improve its aqueous solubility – i.e. providing a higher payload. Both of these properties are of crucial importance to obtain successful W/O/W emulsion based delivery units.

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