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In vitro release of curcumin from vehicles containing alginate and cyclodextrin. Studies of curcumin and curcuminoides. XXXIII

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Combinations of cyclodextrins and alginates were used to solubilize the hydrophobic compound curcumin in aqueous vehicles intended for topical delivery. A careful selection of the excipients is necessary to achieve a sufficient release of curcumin towards a membrane of hydrophilic character, e.g. mucosa. The aim of the study was to investigate the effect of different combinations of cyclodextrins and alginates on curcumin release towards hydrophilic membranes *in vitro*. The curcumin flux through semi-permeable membranes of different molecular weight cut-off was measured to differentiate between the flux of curcumin in its uncomplexed form (restricted flux), its uncomplexed form together with its inclusion complexes (partly restricted flux) and the overall flux of curcumin-cyclodextrin complexes and uncomplexed curcumin (unrestricted flux). A high viscosity of the vehicle was expected to inhibit curcumin flux. Vehicles containing 3% alginate were found to have lower unrestricted flux than the vehicles containing 0.5% alginate independent of the type of cyclodextrin used. The results indicate that the unrestricted curcumin flux (e.g. in the case of wounded skin) is rather independent of the composition of the hydrophilic vehicle and mostly limited by the viscosity. However, partly restricted and restricted curcumin flux were found to depend on both the viscosity and the composition of the vehicles. The cyclodextrin with the demonstrated lowest solubilisation capacity (i.e. hydroxypropyl-β-cyclodextrin) resulted in the highest values of both the restricted and unrestricted curcumin flux. In conclusion, a combination of hydroxypropyl-β-cyclodextrin and propylene glycol alginate seemed to be the best choice with respect to curcumin solubility and release from the vehicle.

1. Introduction

Curcumin (Cur) is a yellow substance found in the root of *Curcuma longa* L. It is widely used as a colouring agent in food and cosmetics, and is a main constituent in curry powder (Sharma 2005). It has a potential as a pharmaceutical active compound due to its antioxidant, anti-inflammatory and anti-cancer activity (Conney 2003; Duvoix et al. 2005; Kuriakose and Sharan 2006; Kuttan et al. 2007; Li et al. 2002; Menon and Sudheer 2007; Sharma 2005). Cur is highly lipophilic and almost insoluble in water at pH values below 7 (Tønnesen 2006). The solubility is a critical parameter when it comes to bioavailability of Cur as a potential drug. The low aqueous solubility is probably the most important factor contributing to the low absorption of Cur from the gastrointestinal tract and the low bioavailability previously reported (Anand et al. 2007; Sharma 2005). Topical application of the substance therefore offers an interesting alternative in the context of inflammations, infections or cancer in surface tissue (e.g. skin, mouth).

Several studies on topically applied Cur have been published during the recent years (Fang et al. 2003; Gopinath et al. 2004; Tiyaboonchai et al. 2007). Administration of Cur to the cheek pouch of Syrian golden hamsters indicates that Cur has an inhibitory effect against oral carcinogenesis (Li et al. 2002). Inhibitory effects on tumour promotion and tumour initiation after topical administration were also demonstrated (Conney 2003). Cur has a potential as a photosensitizer in PDT, and *in vitro* phototoxicity has been reported in mammalian cells, salivary gland cells, nasopharyngeal carcinoma cells and in bacteria (Bruzell et al. 2005; Dahl et al. 1989, 1994).

A topical formulation of Cur would require modification of the viscosity, and possibly also the bioadhesivity of the preparation. The solubility in the vehicle, the release rate from the vehicle, and possible enhancement of drug penetration through membranes are also important factors in developing topical formulation of drugs with low bioavailability and low solubility (Kikwai et al. 2002). Another challenge when it comes to formulation of Cur is the stability of the compound. Cur dissolves in aqueous solutions at pH values above 7, but is then highly susceptible to hydrolytic degradation (Tønnesen 2006). Cur also degrades under exposure to light (Tønnesen 2002). Previous studies have aimed to stabilize Cur against photochemical

degradation, but this has proved to be a difficult task (Tomren et al. 2007; Tønnesen 2002; Tønnesen et al. 2002; Tønnesen 2006). Approaches to increase the solubility and hydrolytic stability of Cur in aqueous solutions have been more successful and include incorporation of Cur in micelles, cyclodextrin (CD) complexation and use of macromolecules (e.g. alginates) as solubilizing agents (Tomren et al. 2007; Tønnesen 2002; Tønnesen et al. 2002; Tønnesen 2006). Based on previous results, combinations of CDs as solubilizing and stabilizing agents and alginates which are viscosity modifying and bioadhesive, were selected as excipients in the preparation of a topical Cur formulation.

Cyclodextrins (CDs) are cone shaped sugars with a hydrophilic outer surface and a lipophilic interior. These properties enable them to interact through non-covalent interactions with poorly water-soluble substances and form inclusion complexes and non-inclusion complexes (Challa et al. 2005; Gabelica et al. 2002; Loftsson et al. 2003; Polyakov et al. 2004). CD complexation of a lipophilic drug molecule will change some of the physiochemical properties of the drug (e.g. aqueous solubility, stability) without affecting the intrinsic ability to permeate lipophilic biological membranes. Lipophilic Cur will probably have a low affinity towards a hydrophilic layer. This will inhibit membrane permeability when the rate-limiting step is penetration through the unstirred water layer/the hydrophilic mucus layer of a membrane (Masson et al. 1999). However, complexation with CDs can both increase and decrease the drug permeability through a membrane. Under unfavourable conditions i.e. when the amount of CD is too high, a decrease in the drug flux might be observed (Accili et al. 2004; Jarho et al. 1996; Loftsson and Brewster 1996; Loftsson 1998; Loftsson et al. 1999; Loftsson et al. 2002b; Masson et al. 1999).

Alginates are a natural, negatively charged, water-soluble polymers obtained from brown algae (Phaeophyceae) or from bacterial fermentation (Moe et al. 1995). Alginic acid is a linear polymer consisting of L-guluronic acid and D-mannuronic acid. The polymers are biocompatible, mucoadhesive and have the ability to stabilize aqueous systems and to give drug formulations mechanical strength and flexibility through the increased viscosity and gelling properties (Levy 1961; Moe et al. 1995; Tønnesen and Karlsen 2002). Alginates are available as different modifications and salts. Sodium alginate (SA) is probably most frequently investigated, and both SA and propylene glycol alginate (PGA) are used in the present study.

The purpose of the present study was to investigate the effects of CDs combined with alginates on Cur permeability through semi-permeable membranes of different molecular weight cut-offs (MWCO) in an effort to predict the optimal combination of the two excipients in a topical Cur formulation.

2. Investigations, results and discussion

2.1. Cur solubility studies in ethanolic CD solution (without alginate)

The phase-solubility diagrams of curcumin (Cur) as a function of an increasing concentration of hydroxypropyl-β-cyclodextrin (HPβCD) and hydroxypropyl-γ-cyclodextrin (HPγCD) are presented in Figs. 1 and 2. The studies were carried out in alginate-free solutions because the increased viscosity complicated the filtration of the solutions prior to quantification. The studies were performed

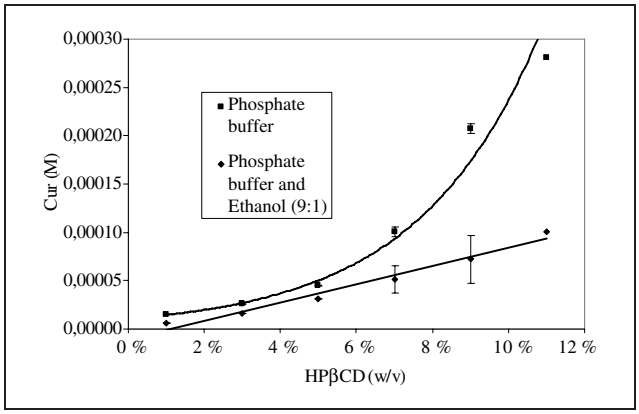

Fig. 1: Phase-solubility diagram of Cur as a function of an increasing HPβCD concentration. *Error bars show ± SD (n ≥ 3)

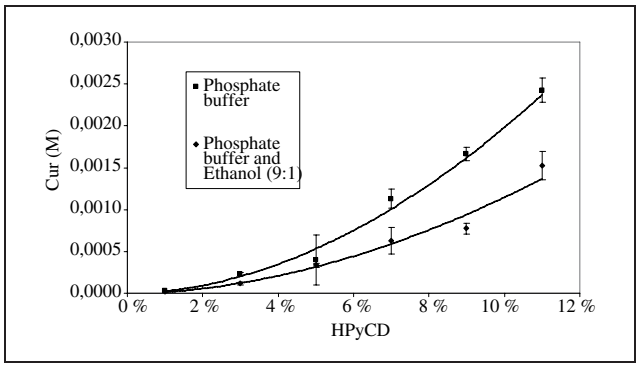

Fig. 2: Phase-solubility diagrams of Cur as a function of an increasing HPγCD concentration. *Error bars show ± SD (n ≥ 3)

to illustrate how the medium affects the CD-Cur complexation and to investigate the solubilization capacity of the selected CDs under the given conditions.

The intrinsic solubility of Cur in phosphate buffer : ethanol (9 : 1) was determined as 3×10^{-7} M (n = 3). The intrinsic solubility of Cur in plain phosphate buffer was below the detection limit of the HPLC system, which has previously been determined to be 3×10^{-8} M (Tønnesen 2002). This value was taken as the solubility in plain buffer. Figures 1 and 2 show that HPγCD has a higher solubilizing effect on Cur than HPβCD. The highest Cur concentration obtained in the present study was 2.4×10^{-3} M as detected in 11% (w/v) HPγCD in phosphate buffer pH 5. The results are slightly different from what is reported previously, e.g. 3.82×10^{-4}M and 1.22×10^{-4} M in 11% HPγCD and HPβCD, respectively (Baglole et al. 2005; Tomren et al. 2007; Tønnesen et al. 2002). The Cur concentration in 10% HPγCD and HPβCD has previously been dermined to be 5.35×10^{-3} M and 1.16×10^{-4} M, respectively, under slightly different conditions (Tomren et al. 2007). The observed differences in solubility may be ascribed to differences in buffer type, ionic strength, crystal form of Cur and degree of substitution of the CD.

The phase-solubility studies of Cur in CD solutions show that the medium affects the stoechiometry of the CD-Cur complexes. This observation is supported by previous studies on Cur-CD complexation. A 2 : 1 host-guest inclusion complex was proposed in distilled water (Baglole et al. 2005) while a 1 : 1 stoechiometry was found in phosphate buffer (Tønnesen et al. 2002). Fig. 1 shows that the phase-solubility diagram of Cur in HPβCD/phosphate buffer : ethanol (9 : 1) is close to linear ($R^2 = 0.97$) and has a slope less than one which indicates a 1 : 1 stoechiometry.

The diagram is, however, different from the phase-solubility diagram obtained in HPβCD/phosphate buffer without ethanol which is of the Ap-type (Higuchi and Connors 1965). The Ap-type of diagram has a positive deviation from linearity and indicates a higher order of complexation between the host and the guest molecule, i.e. a 2 : 1 or 3 : 1 stoechiometri between HPβCD and Cur. Figure 2 shows that in samples containing HPγCD, the Ap-type of phase-solubility diagram is detected both in the presence and absence of ethanol. In both systems the presence of ethanol decreases the overall solubilizing effect of CD. This effect is especially pronounced in samples containing >7% of HPβCD. Alcohol co-solvent can disrupt the Cur-CD complex through competitive binding with the CD cavity, and to a lesser extent increase the bulk hydrophobicity (Huang et al. 1992). On the other hand, alcohols can enhance the stability of inclusion complexes by formation of higher-order complexes. These two effects are governed by the cavity size and size of the guest molecule, and the type and concentration of the alcohol. In this particular case the effect of ethanol is probably caused by competitive binding and displacement of Cur in the CD cavity (Loftsson et al. 1999). The effect of co-solvents on the Cur-CD complexation is under further investigation in our laboratory.

2.2. Cur release studies

The permeation profiles were linear as a function of time in all the systems tested indicating that the diffusion was not limited by the capacity of the membranes. The selected membranes are size-exclusion membranes, where molecules permeate through pores in the membrane. The fundamental equation describing passive diffusion of a drug molecule through a porous membrane is based on Fick's first law of diffusion (Williams 2003):

$$J = (D/\delta)(C_1 - C_2) \quad (1)$$

where the flux (J, μg/cm²/h) is the amount of drug that passes through a unit area per unit time, D is the diffusion coefficient of the drug, C_1-C_2 is the concentration difference between the donor phase (i.e. vehicle, C_1) and the receptor phase (C_2) and δ is the effective thickness of the membrane. Sink-conditions were kept during the experiments and $C_1-C_2 \sim C_1$. Hydrophilic membranes were used to simulate the release of Cur towards a hydrophilic surface (e.g. mucosa). In the present study, all formulations (Table 1) are solutions that contain a fixed Cur concentration. When the drug concentration is kept constant and below saturation, the flux is expected to decrease with an increasing CD concentration assumed that only the free drug molecule can diffuse through the membrane (Loftsson et al. 2002b). The Cur solubility studies in CD solutions without alginate indicated that different Cur-CD complexes might be formed dependent on the solvent (Figs. 1 and 2). Previous studies suggest that drug/CD complexes can self-associate to form water-soluble non-inclusion complexes (Gabelica et al. 2002; Loftsson et al. 2002a). The system becomes even more complicated by addition of co-solvents and macromolecules.

Consequently, it is difficult to predict the stoechiometry of CD-Cur-complexes in the actual formulations which contain alginate, buffer and ethanol in addition to CD.

The following CD-Cur equilibria are postulated:

$$[CD_{free}] + [Cur_{free}] \leftrightarrow [Cur_{incl}] \leftrightarrow [Cur_n : CD_x] \quad (2)$$

where CD_{free} and Cur_{free} are the uncomplexed form of CD and Cur, respectively. Cur_{incl} is the inclusion complex between Cur and CD and $Cur_n : CD_x$ denotes non-inclusion complexes where n and × are the numbers of Cur molecules and CD molecules, respectively. Alginate can stabilize CD-complexes (Loftsson 1998) and Cur_{free}, Cur_{incl} and $Cur_n : CD_x$ can possibly form complexes with alginate. Complexes with alginate are, however, not included in Eq. (2). Membranes with various pore sizes were selected to study the formation of different CD-Cur-complexes (without knowing the exact stoechiometry) as a function of concentration and type of alginate and CD.

HPβCD has an outer diameter of 2 nm and HPγCD is slightly larger (Loftsson et al. 2002b). A membrane with sufficiently large pore size will probably allow both non-inclusion complexes and inclusion complexes between Cur and CD to cross the membrane. The postulated flux through the polypropylene membrane with pore size 0.45 μm is therefore expressed as:

$$J_{PP} = J_1(Cur_{free}) + J_2(Cur_{incl}) + J_3(Cur_n : CD_x) \quad (3)$$

where J_{PP} is the overall Cur flux, $J_1(Cur_{free})$ is the flux of uncomplexed molecules of Cur, $J_2(Cur_{incl})$ is the flux of Cur inclusion complexes and $J_3(Cur_n : CD_x)$ is the flux of the non-inclusion complexes between Cur and CD. In addition to this membrane, two cellulose membranes with a smaller pore size were selected. The cellulose membrane with MWCO 6–8000 Da was used to study the partly restricted release of Cur, i.e. the flux of $Cur_{incl} + Cur_{free}$, assuming that non-inclusion complexes are too large to pass. A similar cellulose membrane with MWCO 1000 Da was used to study the flux of Cur in the uncomplexed form i.e. the flux of Cur_{free} (see Table 2 for details). Cellulose membranes with MWCO 500 and 1000 Da are previously shown to be almost impermeable to β-CD (Ono et al. 1999) and it is therefore assumed that HPγCD, HPβCD and their complexes with Cur do not cross the cellulose membrane with MWCO 1000 Da. None of the membranes were permeable to alginate (data not shown). Finally, a polypropylene membrane containing mucin was prepared in order to study tentative interactions between Cur and mucin. A summary of the membranes, the membrane characteristics and their postulated restrictive effect on Cur release are presented in Table 2. Due to the low aqueous solubility of Cur in the absence of CD it was difficult to determine the flux of Cur without CD in the

Table 1: Formulation composition

		Sodium alginate		Propylene glycol alginate	
		0.5%	3%	0.5%	3%
HPγCD (w/v)	3%	Formulation 1	Formulation 2	Formulation 3	Formulation 4
	10%			Formulation 5	
HPβCD (w/v)	3%	Formulation A	Formulation B	Formulation C	Formulation D
	10%	Formulation E	Formulation F		

Table 2: Summary of the membranes, the membrane characteristics and their postulated restrictive effect on Cur release

Postulated Cur flux	Postulated observations	Membrane	Pore size	Thickness (μm)	Properties
Unrestricted flux = J_{PP}	Free diffusion of Cur_{free}, Cur_{incl} and $Cur_n : CD_x$	GH Polypro®	0.45 μm	114 μm	Hydrophilic polypropylene
Partly restricted flux = $J_1 + J_2$	Free diffusion of Cur_{free} and Cur_{incl}	Spectra/Por® 1	MWCO 6-8000 Da	30–50 μm	Regenerated cellulose Uncharged Hydrophilic Isotropic
Restricted flux = J_2	Free diffusion of Cur_{free}	Spectra/Por® 7	MWCO 1000 Da	60–65 μm	Regenerated cellulose Uncharged Hydrophilic Isotropic "Wet-membrane"
Unrestricted flux = J_{PP}	Free diffusion of Cur_{free}, Cur_{incl} and $Cur_n : CD_x$	GH Polypro® prepared with mucin	0.45 μm	>114 μm	Hydrophilic polypropylene with mucin

Abbreviations: Cur_{free} = uncomplexed Cur
Cur_{incl} = Cur inclusion complex
$Cur_n : CD_x$ = Cur non-inclusion complex
MWCO = molecular weight cut-off

donor phase. It was also difficult to compare results from different filters due to differences in effective filter thickness and number of pores per unit surface area, the latter which is unknown.

2.2.1. Unrestricted Cur flux (J_{PP}) from vehicles containing 3% CD in combination with 0.5% or 3% alginate

Figure 3 presents the Cur flux from vehicles containing 3% CD and 0.5 or 3% alginate, respectively through the membrane with the largest pore size (0.45 μm). The observed flux (J_{PP}) is postulated to include both free and complexed Cur according to Eq. (3).
Formulations containing 3% alginate have a lower J_{PP} compared to the formulations containing 0.5% alginate, the former being much more viscous than the latter. The consistency coefficient (K) for selected formulations are presented in Table 3 as an expression of the viscosity. The relationship between shear stress (τ) and shear rate ($\dot{\gamma}$) according to the power law representation is given by:

$$\tau = K\dot{\gamma}^n \quad (4)$$

where K is the consistency coefficient and n is the flow behaviour index. This equation can be differentiated to give the dynamic viscosity, η, thus:

$$\eta = \frac{d\tau}{d\dot{\gamma}} = nK\dot{\gamma}^{n-1} \quad (5)$$

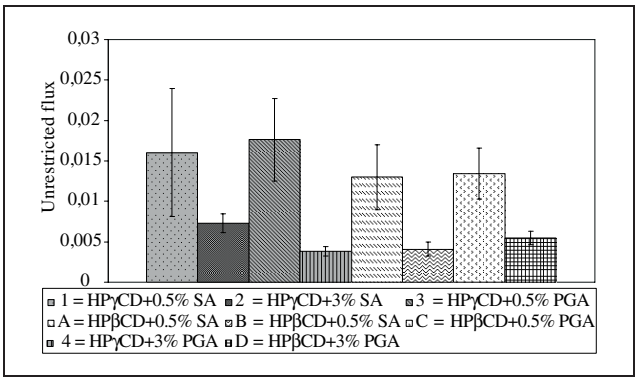

Fig. 3: The unrestricted Cur flux (J_{PP}, μg/cm²/h) through a polypropylene membrane (0.45 μm) at steady-state. Error bars show ± SD. CD concentration is 3% (w/v)

There is proportionality between K and η at constant shear rate.
The diffusion coefficient (D) (Eq. (1)) is inversely proportional to the viscosity of the diffusion medium (vehicle) according to the Stokes-Einstein equation (Florence and Attwood 1998). There is no significant difference in J_{PP} between formulations containing 0.5% alginate or between formulations containing 3% alginate, independent of type of CD and alginate. A hydrophilic membrane with such a large pore size will act as an unstirred water layer, i.e. the main barrier in a diffusion controlled membrane penetration *in vivo* (Loftsson et al. 2006a, b). The results indicate that the unrestricted Cur flux (e.g. in the case of wounded skin) is independent of the composition of the hydrophilic vehicle and mostly limited by the viscosity in case of preparations with Cur concentrations below saturation. However, in Cur saturated systems the type and amount of CD will be of importance as e.g. HPγCD can solubilize approximately 10 times more Cur than HPβCD, thereby enhancing the permeation through an aqueous diffusion layer (Loftsson et al. 2006a, b). A small increase in Cur

Table 3: Consistency coefficient (K) and Flow behaviour index (n) expressed as an average value of 3 replicates (± SD) of selected formulations

	K (Pa · s^n)	n (–)
3% HPγCD and 0.5% SA (Formulation 1)	0.019 (± 0.001)	0.975 (± 0.003)
3% HPγCD and 3% SA (Formulation 2)	1.585 (± 0.123)	0.819 (± 0.007)
3% HPγCD and 0.5% PGA (Formulation 3)	0.048 (± 0.024)	0.881 (± 0.013)
3% HPγCD and 3% PGA* (Formulation 4)	16.73 (± 4.316)	0.495 (± 0.015)
3% HPβCD and 0.5% SA (Formulation A)	0.020 (± 0.001)	0.984 (± 0.027)
3% HPβCD and 3% SA (Formulation B)	2.038 (± 0.061)	0.806 (± 0.003)
3% HPβCD and 0.5% PGA (Formulation C)	0.043 (± 0.004)	0.918 (± 0.007)
3% HPβCD and 3% PGA (Formulation D)	20.26 (± 5.517)	0.475 (± 0.028)

* Only two parallells

flux was observed by an increase in HPβCD concentration to 10% in non-saturated samples containing SA (Formulations E and F Table 1; data not shown), but the increase was not significant.

2.2.2. Partly restricted Cur flux from vehicles containing 3% CD in combination with 0.5% or 3% alginate

Figure 4 presents the Cur flux from vehicles containing 3% CD and 0.5 or 3% alginate, respectively, through the membrane with MWCO 6–8000 Da. The observed flux (Table 2) is postulated to include both free Cur and Cur-CD complexes, but to exclude non-inclusion complexes.

In general, the partly restricted Cur flux of formulations containing HPβCD (Formulations A–D) was markedly higher than the partly restricted Cur flux of formulations containing HPγCD (Formulations 1–4). The molecular weights of HPγCD and HPβCD are quite similar, reported by the producer to be 1576 and 1380–1500 Da, respectively. The observed differences in the Cur flux might therefore be caused by interactions between Cur-CD and alginate, and differences in Cur_{incl} stochiometry rather than differences in molecular weight of the CD. Only small, but significant differences in the partly restricted flux from formulations containing HPβCD were observed even though the formulations have quit different viscosities (Table 3). Formulation A shows a significantly lower flux than Formulations C and D, which both contain PGA. It seems that the amount and type of alginate have limited influence on the partly restricted flux from these formulations in this model. Viscosity independent drug flux has previously been reported (Loftsson et al. 2002b) on a cellophane membrane with a MWCO 500. The alginate amount and to a lesser extent the type, seem however, to affect the partly restricted flux from formulations containing HPγCD (Formulations 1–4). The formulations containing 3% alginate have a significantly lower partly restricted flux than the corresponding formulations containing only 0.5% alginate, consistent with the differences in viscosity (Table 3). The difference between the partly restricted flux from formulations containing 0.5% alginate in combination with HPγCD, i.e. Formulations 1 and 3, could also be ascribed to differences in viscosity. By a further increase in CD concentration to 10% the flux was reduced compared to 3% CD (Formulations 5 and E, Table 1; data not shown), but the decrease in flux was only significant in case of HPβCD. This is consistent with previous observations (Loftsson et al. 2006b).

2.2.3. Restricted Cur flux from vehicles containing 3% CD in combination with 0.5% or 3% alginate

Figure 5 presents the Cur flux from vehicles containing 3% CD and 0.5 or 3% alginate respectively, through the membrane with MWCO 1000 Da. The observed flux (Table 2) is postulated to include only free Cur and to exclude Cur-CD complexes and non-inclusion complexes.

The results emphasize the importance of the type of CD as the flow becomes more restricted. The formulations containing HPγCD (Formulations 1–4) show significant lower flux than formulations containing HPβCD (Formulations A–D), independent of type and amount of alginate. This is consistent with the results obtained in the partly restricted flow model above. A possible explanation for the observed differences in the restricted flux between samples containing HPγCD and HPβCD is the difference in the complex binding constant between Cur and the two CDs. The apparent stability constant was not determined from the phase solubility diagrams because of non-linear curves. The difference in slope between the solubility curves (Figs. 1 and 2) does, however, give an indication of the relative affinity for the different CD derivatives. Cur may have approximately a 10-fold higher affinity for HPγCD compared to HPβCD. All the samples are unsaturated with respect to Cur. A higher Cur-CD complex binding constant will reduce the equilibrium concentration of free Cur in the sample, and thereby the concentration gradient which is the driving force in the restricted diffusion process (Eq. (1)). The alginate amount and type also seem to affect the restricted flux from the formulations. The formulations containing 3% alginate have a lower restricted flux than the corresponding formulations containing only 0.5% alginate, consistent with the differences in viscosity (Table 3). The difference is significant in the case of PGA (Formulations 1, 3 and A, C vs Formulations 4 and B, D). Alginates can solubilize Cur and interact with Cur via intermolecular H-bonds. This interaction leads to the formation of a gel above certain Cur-concentrations in the presence of both SA and PGA (Tønnesen 2006). SA has apparently a better solubilizing effect on Cur than PGA, at least at a 0.5% concentration (Tønnesen 2006). A lower affinity for the vehicle polymer combined with a lower CD-complex binding constant might partly explain the unexpected high flow of free Cur from Formulation C (Lee and Lin 2002).

The results indicate that the restricted Cur flux (e.g. from a vehicle through a viscous, thick mucus diffusion barrier adjacent to a membrane) is dependent of the composition of the hydrophilic vehicle. The type of CD (i.e. the complex binding constant) is of major importance in non-satu-

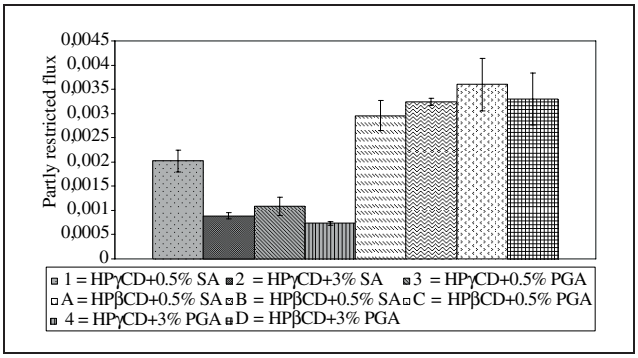

Fig. 4: The partly restricted Cur flux (μg/cm²/h) through a cellulose membrane (MWCO 6-8000Da) at steady-state. Error bars show ± SD. CD concentration is 3% (w/v)

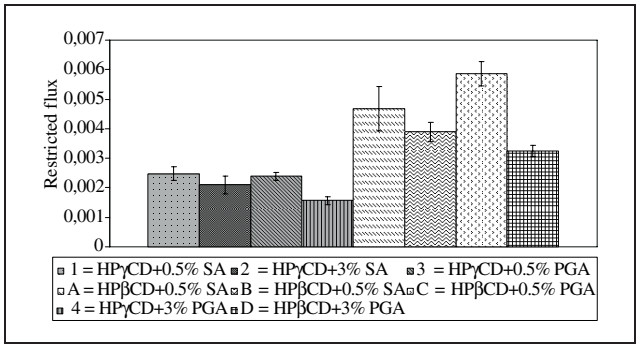

Fig. 5: The flux of uncomplexed Cur (μg/cm²/h) through a cellulose membrane (MWCO 1000Da) at steady-state. Error bars show ± SD. CD concentration is 3% (w/v)

rated systems of a highly lipophilic substance like Cur. A CD with a sufficient but not too high solubilizing capacity should be selected in this case. The flux might be enhanced by selection of a polymer with a sufficient but not too high solubilizing capacity as a viscosity modifying agent. In the case of Cur a combination of HPβCD and PGA seemed to be the best choice.

2.2.4. Unrestricted Cur flux (J_{PP}) on a membrane covered with mucin

Mucus consists of a complex viscoelastic mixture of mucins, lipids, bacteria, proteins, electrolytes and cells, which may interact with drugs and reduce absorption over a membrane (Khanvilkar et al. 2001). Mucin is the main glycoprotein in mucus, having the potential to interact with drugs. Interaction between Cur and proteins might be expected, as binding between Cur and gelatine and various positively charged amines has been observed (Tønnesen 2006). Mucin has, however, an overall negative charge due to the content of sialic acid, but contains amines which might affect the release of Cur. An experiment with dried mucin covering a polypropylene membrane was thus performed.

Only formulations containing 0.5% alginate were tested (Formulations 1, 3, A and C respectively). J_{PP} through the polypropylene membrane covered with mucin was similar to J_{PP} through an untreated polypropylene membrane (results not shown). The similar unrestricted Cur flux through an untreated membrane and a membrane covered in mucin shows that the flux is unaffected by the presence of mucin *in vitro*. Although these results cannot exclude any interactions between Cur and mucin affecting Cur release *in vivo*, the test indicates a low probability of such an interaction.

2.3. Stability studies

2.3.1. Physical appearance of the formulations

A gel-like structure was observed in Formulations 1 and A (0.5% SA) after storage in the dark for one month. The other formulations listed in Table 1 (except Formulations E and F which were not tested) appeared physically stable after storage.

2.3.2. Cur stability after storage

The Cur concentration in selected formulations after storage was evaluated by the absorbance at 500nm. The major hydrolytic degradation products of Cur (feruloyl methane, ferulic acid and vanillin), do not absorb at 500nm and are unlikely to interfere with the absorption measurements (Tønnesen et al. 2002). A significant decrease in Cur absorbance ($\leq$16%) was detected in three of the samples after storage (i.e. Formulations 1, A, C). Further HPLC analysis of a filtered and diluted sample of Formulation 1 did not confirm the presence of any degradation products. Cur has previously been demonstrated to have a reasonably hydrolytic stability at pH 5 in a 10% HPβCD-solution with a half life of >100 h (Tomren et al. 2007). The degradation rate of Cur depends however, on the degree of protection by the CD and on the pH of the solution. It has previously been observed that the degradation rate of Cur at pH 10 in HPγCD is significantly higher than in HPβCD (Tomren et al. 2007). Further data on the stability of selected formulations need to be obtained in future product development.

Table 4: Effect of storage on the consistency coefficient (K) (average ± SD, n = 3) for selected formulations

	Fresh K ($Pa \cdot s^n$)	Stored 1 month K ($Pa \cdot s^n$)
3% HPγCD and 0.5% SA (Formulation 1)	0.019 ± 0.001	0.024 ± 0.005
3% HPγCD and 0.5% PGA (Formulation 3)	0.048 ± 0.024	0.033 ± 0.009
3% HPβCD and 0.5% SA (Formulation A)	0.020 ± 0.001	0.018 ± 0.002
3% HPβCD and 0.5% PGA (Formulation C)	0.043 ± 0.004	0.025 ± 0.001

2.3.3. Vehicle stability – Effect of storage on shear viscosity

Table 4 presents the mean consistency coefficient (K) as an expression of the viscosity measured on freshly prepared Formulations 1, 3, A and C and on the same batches after one month of storage in the dark at ambient temperature.

No apparent difference in K after one month storage was detected in Formulations A, 1 and 3, which indicates stable vehicles. A significant decrease in viscosity was detected in Formulation C, which indicates a degradation of the PGA network. The reduction in the viscosity of alginate solutions reflect the chain scission of the polymer chains (Levy 1961; Moe et al. 1995). The glycoside linkages are susceptible to both acid and alkaline degradation and oxidation by free radicals. The degree of chain scission is dependent on pH with a minimum degradation rate around neutrality. At pH values less than 5, alginates are susceptible to a proton-catalyzed hydrolysis, which is dependent on time, pH, and temperature (Tønnesen and Karlsen 2002). All the selected formulations in this study contain phosphate buffer (pH 5). The expected degradation by proton-catalyzed hydrolysis was only detected to a smaller extent in Formulations C by the use of rheological measurements.

3. Experimental

3.1. Materials

Cur was synthesized according to the method of Pabon (1964). Two types of alginate; sodium alginate (Protanal LF10/60 LS, lot number s17261), and propylene glycol alginate (Protanal ester SD-LB, lot no. SLP3908) were generously provided by FMC Biopolymers, Drammen, Norway. The cyclodextrins were purchased from Wacker Chemie AG, München, Germany and were as follows: 2-hydroxypropyl-beta-cyclodextrin (Cavasol® W7 HP, Mw∼1380–1500) and 2-hydroxypropyl-γ-cyclodextrin (Cavasol® W8 HP, Mw∼1576). Mucin from porcine stomach, type II with 1% bound sialic acid was delivered by Sigma-Aldrich. The phosphate buffer of pH 5 (0.05 M) was prepared in distilled water from sodium dihydrogen phosphate and disodium hydrogen phosphate. Sodium chloride was used to adjust the ionic strength ($\mu = 0.085$). The mobile phase for detection in HPLC was prepared from acetonitrile (4 parts) and 0.5% citric acid pH 3 (6 parts). The citric acid was prepared from citric acid monohydrate and adjusted to pH 3 with a 10% potassium hydroxide solution in distilled water. All reagents were of analytical grade.

3.2. Release studies

The permeability of Cur from the various formulations (Table 1) through selected membranes (Table 2) was studied using Franz diffusion cells (PermeGear, Hellertown, PA, USA). The diffusions area was 1 cm^2 and the receptor volume was 8 ml. The formulations (donors) were prepared the day prior to the release experiment as described below. The receptor chamber was filled with 8 ml of CD-solution in phosphate buffer : ethanol (9 : 1) were the CD concentration was equivalent to the donor sample (i.e. 3% or 10% respectively). The membrane was mounted between the donor and the recep-

tor chamber. The actual formulation (1.5 ml) was applied on to the membrane and the sample holder was covered with aluminium foil. The receptor solution was continuously stirred at speed 4 (KIKA® WERKE magnetic stirrer). Samples of 200 µl were withdrawn through the sampling port after 2, 3, 4, 5 and 6 hours (polypropylene membrane/polypropylene membrane with mucin) or after 5, 6, 7, 8, 9, and 10 hours (cellulose membranes) and diluted to a total volume of 400 µl in ethanol. The volume withdrawn from the cell was immediately replaced with a corresponding CD-solution. Sink-conditions were kept during the experiment and the temperature was 21 ± 1 °C. The amount of Cur in the receptor phase was quantified by HPLC as previously described (Tønnesen et al. 2002). The system was equipped with a Nova Pak® C18 column (Waters, Milford MA). The detection wavelength was 420 nm. The mobile phase was composed of 0.5% citric acid adjusted to pH 3 with KOH and acetonitrile (6 : 4). A flow rate of 1 ml/min was used. The retention time of Cur was approximately 9 min under the given conditions. Cur in a corresponding CD-solution was used for the preparation of a standard curve. A new stock solution was prepared every week. The cumulative amount of the permeated Cur per unit surface area was plotted versus time and the cumulative flux (J_{ss}, µg/cm²/h) was calculated from a steady-state slope. Steady-state was assumed when the obtained curve was linear ($R^2 > 0.95$). The average fluxes from at least four parallels were analysed with one-way ANOVA (Minitab 15) using Fisher 95% individual confidence intervals and pair-wise comparisons to determine if statistically significant differences were observed between the variables.

3.2.1. Membrane model

Table 2 presents the characteristics of the membranes used. The postulated effects of the membranes on the Cur release are included.
Three hydrophilic membranes of different pore sizes were selected to study the release of Cur. One of the membranes was covered with mucin prior to use. The membranes were as follows: regenerated cellulose membrane, Spectra/Por® 1, molecular weight cut-off (MWCO) 6-8000 Da (Spectrum Europe B.V.), regenerated cellulose membrane, Spectra/Por® 7 dialysis membrane, MWCO 1000 Da (Spectrum Europe B.V.), polypropylene membrane, GH Polypro®, pore size 0.45 µm (Pall Corporation, Michigan, USA) and the same polypropylene membrane treated with mucin. The polypropylene membrane containing mucin was prepared by the following method: A volume of 100 µl freshly made mucin dispersion 3% (w/v) in phosphate buffer (pH 5) was added to the surface of the polypropylene membrane mounted to the upper part of a diffusion cell. The membrane was left to dry at 35 °C overnight before use.

3.2.2. Cur solubility studies in CD-solutions (without alginate)

A measure of Cur solubility as a function of CD concentration was performed according to the method described by Higuchi and Connors (1965). The samples were protected from light. Experiments were performed by adding an excess amount of Cur powder (>4 mg/5 ml) to CD-solutions (1, 3, 5, 7, 9 and 11% w/v) prepared in phosphate buffer (pH 5) containing 10% (v/v) ethanol or in plain phosphate buffer. The suspension formed was agitated for 7 days prior to filtration (Acrodisc® 13 mm, 0.8 µm, Versapore® membrane) followed by quantification by HPLC as previously described. Cur in ethanol was used for the preparation of a standard curve, and the tests were preformed in triplicate. The solubility of Cur in buffer : ethanol (9 : 1) was determined according to the same procedure. The solubility in plain buffer was set to the analytical HPLC detection limit (3×10^{-8} M) because the actual solubility is below the detection limit (Tønnesen 2002). Phase diagrams were constructed by plotting the molar concentration of solubilized Cur against the concentration of CD (%, w/v).

3.2.3. Preparation of alginate-containing formulations with Cur

The composition of the samples is presented in Table 1. Nine parts of CD stock solution in phosphate buffer (e.g. 3.33% w/v) was added to one part of Cur in ethanol (0.3 mg/ml) to give a total CD concentration of 3 or 10% (w/v) and a Cur-concentration of 0.03 mg/ml (8.14×10^{-5} M) in the final solutions. An amount of 0.05 or 0.3 grams of alginate was then added to 10 ml of the solution (or the suspension) and manually agitated for a short time before agitation at 150 rpm for 19–20 h (Edmund Bűhler, HOUM AS, Oslo, Norway) at room temperature protected from light.

3.3. Stability studies

3.3.1. Physical appearance of the formulations

The physical appearance of the formulation presented in Table 1 was visually examined after one month of storage protected from light at ambient temperature.

3.3.2. Cur stability after storage

Formulations containing 0.5% alginate and 3% CD (Formulations A, C, 1 and 3; Table 1) were prepared as previously described and stored at ambient temperature, protected from light.

The Cur concentration in the formulations should preferentially be quantified by HPLC but this proved to be very difficult due to the presence of alginate. A spectrophotometric method was therefore selected. The absorbance of Cur was detected spectrophotometrically on a Shimadzu UV-2101 PC UV-VIS scanning spectrophotometer at 500 nm. The detection wavelength is at the red-end tail of the Cur absorption spectrum and was selected for two reasons. The samples had a very high absorbance (>2) at the Cur absorption maximum (~430 nm). The content of alginate made it difficult to withdraw and dilute the samples, and the detection at 500 nm allowed for a direct quantification of the samples. Secondly, the known hydrolytic degradation products do not interfere at this wavelength (Tønnesen and Karlsen 1985). The method was linear in the concentration range 0.028–0.28 mg/ml, Reg = 0.99. Samples stored for 1 month were compared to freshly prepared samples. The absorbance was calculated as the mean average absorbance of three replicates, and the change in absorbance at 500 nm was used as an expression of Cur degradation. The change in absorbance was analysed with the same statistical tool as described above.

3.3.3. Rotational viscosity measurements

The shear viscosity was measured on all the formulations containing 3% CD (Table 1). To investigate the physical stability of the vehicle over time, viscosity measurements were performed on selected, freshly prepared formulations (Formulations A, C, 1 and 3) and on the same batches after one month of storage at ambient temperature protected from light. All the measurements were performed on a Physica UDS 200 (Paar Physica) rheometer equipped with a cone-plate (45 mm, 1 degree angle, MK 24) at 21 ± 0.05 °C. A sample volume of about 4 ml was gently poured into the selected geometry and any excess sample was removed before application of silicon oil to avoid evaporation from the samples. The samples were allowed to rest in the rheometer for 15 min prior to application of shear rates from 0.5 to 100 1/s in rotation. The Ostwald model (Power law) was used to describe the flow and viscosity curves obtained (Mezger 2002) and the results were reported as the mean average consistency coefficient (K) and the mean average flow index (n) (±SD) of three replicates ($R^2 > 0.95$). The viscosity (η) relates to K and n through Eq. (5). The significance of the results from the vehicle stability test were analysed with the same statistical tool as described above.

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