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Formulation and evaluation of release and swelling mechanism of a water-in-oil emulsion using factorial design

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Abstract

Water-in-oil emulsions have a potential as a parenteral prolonged release system for hydrophilic drugs. A consistent challenge when developing an emulsion drug delivery system is to obtain a proper release characteristic of the entrapped drug. The aim of the present study was to study the release mechanism from water-in-oil emulsions. Secondly, to study the effects of droplet size, phase ratio and osmotic pressure on the release rate of glucose from water-in-oil emulsions in a factorial experimental design. The release mechanism of glucose was deduced from the release kinetics of two coentrapped marker molecules, glucose and inulin, with a molecule weight of 180 and 5000 g/mol, respectively. The results indicate that release of glucose was dominated by diffusion through the oily barrier as opposed to membrane rupture. Using statistical methodology, the release rate of glucose could be varied 8 fold in a controlled manner with osmotic pressure as the most important parameter. The osmotic behaviour of the emulsions was further studied in a dynamic swelling study. These results show that the release of entrapped hydrophilic drug can be controlled within certain limits using pharmaceutical formulation principles. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: W/o emulsion; Prolonged release; Release mechanism; Osmotic behaviour; Factorial design

1. Introduction

Many new biotech drugs are often characterised by a poor gastro-intestinal bioavailability, high potency and short plasma half-lives. Thus, the need for parenteral controlled/prolonged release systems is increasing.

Water-in-oil (w/o) emulsions and the related water-in-oil-in-water (w/o/w) emulsions have for a long time been considered as attractive prolonged release systems for hydrophilic drugs with oil and surfactant layers acting as a release barrier (Davis et al., 1985). However, only relatively few articles suggest the use of w/o emulsions for parenteral use probably because of a non-optimal stability and toxicological profile. The emulsions prepared in this study are viewed as potential parenteral drug carriers. The emulsions consist of bioaccept-

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able compounds (medium-chain triglycerides, polyglycerol polyricinolate, sorbitan monooleate). Furthermore, the w/o emulsions have a good injectability as a result of a low viscosity and a good physical stability (Bjerregaard et al., 1999).

W/o emulsions are easy to prepare and offer a wide range of opportunities for modifying/controlling release properties by varying formulation or process parameters. The physical-chemical parameters influencing the release rate of drugs from w/o and w/o/w emulsions have been evaluated in many studies. In an early study, Windheuser et al. (1970), demonstrated that parameters such as partition coefficient of the drug, viscosity of the oil phase, and phase volume ratios could be used to control release rates of benzocaine qualitatively from a w/o emulsion. Furthermore, it has been shown that the nature of the surfactant (Omotosho et al., 1989; Jager-Lezer et al., 1997), surfactant concentration (Lin et al., 1992; Garti et al., 1994; Sela et al., 1995; Jager-Lezer et al., 1997), droplet size (Omotosho et al., 1989; Okochi and Nakano, 1996; Cole and Whateley, 1997), interfacial complexation between albumin and Span 80 (Florence et al., 1985; Omotosho et al., 1986; Garti et al., 1994) and osmotic imbalance (Brodin et al., 1978; Adeyeye and Price, 1990; Hino et al., 1995; Geiger et al., 1998) are determinant parameters for drug release.

In order to develop a prolonged release system with general applicability it is of great importance to understand the release mechanisms and kinetics. Systems influenced by more than one factor are best studied by statistically experimental design, because this methodology enables one to study all factors together at the same time. However, a survey of literature reveals no studies employing factorial design to assess the effect of various parameters on release from simple w/o emulsions.

The aim of the present study was to determine the release mechanism for a w/o emulsion and to identify formulation/process parameters with significant effect on release rate of a hydrophilic marker, glucose, including important interactions between parameters using factorial design of the experiments.

2. Materials and methods

2.1. Materials

Fractionated coconut oil (Viscoleo) was supplied from H. Lundbeck A/S (Denmark). The polymeric surfactant, polyglycerol polyricinolate (PG PR), was kindly donated by Danisco Ingredients (Denmark), while the cosurfactant, Span 80 (sorbitan monooleate), was obtained from Sigma Chemical Company (USA).

HPLC purified D-[6-^{3}H]glucose (M_w: 180 g/mol) and [carboxyl-^{14}C]inulin (M_w: 5000 g/mol) were obtained from New England Nuclear, USA. Tritium labelled water, $^{3}H_2O$, was obtained from Packard (the Netherlands). To verify the radioactive purity and identity of the tritium labelled glucose, a TLC test, as specified in the 2nd European Pharmacopoeia, was performed prior to the release experiments. The radioactive purity of [^{14}C]inulin was verified by gel chromatography on a 20×1 cm column packed with Sephadex G-15 (Pharmacia, Sweden). Isopropylmyristat was obtained from Merck, Germany. All other chemicals used were of reagent grade.

2.2. Equipment

An Ultra Turrax homogeniser (Janke and Kunkel, Germany) and a Branson Sonifier ultrasound probe equipped with a microtip (Branson, USA) were used to prepare the emulsions. The photon correlation spectroscopy (PCS) system consisted of a Malvern Zetasizer 4 (Malvern Ltd., UK) with a helium-neon laser (wavelength = 633 nm) and a 7032 Multi-8 correlator. All sizes given are z-average-mean values. Liquid scintillation was performed with Tricarb counter (Packard, the Netherlands). A K10 tensiometer with a Du Noüy ring (Krüss GmbH, Germany) thermostatically controlled at 37°C was used to determine interfacial tension. Osmolality was measured with an Osmomat 030-D (Gonotec Gmbh., Germany).

2.3. Emulsion preparation

The w/o emulsions were prepared by dropwise addition of the aqueous phase to the oil phase

under agitation with a magnetic stirrer. The composition of the emulsions is given in Table 1. Emulsification was achieved with an Ultra Turrax equipped with a dispersing element, S25N-25G, agitating at 13 500 rpm for 3×1 min giving emulsion droplets with a mean droplet diameter of 560 (± 43) nm. Some emulsions were further treated with an ultrasound probe (Branson, USA) equipped with a microtip at an output value of 5 for 3×1 min. Ultrasonication further reduced the mean droplet diameter to 210 (± 11) nm.

2.4. Droplet size determination

The average mean droplet size of the dispersed aqueous phase was determined with PCS. About 1–3 μl of emulsion was dispersed in 1 ml of isopropylmyristate (viscosity = 4.9 mPa s) previously saturated with water. Measurements were carried out at 25°C and at a scattering angel of 90°.

2.5. Release experiments

The hydrophilic marker molecules, [^{3}H]glucose and [^{14}C]inulin, were released into buffers with various osmolarities using a Hanson Transdermal diffusion cell system (Hanson Research Co., USA). A weighed amount of emulsion (approximately 200 mg) was placed on 3.7 ml of 0.05 M phosphate buffers pH 7.4 without any membrane separating emulsion and buffer. The release buffer was adjusted to various osmolarities with NaCl ranging from 300 to 2500 mOsm/kg and contained 0.05% NaN_3 as a preservative. Serial sampling was performed and the amount of glucose and inulin released was determined by liquid scintillation. The sample volume was replaced with fresh release buffer. The apparent permeability, P_{app} (cm/s), of marker molecules from the emulsions was determined from the linear part of plots with hydrophilic marker released per cm^2 versus time according to the equation:

$$P_{app} = dQ/dt \times 1/(A \times C_0) \quad (1)$$

where dQ = quantity of substance released during the time, dt, A = interfacial area, C_0 = initial marker concentration in emulsion droplets.

2.6. Swelling studies

The specific uptake of tritium labelled water in two emulsions (mean droplet diameter of 185 and 716 nm, respectively) was studied at various osmotic gradients. A weighed amount of emulsion (500 mg) with an internal aqueous phase adjusted to an osmotic strength of 2150 mOsm/kg was placed on various 0.05 M phosphate buffers labelled with tritiated water in closed vials termostated at 37°C. The osmolarities of the buffers ranged from 150 to 3050 mOsm/kg. Samples of 50 μl of emulsion was consecutively withdrawn and the amount of tritium uptake quantified by scintillation.

2.7. Factorial design

The effect of droplet size (homogenisation method), osmotic gradient and phase ratio between the aqueous and oily phase on release kinetics were studied by a two level full factorial design with one center point and one replicate. The levels of the factors are listed in Table 2. These factors are easy to control. Furthermore, they were expected to have a pronounced effect on release rate glucose from the w/o emulsion,

Table 1
Composition of w/o emulsions (% w/w)

	I	II	III
Phosphate buffered saline with 40–280 mM glucose and/or 0.4 mM inulin	30.0	45.0	60.0
Sorbitan monooleate	1.0	1.0	1.0
Polyglycerol polyricinolate	3.5	3.5	3.5
MCT-oil	65.5	55.5	45.5

Table 2
Factorial design: variables and factor levels

Factor	Factor level		
	Low (−)	Center (0)	High (+)
X_{size}: size/mean droplet diameter (nm)	210	–	560
$X_{\Delta\pi}$: osmotic gradient, $\Delta\pi$, relative to release buffer (mOsm/kg)	0	625	1250
X_{ϕ}: Amount of aqueous phase (% w/w)	30	40	50

expressed as the apparent permeability coefficient, P_{app}.

Release data from the factor experiment were analysed with Modde 4 software (Umetri AB, Sweden) using a multiple linear regression model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + e$$

where Y is the response parameter of interest, i.e. the apparent permeability coefficient of glucose, X_1 to X_3 are independent process or formulation variables, β_0 is a constant (arithmetic mean), β_1 to β_{23} are model coefficients determined by the multiple linear regression analysis and e is the residual error.

3. Results and discussion

3.1. Evaluation of release mechanism

Generally, two main release mechanisms of w/o emulsion and the related w/o/w emulsions can be distinguished, diffusion and emulsion breakdown/membrane rupture (Florence et al., 1985; Magdassi and Garti, 1986; Laugel et al., 1996). Diffusion-controlled release will be dependent upon polarity and molecular weight of the drug and surfactant type/concentration in case of reverse micellar transport, while membrane rupture will be dependent upon the physical stability of the emulsion. To discriminate between the two release mechanisms, two hydrophilic marker molecules, [^{3}H]glucose and [^{14}C]inulin, were co-entrapped in the aqueous phase of the emulsions. The concentration of glucose was varied between 40 and 280 mM, while the inulin concentration was fixed at 0.4 mM. The osmolality of the release media was adjusted to the same value as the disperse phase of the emulsion.

Results from these release experiments are shown in Fig. 1. The release rates of glucose and inulin were almost linear. This pseudo zero order release behaviour can be explained by the small fraction of glucose released during the time course of the study and as a result of sink conditions. The true release kinetics is properly first order as proposed for w/o/w emulsions by Florence and Whitehill (1982) and Ohwaki et al. (1993). The relative release rates of glucose from different emulsions are almost identical, which show that the concentration of glucose can be varied between wide boundaries without any effect on release kinetics. The same tendency is observed with inulin, although a greater variability in the release data is observed. This variability properly reflects that inulin to a high degree is also released by membrane rupture. Hence, the variability might be a result of small differences in physical stability between the various emulsions. Other studies concerning thick gel-like w/o emulsions with high volume fraction of dispersed phase (Koizumi and Higuchi, 1968a,b) show that the released fraction is linearly dependent on the square root of time. In concentrated emulsions, the movement of emulsion droplets is highly restricted, because of a tight packing of emulsion droplets. Higuchi's model of release kinetics assumes that when one layer of emulsion droplets is depleted for drugs, release from the next layer starts. Higuchi's model has successfully been adapted to w/o/w emulsions (Magdassi and Garti, 1986; Cole and Whateley, 1997). However, it can not be adapted to the emulsions in this study, because the droplets move

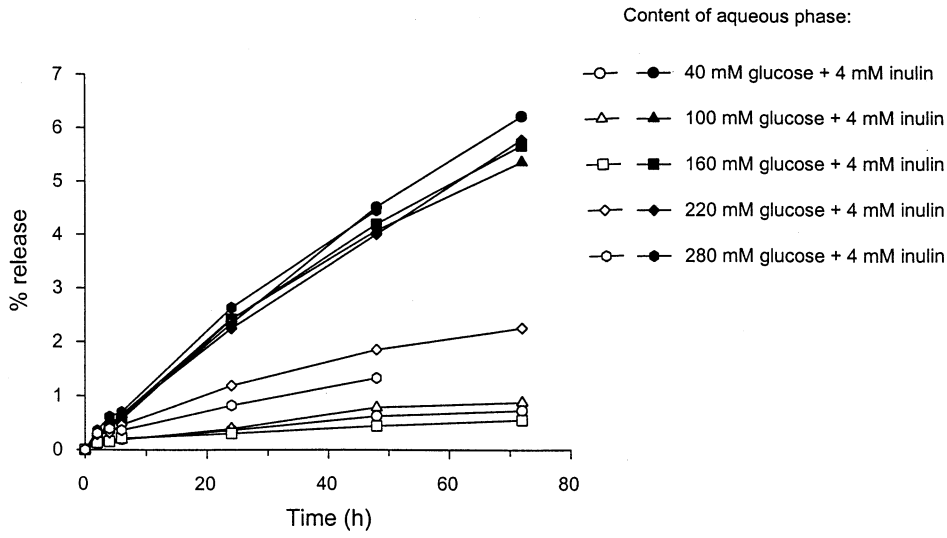

Fig. 1. Release profile of glucose (M_w: 180 g/mol) and inulin (M_w: 5000 g/mol) from w/o emulsions with various amounts of entrapped solutes ($n = 3$). The water phase in all emulsions was all adjusted to the same osmolality with sodium chloride. Filled and open symbols indicate released glucose and inulin, respectively. Standard deviations are not shown for clarity.

relatively freely in the w/o emulsions. From the linear part of the slopes in Fig. 1, the apparent permeability coefficients of glucose and inulin can be calculated. These values are listed in Table 3. The apparent permeability coefficients for glucose are significant larger than for inulin, which properly reflects the different molecular weights of glucose and inulin on 180 and 5000 g/mol, respectively. Consequently, release is dominated by diffusion of solutes through the oil phase (or a thin oil lamellae separating droplets from release media) rather than membrane rupture. Magdassi and Garti (1986) and Laugel et al. (1996), also observed that the diffusion release was the dominating release mechanism from w/o/w emulsion formulations. If the release process was dominated by membrane rupture, the P_{app} of the two hydrophilic marker molecules would be similar as a result of simultaneous release of glucose and inulin.

3.2. Factorial design

In this study, P_{app} of glucose was varied between 0.6×10^{-8} and 4.8×10^{-8} cm/s. The results from the factorial experiment (Table 4) can

Table 3
Results from release mechanism study: the apparent permeability coefficient of glucose and inulin at various concentrations of entrapped glucose ($\pm$ S.D., $n = 3$)

Emulsion ID	Concentration of solute		$P_{app}(I) \times 10^8$, glucose (cm/s)	$P_{app}(II) \times 10^8$, inulin (cm/s)	Ratio, $P_{app}(I)/P_{app}(II)$
	Glucose (mM)	Inulin (mM)			
1	40	4	2.96 ± 0.70	0.34 ± 0.02	8.7
2	100	4	3.03 ± 0.51	0.51 ± 0.21	6.0
3	160	4	2.73 ± 0.60	0.29 ± 0.11	9.5
4	220	4	2.78 ± 0.04	1.10 ± 0.14	2.5
5	280	4	3.16 ± 0.08	0.94 ± 0.20	3.4

Table 4
Experiments of the factorial design: setup and results ($\pm$S.D., $n = 2$)

Emulsion ID	Amount of water phase, % (w/w)	Mean droplet diameter (nm)	Osmolality (mOsm/kg)	P_{app} of glucose $\times 10^8$ (cm/s)
A	30	210	0	3.21 ± 0.09
B	30	210	1260	1.26 ± 0.16
C	30	560	0	1.15 ± 0.18
D	30	560	1260	0.62 ± 0.06
E	40	210	630	2.52 ± 0.07
F	50	210	0	4.84 ± 0.64
G	50	210	1260	1.46 ± 0.35
H	50	560	0	2.03 ± 0.04
I	50	560	1260	1.10 ± 0.00

be well explained by a multiple linear regression model ($R^2 = 0.9657$) as shown in Fig. 2. The model has good predictive power ($Q^2 = 0.9190$), which shows that the data are not overinterpretated by the model. Even the center point, E (Fig. 2) is well explained with the model. So, a simple linear model might be sufficient to describe the effect of osmotic gradients and phase ratio on release rate within the chosen levels. Fig. 3 illustrates that all three parameters (size, osmotic gradient and phase volume of the disperse phase) have statistically significant effects ($P < 0.001$). In addition, two interaction terms (size-osmotic gradient and osmotic gradient-phase ratio) have statistically significant effects ($P < 0.05$). The P_{app} of glucose can then be described by Eq. (2):

$$P_{app} \times 10^8 = 1.94 - 0.72 \times X_{size} - 0.85 \times X_{\Delta\pi} + 0.40 \times X_{\phi} + 0.48 \times X_{size}X_{\Delta\pi} - 0.23 \times X_{\Delta\pi}X_{\phi} \quad (2)$$

3.2.1. Phase ratio of w/o emulsion

As expected, the release rate increased with increasing volume of aqueous phase. A possible explanation is that an increase in water content leads to a proportional increase in droplet number (droplets per gram of emulsion) according to Eq. (3) and consequently a higher concentration of emulsion droplets at the oil/release buffer interface.

$$N = 3\phi/4\pi r^3 \quad (3)$$

where N = droplet number, ϕ = volume fraction of dispersed phase; r = droplet radius.

The effect of increasing the volume fraction of dispersed phase with 68% is an increase of the release rate of glucose with 41%. Consequently, the droplet number and release rate are not strictly proportional.

3.2.2. Size of w/o emulsion

The effect of emulsification method, and thereby size, was pronounced as shown in Fig. 3. A reduction in mean droplet size from 560 to 210 nm by sonication increased the P_{app} of glucose with approximately 74% (keeping the other

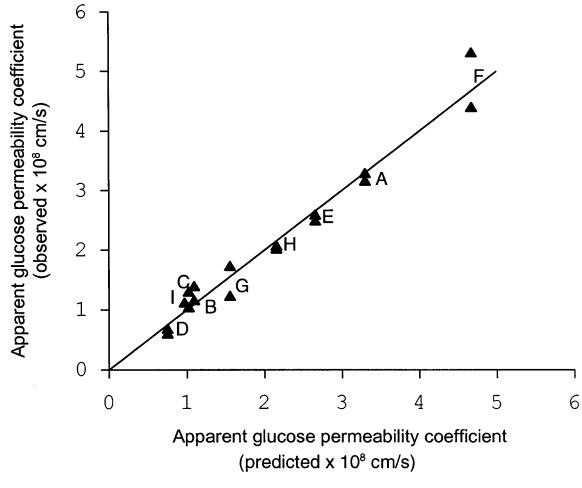

Fig. 2. Results from the factorial experiments. Correlation between measured and predicted values of the apparent permeability coefficients of glucose from w/o emulsions, $R^2 = 0.9657$ (letters refer to the identification of the emulsions, see Table 4).

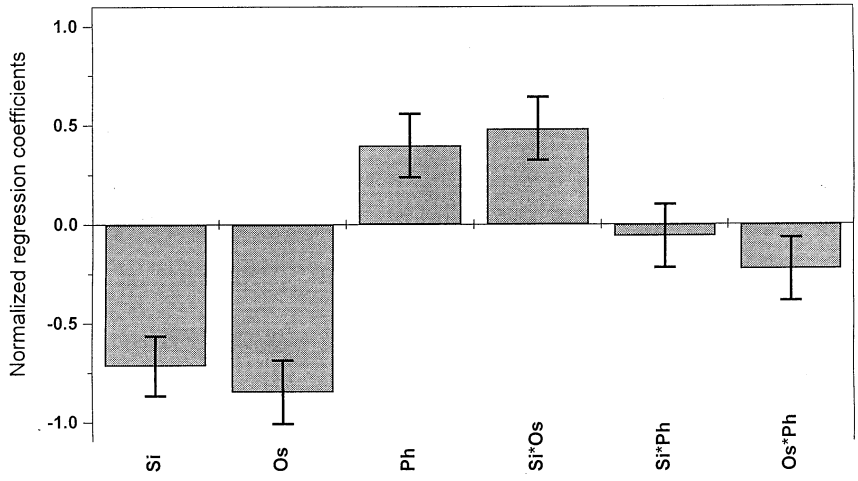

Fig. 3. Graphical representation of regression coefficients from the multiple linear regression analysis. The regression coefficients indicate the effect on P_{app} of glucose by changing one of the factors from a center to a high level value. Abbreviations: Si = size of emulsion droplets; Os = osmotic gradient between emulsion droplets and release buffer; Ph = volume fraction of water phase; and an asterisk (*) indicate an interaction term between two factors.

factors on a center level). A relatively higher surface area of the emulsion droplets can possibly explain this increase. A larger specific surface area is reflected in a relatively larger area occupied by water droplets at the emulsion/release buffer interface. The specific surface area of the emulsion droplets can be calculated from Eq. (4):

$$S = 3\phi/r \quad (4)$$

where S = specific surface area of emulsion droplets; ϕ = volume fraction of dispersed phase; r = droplet radius.

According to Eq. (4), a reduction of the mean droplet diameter from 560 to 210 nm corresponds to an increase in specific surface area of approximately 270%.

An additional explanation is that a larger specific surface area depletes the interface for surfactants and destabilizes the emulsion droplets. Destabilization favours release by membrane rupture, which seems not to be the case, taking the results from the release mechanism-study into consideration. The lowest polyglycerol polyricinolate concentration needed to form a saturated monolayer at the interface (when the mean droplet diameter = 210 nm and volume fraction of dispersed phase = 0.5) corresponds to approximately 3.5% w/w calculated according to Jager-Lezer et al. (1997):

$$n = 3\phi/r^3 a_s L \quad (5)$$

where n = molar concentration; ϕ = volume fraction of dispersed phase; r = droplet radius; a_s = molecular area occupied by lipophilic surfactant (approximately 100 Å^2 for polyglycerol polyricinolate); L = constant of Avogadro.

3.2.3. Osmotic behaviour

In w/o emulsions, the oil phase may be regarded as a semipermeable membrane allowing transport of water. Flux of water (J_w) into the aqueous phase of the emulsion may then be induced by an osmotic gradient, $\Delta\pi$, between the emulsion droplets and an exterior water phase, as described by Matsumoto and Kohda (1980), for w/o/w emulsions:

$$J_w = -L_p ART(g_2c_2 - g_1c_1)/V \quad (6)$$

where L_p = hydrodynamic coefficient of the oily membrane, A = area of the membrane, T = absolute temperature, g_1 = osmotic coefficients of electrolyte solutions of concentrations c_1; V = partial molar volume of water. Eq. (6) can be reduced:

$$J_w = -P_o A(g_2c_2 - g_1c_1) \quad (7)$$

where P_o is the osmotic permeability coefficient (Matsumoto and Kohda, 1980).

In this study, release of glucose from the emulsion droplets was reduced when the droplets were hyperosmotic compared to the receptor buffer (Fig. 3). These results are consistent with a recent study of Bjerregaard et al. (1999), where a linear relation between the magnitude of the hyperosmolality of emulsion droplets and P_{app} of glucose from a w/o emulsion were demonstrated. This osmotic (swelling) down-regulation of release has also been reported with some w/o/w emulsions (Brodin et al., 1978; Omotosho et al., 1986; Ohwaki et al., 1993; Hino et al., 1995; Kumbaradzi et al., 1996; Jager-Lezer et al. 1997). The down-regulation has been explained as a salting out effect of the surfactant layer which rigidifies the oil/water interface (Brodin et al., 1978; Hino et al., 1995). However, there seems to be some controversy over the salting out phenomenon. Opawale and Burgess (1998), showed that sodium chloride (0.01–0.1 M) decreased the interfacial elasticity of some Span surfactants and destabilized w/o emulsions prepared with these surfactants. In the present study the osmotic gradient was created by reducing the salt concentration in the release buffer. Hence, the swelling down-regulation of drug release can not be explained by the salting out phenomenon. Instead, the swelling down-regulation might be explained by a reverse solvent drag and/or by a reduced drug concentration gradient after swelling of the emulsion droplets.

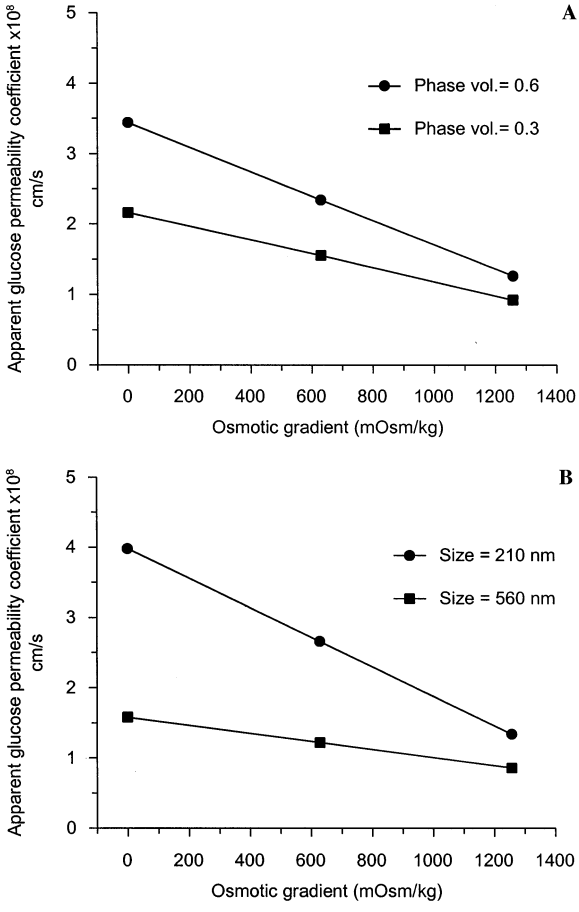

Fig. 4. Interaction plot. Effect of osmotic gradients on the apparent permeability coefficients, P_{app}, of glucose at various (A) phase ratios; and (B) mean droplet sizes.

The osmotic down-regulation seems to be dependent on the swelling degree of the emulsion droplets (Jager-Lezer et al., 1997; Bjerregaard et al., 1999). The more the swelling, the lower the release. The swelling capacity is strongly related to the nature and concentration of lipophilic surfactant (Jager-Lezer et al., 1997; Geiger et al., 1998). In addition, lipophilic surfactants have very different sensitivities towards the salting out phenomenon, which is reflected in the interfacial elasticity (Opawale and Burgess, 1998).

After a critical swelling point, the oily membrane separating the emulsion droplets from release media breaks and the droplets are expelled from the primary emulsion. This swelling-breakdown behaviour has recently been described by Jager-Lezer et al. (1997) and Geiger et al. (1998) for w/o/w emulsions. However, even with a high volume fraction of disperse phase (0.5), no acceleration of glucose release, as a result of an interaction effect between a high osmotic gradient and a large phase ratio, is observed. Instead, this interaction term is negative as shown in Fig. 3. This indicates that the w/o emulsions with high volume fractions of dispersed phase have a higher swelling ability than emulsions with small volume fractions of dispersed phase (Fig. 4a).

An important interaction between droplet size and osmotic gradient is also observed. The w/o emulsion with small emulsion droplets is more

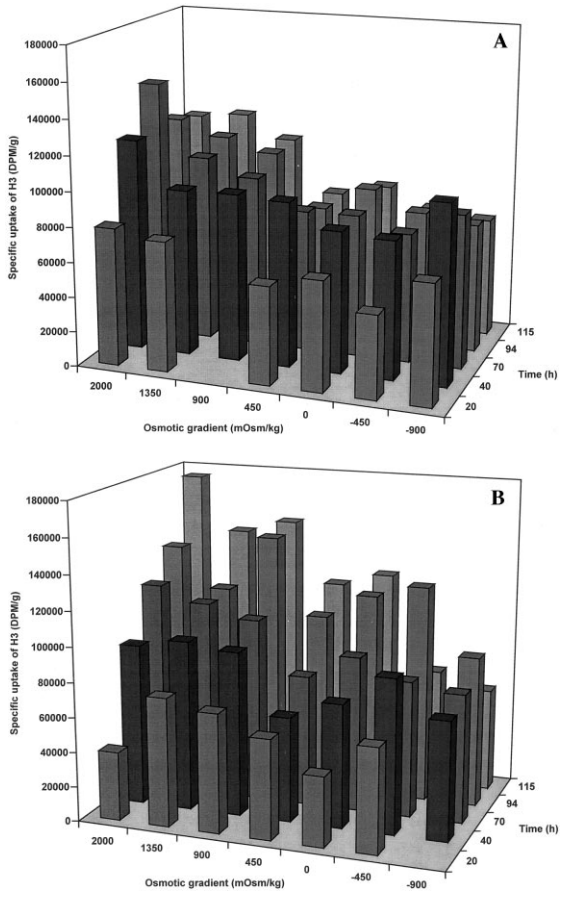

Fig. 5. Dynamic swelling studies at various osmotic gradients between emulsion droplets in the w/o emulsions and an external aqueous phase. Positive and negative values correspond to hyperosmotic and hypoosmotic emulsion droplets, respectively. Results from w/o emulsions with a mean droplet diameter of 185 and 716 nm are depicted in Fig. 5A and 5B, respectively.

responsive towards osmotic pressure than the larger emulsion droplets with regard to release rate as shown in Fig. 4b.

The interfacial tension between the oily phase and the aqueous phase with 0.278 mM glucose was measured to 1.2 ($\pm$ 0.1) mN/m. The Laplace pressure for droplets with a mean droplet diameter of 210 nm and 560 nm can be calculated from Eq. (8) to 2.3×10^4 and 0.8×10^4 Pa, respectively. These values are negligible compared to the osmotic pressure, which for an osmotic gradient of 1250 mOsm/kg can be calculated to 3.6×10^6 Pa. Hence, no major difference in swelling kinetics is expected.

The Laplace pressure, Δp, is given by the Young-Laplace equation:

$$\Delta p = \gamma(1/r_1 + 1/r_2) \quad (8)$$

where γ = interfacial tension and r_i = principal radii of curvature.

3.3. Swelling studies

The interaction between osmotic gradients and droplet size was further investigated in dynamic swelling studies of w/o emulsions with a mean droplet diameter of 185 ($\pm$ 3) and 716 ($\pm$ 28) nm, respectively (Fig. 5). There is a tendency that larger emulsion droplets have a higher swelling ability than smaller emulsion droplets. From Fig. 6, the osmotic permeability coefficient, P_o, can be calculated from the linear parts of the curves, setting the interfacial 'membrane' area to A, and the temperature to 310 K.

W/o emulsions with a mean droplet diameter of 716 nm have a P_o of 10.6×10^{-5} cm/s while the w/o emulsions with a mean droplet diameter of 185 nm have a P_o of 6.8×10^{-5} cm/s. These

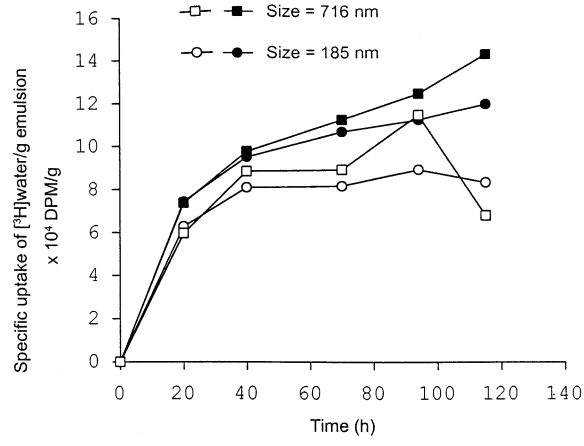

Fig. 6. Dynamic swelling studies with isoosmotic and hyperosmotic w/o emulsions with a mean droplet diameter of 185 (circles) and 716 nm (squares), respectively (data from Fig. 5). Filled and open symbols indicate an osmotic gradient, $\Delta\pi$, of 1350 and 0 mOsm/kg, respectively. The osmotic permeability coefficient of water, P_o, was calculated from the linear part of the curves.

values are close to the value obtained by Florence and Whitehill (1981), with w/o/w emulsions ($P_o = -6.3 \times 10^{-5}$ cm/s). The reason for the size dependent variability in osmotic permeability coefficients determined in this study is not the different magnitudes of the Laplace pressure, because this type of pressure is very small compared to the osmotic pressure in this study. However, it should be kept in mind from the release studies that a size reduction also increase the release of entrapped hydrophilic solutes such as electrolytes from the w/o emulsions, thereby reducing the effective osmotic gradient.

4. Conclusion

The two marker molecules, glucose and inulin, showed different release kinetics strongly indicating a diffusion mediated release mechanism of the w/o emulsion. Consequently, drug release is dependent upon molecular weight and polarity of the molecule. Moreover, release properties of w/o emulsions can be controlled within certain limits by control of droplet size, phase ratio between the oily and aqueous phase and osmotic gradient.

The effect of osmotic gradients on release rate from w/o emulsions and swelling of emulsion droplets is non-trivial. Hence, larger emulsion droplets have a larger swelling ability but are not as responsive to osmotic gradients regarding release rate as small droplets.

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References

Adeyeye, C.M., Price, C.J., 1990. Effect of dextrose in the internal aqueous phase on formation and stability of w/o/w multiple emulsions. Drug Dev. Ind. Pharm. 16 (6), 1053–1071.

Bjerregaard, S., Söderberg, I., Vermehren, C., Frokjaer, S., 1999. The effect of controlled osmotic stress on release and swelling properties of a water-in-oil emulsion. Int. J. Pharm., 183, 17–20.

Brodin, A.F., Kavaliunas, D.R., Frank, S.G., 1978. Prolonged drug release from multiple emulsions. Acta Pharm. Suec. 15 (1), 1–12.

Cole, M.L., Whateley, T.L., 1997. Release rate profiles from theophylline and insulin from stable multiple w/o/w emulsions. J. Controlled Release 49, 51–58.

Davis, S.S., Hadgraft, J., Palin, J.K., 1985. Medical and pharmaceutical applications of emulsions. In: Becher, P. (Ed.), Encyclopedia of Emulsion Technology, vol. 2. Marcel Dekker, New York, pp. 159–238.

Florence, A.T., Whitehill, D., 1981. Some features in water-in-oil-inwater multiple emulsions. J. Colloid Interface Sci. 79 (1), 243–256.

Florence, A.T., Whitehill, D., 1982. Formulation and stability of multiple emulsions. Int. J. Pharm. 11, 277–308.

Florence, A.T., Law, T.K., Whateley, T.L., 1985. Nonaqueous foam structures from osmotically swollen w/o/w emulsion droplets. J. Colloid Interface Sci. 107, 584–588.

Garti, N., Aserin, A., Cohen, Y., 1994. Mechanistic considerations on the release of electrolytes from multiple emulsions stabilized by BSA and nonionic surfactants. J. Controlled Release 29, 41–51.

Geiger, S., Tokgoz, N.S., Fructus, A., Jager-Lezer, N., Seiller, M., Lacombe, C., et al., 1998. Kinetics of swelling-breakdown of a w/o/w multiple emulsion: possible mechanisms for the lipophillic surfactant effect. J. Controlled Release 52, 99–107.

Hino, T., Takeuchi, H., Niwa, T., Kitagawa, M., Kawashima, Y., 1995. Analysis of drug release from diluted water/oil/water emulsions by a model of the rupture of oil membrane. J. Pharm. Pharmacol. 47, 1–7.

Jager-Lezer, N., Terrisse, I., Bruneau, F., Tokgoz, S., Ferreira, L., Clausse, D., et al., 1997. Influence of lipophilic surfactant on the release kinetics of water-soluble molecules intrapped in a w/o/w multiple emulsion. J. Controlled Release 45, 1–13.

Koizumi, T., Higuchi, W.I., 1968a. Analysis of data on drug release from emulsions II. Pyridine release from water-in-oil emulsions as a function of pH. J. Pharm. Sci. 57, 87–92.

Koizumi, T., Higuchi, W.I., 1968b. Analysis of data from emulsions III. Boundary effect on pyridine release from water-in-oil emulsions as a function of pH. J. Pharm. Sci. 57, 93–97.

Kumbaradzi, E.F., Pavlovska, E.P., Simov, A., Coracinova, K., 1996. Phase inversion of multiple water/oil/water into a water/oil emulsion caused by osmotic pressure gradients. Acta Pharm. 46, 177–185.

Laugel, C., Chaminade, P., Baillet, A., Seiller, M., Ferrier, D., 1996. Moisturizing substances entrapped in w/o/w emulsions: analytical methodology for formulation, stability and release studies. J. Controlled Release 38, 59–67.

Lin, S.Y., Wu, W.H., Lui, W.Y., 1992. In vitro release, pharmacokinetic and tissue distribution studies of doxorubicin hydrochloride (Adriamycin HCl) encapsulated in lipiodolized w/o emulsions and w/o/w multiple emulsions. Pharmazie 47, 439–443.

Ohwaki, T., Nakamura, M., Ozawa, H., Kawashima, Y., Hino, T., Takeuchi, H., et al., 1993. Drug release from the water-in-oil-in-water multiple emulsion in vitro. II. Effects of the addition of hydrophilic surfactants to the internal aqueous compartment on the release rate of secretin. Chem. Pharm. Bull. 41 (4), 741–746.

Okochi, H., Nakano, M., 1996. Basic studies on formulation, method of preparation and characterization of water-in-oil-in-water type multiple emulsions containing vancomycin. Chem. Pharm. Bull. 44, 180–186.

Omotosho, J.A., Whateley, T.L., Law, T.K., Florence, A.T., 1986. Nature of the oil phase and the release of solutes from multiple (w/o/w) emulsions. J. Pharm. Pharmacol. 38, 865–870.

Omotosho, J.A., Whateley, T.L., Florence, A.T., 1989. Methotrexate transport from the internal phase of multiple w/o/w emulsions. J. Microencap. 6 (2), 183–192.

Opawale, F.O., Burgess, D.J., 1998. Influence of interfacial properties of lipophilic surfactants on water-in-oil emulsion stability. J. Colloid Interface Sci. 197, 142–150.

Magdassi, S., Garti, N., 1986. A kinetic model for release of electrolytes from w/o/w multiple emulsions. J. Controlled Release 3, 273–277.

Matsumoto, S., Kohda, M., 1980. The viscosity of W/O/W emulsions: An attempt to estimate water permeation coefficient of the oil layer from the viscosity changes in diluted systems on aging under osmotic pressure gradients. J. Colloid Interface Sci. 73 (1), 13–20.

Sela, Y., Magdassi, S., Garti, N., 1995. Release of markers from the inner water phase of W/O/W emulsions stabilized by silicone based polymeric surfactants. J. Controlled Release 33, 1–12.

Windheuser, J.J., Best, M.L., Perrin, J.H., 1970. Evaluation of sustained-action parenteral emulsions. Bull. Parenter. Drug Assoc. 24, 286–294.