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Degradation kinetics of hydrolytically susceptible drugs in O/W emulsions—Effects of interfacial area and lecithin

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Abstract

To investigate the influence of the interfacial area and the emulsifier lecithin on the degradation rate of drugs prone to hydrolysis in parenteral lipid O/W emulsions we measured the degradation kinetics of phenyl salicylate in systems consisting of Miglyol as oil, buffered and isotonized aqueous phase and lecithin as emulsifier. Two-layer oil over water systems and emulsions of different oil droplet diameters and emulsifier contents were tested and a kinetic model was developed to interpret the results. The measurements showed a complex influence of interfacial area and liposomal concentration on the hydrolysis of phenyl salicylate. The interface between oil and water does not act as a diffusion barrier for phenyl salicylate, neither without nor with an interfacial layer of emulsifier. However, the presence of the layer and the formation of liposomes by the emulsifier lead to an overall acceleration of the hydrolysis. Three effects, partially counteracting each other, could be distinguished: the increase of phenyl salicylate concentration in the aqueous phase with increasing emulsifier concentration, the acceleration of hydrolysis with increasing interfacial area and the protection from hydrolysis by incorporation of phenyl salicylate into the emulsifier liposomes.

Keywords: Parenteral lipid emulsions; Phenyl salicylate; Kinetic of hydrolysis; Interfacial area; Lecithin; Liposomes

1. Introduction

Lipid oil in water (O/W) emulsions may be used as parenteral formulations to increase, by incorporation into the organic phase, the content of poorly water-soluble drug substances in preparations. This in addition will have an impact on the chemical stability of the active ingredient, which holds especially true for hydrolytically susceptible drugs. The stabilizing effect of emulsions is controversial in the literature. While Dingler et al. (1999) deny the potential of O/W emulsions to prolong the shelf-life of a drug, other authors like Prankerd and Stella (1990) claim an improvement of chemical stability when drug substances are formulated in emulsions. Certainly, the protection from degradation in the oil phase will never be total because of the equilibrium between organic and aqueous phase. The influence of the aqueous phase on chemical degradation can only be reduced, not annihilated.

Little is known about the physicochemical parameters of emulsions affecting the degradation rate of drug substances. So far only the influence of the distribution coefficient of the solute between aqueous and organic phase and the surface charge of the oil droplets in O/W emulsions has been described (Pongharoenkiat et al., 2002). On the one hand, the degradation rate of hydrolytically susceptible drugs decreases with increasing distribution coefficient as the concentration in the aqueous phase is reduced. On the other hand, the surface charge of the oil droplets affects the pH value around the interface. Since a pH sensitive solute degrades at a rate depending on the pH of its microenvironment, the surface charge can influence the degradation rate. This was observed, however, only for low lipophilic compounds whereas the degradation of highly lipophilic solutes was found to be independent of the surface charge.

Kinetic effects of the interfacial area and the presence of emulsifiers have not been reported yet. Lecithin, for example, modifies the interface between the oil droplets and the aqueous phase and excess lecithin would form liposomes in water (Férézou et al., 2001, 1994b; Rotenberg et al., 1991). The impact of these effects on the kinetics of hydrolysis is the object of

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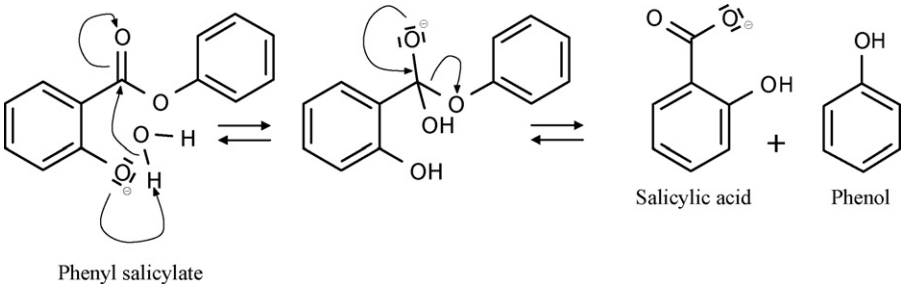

Fig. 1. Intramolecular base catalyzed hydrolysis of phenyl salicylate.

the investigations presented here. Phenyl salicylate was used as drug substance prone to hydrolysis for kinetic measurements in a cylindrical two-layer oil over water system as well as in emulsions produced under different conditions to quantify the influence of interfacial area and emulsifier concentration. Phenyl salicylate is known to saponify in water to form the degradation products salicylic acid and phenol (Capon and Ghosh, 1966; Kahn et al., 1983). The reaction is triggered by the intermolecular base catalysis of the hydroxyl group, as depicted in Fig. 1. This characteristic instability in water makes phenyl salicylate an ideal compound for kinetic measurements. In addition, having a poor solubility in water of just 9 μg/mL, it represents a typical example for drugs that may require to be formulated in parenteral lipid emulsions (Prankerd and Stella, 1990; Müller and Hildebrand, 1998; Singh and Louis, 1986).

2. Materials

Phenyl salicylate was obtained from Lancaster (Eastgate, United Kingdom). The non-ionic isotonizer glycerol was purchased from Merck (Hohenbrunn, Germany). As oil phase middle chain trigycerides, supplied under the trade name Miglyol by Cognis Germany GmbH (Düsseldorf, Germany), was used. The emulsifier chosen was Lipoid E80® obtained from Lipoid KG (Ludwigshafen, Germany). Phosphate-buffer (pH 7.4) was prepared by dissolving 13.61 g potassium dihydrogen phosphate in 1 L double distilled water. Sodium hydroxide solution and hydrochloric acid (both 0.1 mol/L) were used for pH adjustment. All three chemicals for buffer preparation were purchased from Merck. The buffer had an osmolality of 100 mOsm/kg. For HPLC analysis acetonitrile obtained from Fluka Chemie GmbH (Buchs, Switzerland) was used. The standard buffer solutions for the calibration of the pH-meter were supplied from Sigma–Aldrich GmbH (Seelze, Germany). All chemicals were of reagent grade or higher.

3. Methods

Kinetic studies were carried out by observing the concentrations of the three compounds, phenyl salicylate, phenol and salicylic acid, in different systems over a period of approximately 30 days.

All measurements were carried out at ambient temperature. The storage temperature was 20 ± 3 °C.

3.1. Two-layer systems

The experimental setup is shown in Fig. 2. In a glass cylinder C, a layer of oil was placed over an isotonized, buffered aqueous phase. A two-blade agitator (A), one blade for each phase, was operated at a low rate of 15 rpm just to provide for a homogeneous material distribution within the phases. Two syringes were attached to the wall of the glass cylinder for sampling from the aqueous (S1) and the oil (S2) phase separately. Glass cylinders 8.4, 13.6 and 18.6 cm in diameter were employed, providing for interfacial areas of 55.1, 145.0 and 271.7 cm^2.

At each time point samples of 0.5 and 0.1 mL were taken from the aqueous and oil phase respectively. These volumes were allowed for when calculating the concentrations of the three compounds phenyl salicylate, phenol and salicylic acid.

3.1.1. Buffered, isotonized aqueous phase

The aqueous phase was buffered at pH 7.4 with phosphate. The concentration of the isotonizer glycerol was adjusted so as to obtain the physiological osmotic pressure of 300 mOsm/kg. Given the osmolality of 100 mOsm/kg for the phosphate buffer the final osmolality of the aqueous phase was around 400 mOsm/kg.

Two-layer systems were studied with and without the addition of lecithin as emulsifier. The latter was used to approach the conditions of O/W emulsions. The compositions of the systems are given in Table 1. The volume ratio of oil to water is that of a

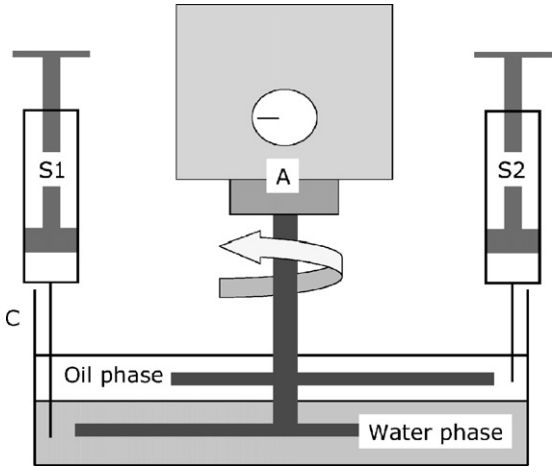

Fig. 2. Drawing of the agitated cylindrical two-layer oil over water system. C: glass cylinder; A: two-blade agitator; S1 and S2: syringes.

Table 1
Components of the cylindrical two-layer system

Component	System (O) without lecithin	System (L) with lecithin
Phenyl salicylate (g)	1.5	1.5
Miglyol (mL)	60.0	60.0
Lipoid E 80® (mg)	–	3
Isotonized buffer solution (mL)	540.0	540.0

10% lipid emulsion. Phenyl salicylate has a total concentration of 2.5 mg/mL whereas the lecithin quantity corresponds to its CMC, which was chosen to obtain an interface saturated with emulsifier but avoid the formation of liposomes in the aqueous phase.

The systems were prepared by dissolving phenyl salicylate and, if required, lecithin in the oil phase and using this solution to cover the isotonized buffer solution in the relevant glass cylinder.

3.1.2. Liposomal dispersions as aqueous phase

Excess emulsifier is generally required for the preparation of lipid emulsions so that the stabilisation of the interface is accompanied by the formation of liposomes in the aqueous phase. The presence of these liposomes may influence the degradation kinetics. To test and quantify this influence, liposomal dispersions of four different lecithin concentrations were prepared and the hydrolysis of phenyl salicylate in these dispersions was observed. The lecithin concentrations were 22.5, 45, 67.5 and 90 mg/mL. Each concentration was tested in triplicate.

The dispersions were prepared by mixing lecithin and the buffer solution with an Ultra-Turrax T25 (Jahnke und Kunkel, Staufen, Germany) at a rate of 8000 rpm until a homogeneous premixture was obtained. This premixture was homogenized with a high-pressure homogenizer (LAB 1000, APV, Lübeck, Germany). Five homogenisation cycles at 500 bar and a temperature of 40 °C were applied.

Finally, 540 mL of each liposomal dispersion was placed in the 145.0 cm^2 glass cylinder and covered with 60 mL of Miglyol containing 1.5 g phenyl salicylate.

3.1.3. Partition coefficients

The concentrations of the compounds were determined separately in both phases. Hence, the data could not only be used to study the degradation kinetics but also to calculate the partition coefficients of phenyl salicylate and its degradation products between the two phases—either oil over isotonized buffer solution or oil over liposomal dispersions. These results from the two-layer systems were important for the evaluation of emulsion studies. Since emulsions could not be phase-separated for analysis, only the compounds' total concentrations were accessible to determination. Using the partition coefficients, however, the concentrations in the aqueous phase of the emulsions could be calculated from the total concentration.

Table 2
Composition of 100 mL of lipid emulisons

Components	0.6% lecithin	1.2% lecithin	2.4% lecithin
Phenyl salicylate (g)	0.25	0.25	0.25
Miglyol (mL)	10.0	10.0	10.0
Lipoid E 80® (g)	0.6	1.2	2.4
Sodium oleat (g)	0.03	0.03	0.03
Isotonized buffer solution (mL)	90.0	90.0	90.0

3.2. Emulsions

250 mL volumes of O/W emulsion with different lecithin concentrations were prepared in triplicate under different homogenisation conditions to vary the droplet size and hence the interfacial area. The compositions of the emulsions are given in Table 2. Miglyol, phenyl salicylate and lecithin were mixed and heated to 40 °C until the drug substance and the emulsifier were dissolved. Separately, the isotonized buffer solution and the sodium oleat were mixed at 40 °C.

The two phases were first homogenized with an Ultra-Turrax T25 for 3 min at 8000 rpm to produce a pre-emulsion. This premixture was then passed through a high-pressure homogenizer (LAB 1000, APV, Lübeck, Germany) at 40 °C. Table 3 gives the pressures and number of homogenisation cycles of the different preparations.

3.3. Particle characterisation

3.3.1. Photon correlation spectroscopy (PCS)

The droplet size of the emulsions was determined by photon correlation spectroscopy using a Zetasizer Nano-ZS (Malvern, Malvern, United Kingdom). Before performing the measurement the samples were diluted with distilled water to approximately one part in one thousand until the Tyndall effect was apparent. PCS provides the mean hydrodynamic diameter, z-average (z_{av}), of the bulk population and, through the polydispersity index (PI), the width of the distribution. The z-average values were used to calculate the interfacial areas of the emulsions.

3.3.2. Laser diffractometry (LD)

Droplets in the micrometer range were detected by laser diffractometry using a Coulter LS 230 (Coulter Electronics, Krefeld, Germany). The volume distributions obtained for the droplet size were calculated using the Mie theory in connection

Table 3
Emulsion preparation—homogenisation conditions

Lecithin content (%)	Pressure		
	800 bar	600 bar	400 bar
0.6	/	3	3
1.2	2	2	2
2.4	2	2	2

Values denote number of cycles.

with PIDS. The parameters found to be suitable for parenteral lipid emulsions (Müller and Schuhmann, 1997) are 1.46 for the real part and 0.01 for the imaginary part of the refractive index.

3.3.3. Zeta potential (ZP)

The Zetasizer Nano-ZS used for determining the droplet size was also employed to measure their zeta potential, applying a field strength of 20 V/cm and diluting the sample to the same volume ratio as for the measurement of the size distribution. The zeta potential was calculated by means of the Helmholtz–Smoluchowski equation. A well-defined conductivity and pH value of the measuring solution are crucial to avoid fluctuations of the results due to varying conditions (Müller, 1996). Therefore, the measuring medium was mixed from a sodium hydroxide and a hydrochloric acid solution, both at concentrations affording a conductivity of 50 μS/cm, to adjust the pH value at 7.4.

3.3.4. Surface tension

The interfacial tension between oil and aqueous phase was measured applying the ring method on a Lecompte du Noüy tensiometer (Digitial tensiometer KIO, Krüss, Hamburg, Germany). 20 mL of isotonized buffer solution was covered with 20 mL of Miglyol containing different concentrations of lecithin. Prior to the measurement all samples were allowed to equilibrate for 2 h. The ring was then positioned on the interface to determine the surface tension. The measurements were performed at 20 °C.

3.3.5. pH measurement

A pH-meter (pH-meter 766 calimatic, Knick, Germany) was used with a micro-glasselectrode (InLab® 442, Mettler Toledo, Switzerland) to measure the pH value. The equipment was calibrated with standard buffer solutions of pH 4, 7 and 9. The results were read when the displayed value did not change for at least 1 min.

3.3.6. Tonicity measurement

The osmotic pressure was measured by means of a vapor pressor osmometer (Vapor pressor osmometer 5520, Wescor, USA). The sample volume was 10 μL.

3.3.7. HPLC measurement

The concentrations of the three compounds, phenyl salicylate, phenol and salicylic acid, were determined via HPLC using standard substances for calibration. The HPLC system consisted of a pump (Dionex, model P580), an automated sample injector (Dionex, model ASI 100), a separation column Xterra® RP8, 3.5 μm, of 4.6 mm internal diameter and 150 mm length (Waters, USA) and a photodiode array detector (Dionex, model PDA 100). The mobile phase was mixed from a 0.1% aqueous solution of acetic acid (eluent A) and acetonitrile (eluent B) to produce the following gradient: linear from 100% of A to 80% of B in 20 min, then 100% of B for 10 min and finally 100% of A for another 10 min. The chromatography was performed at room temperature with an injection volume of 60 μL. Under these conditions phenol has a retention time of 10 min, salicylic acid of 14 min and phenyl salicylate of 22 min. The detection wavelengths were 270 nm for phenol and 301 nm for phenyl salicylate and salicylic acid.

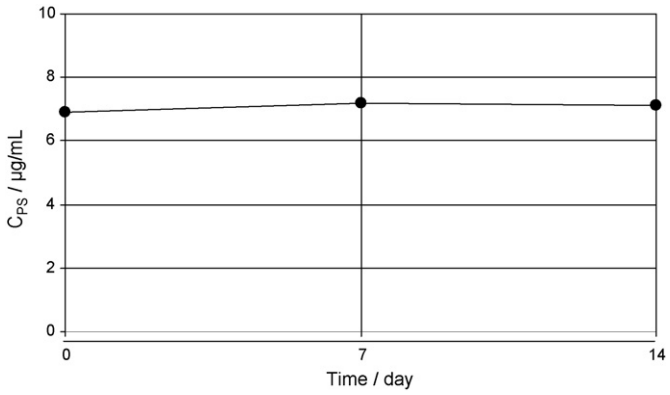

Fig. 3. Concentration of phenyl salicylate, C_{PS}, in Miglyol vs. time of storage at 60 °C.

4. Results

The experiments were designed to elucidate separately the effects of the interfacial area and the emulsifier concentration on the saponification kinetics of phenyl salicylate in O/W emulsions. Basic features were first studied in the two-layer system and the results were then used to explain the findings obtained with emulsions.

4.1. Monophase systems

Before investigating the two-layer systems, the degradation of phenyl salicylate was studied separately in oil and water. The time plots in Figs. 3 and 4 confirm that it is only the aqueous phase where the drug saponifies.

4.2. Two-layer system

Starting with the basic System (O) without emulsifier (see Table 1), the concentrations of phenyl salicylate and the

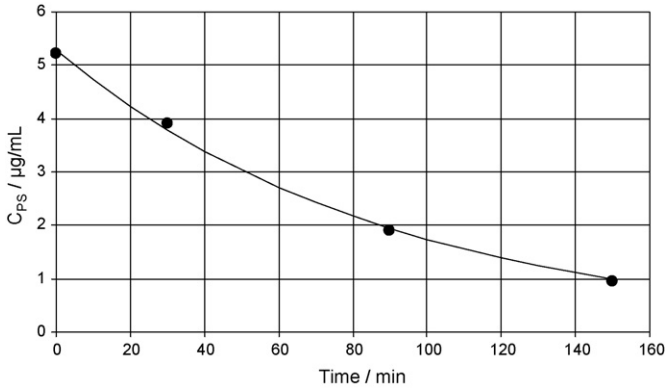

Fig. 4. Concentration of phenyl salicylate, C_{PS}, in aqueous buffer pH 7.4 vs. time of storage at 60 °C.

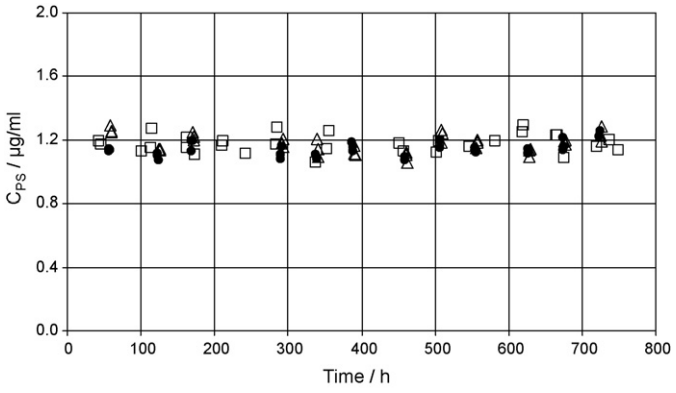

Fig. 5. Concentrations of phenyl salicylate, C_{PS}, vs. time in the aqueous phase of the basic System (O) described in Table 1. The interfacial areas were 55.1 cm^2 (●), 145.0 cm^2 (△) and 271.7 cm^2 (□) ($n = 3$).

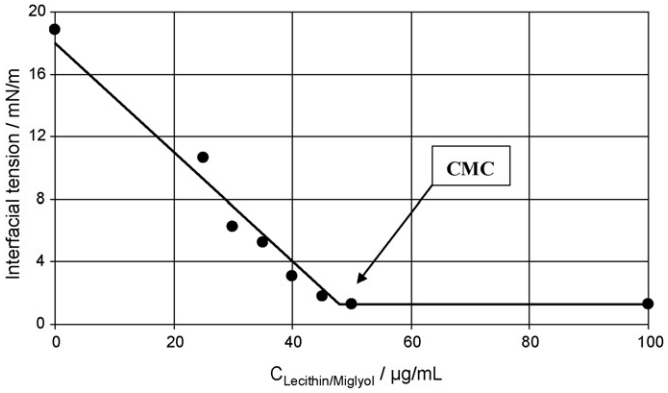

Fig. 7. Interfacial tension vs. lecithin concentration in the oil.

degradation product phenol were observed in the aqueous phase for the three interfacial areas 55.1, 145.0 and 271.7 cm^2. The time plots over 720 h are depicted in Figs. 5 and 6. Three findings can be derived: first, the concentration of phenyl salicylate in the aqueous phase does not change over time; second, the phenol concentration increases linearly with time; and third, the interfacial area obviously makes no significant difference regarding the slope of the plots.

Following this finding, all further two-layer experiments were confined to the interfacial area of 145.0 cm^2. The next stage of the experimental design was the addition of the emulsifier lecithin to simulate the stabilisation of the interface between oil and aqueous phase as required for the preparation of emulsions. For the kinetic measurements in the two-layer system, the lecithin concentration was first adjusted at its CMC which was determined by plotting the interfacial tension versus lecithin concentration in Miglyol.

As shown in Fig. 7, the interfacial tension decreases with increasing lecithin concentration until the interface is saturated with a monomolecular layer of emulsifier at 50 µg/mL. Further addition of lecithin does not change the interfacial tension anymore but would lead to the formation of liposomes in the aqueous phase.

The CMC of 50 µg/mL was now chosen to prepare the System (L) described in Table 1 and compare the degradation rate of phenyl salicylate with that of System (O). Fig. 8 shows the linear increase of the phenol concentration for both systems.

No significant difference of the degradation rate was observed.

Excess amounts of emulsifier were added to the two-layer system on the third experimental stage. This, in the first place, caused an increase of the phenyl salicylate concentration in the aqueous phase as demonstrated by Fig. 9. The relation between lecithin and phenyl salicylate concentration is obviously linear.

Given the initial concentration of 25 mg/mL of phenyl salicylate in the oil phase the concentrations in the aqueous phase were used to calculate the partition coefficients. The same was done for phenol from the concentrations measured separately in the oil and aqueous phase during hydrolysis. Table 4 provides the partition coefficients obtained for the different lecithin concentrations. The concentration of the second degradation product, salicylic acid, in Miglyol was too low to be detected by the applied HPLC method.

The increase in the phenyl salicylate concentration with lecithin content is accompanied by an acceleration of the degradation rate as can be seen in Fig. 10. Apart from this acceleration,

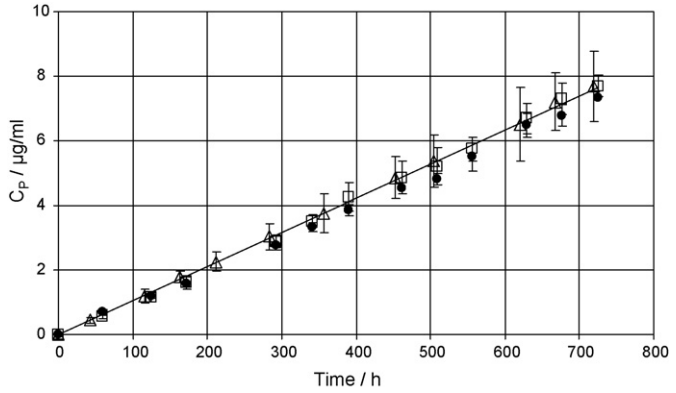

Fig. 6. Concentrations the degradation product phenol, C_P, vs. time in the basic System (O) described in Table 1. The interfacial areas were 55.1 cm^2 (●), 145.0 cm^2 (△) and 271.7 cm^2 (□) ($n = 3$).

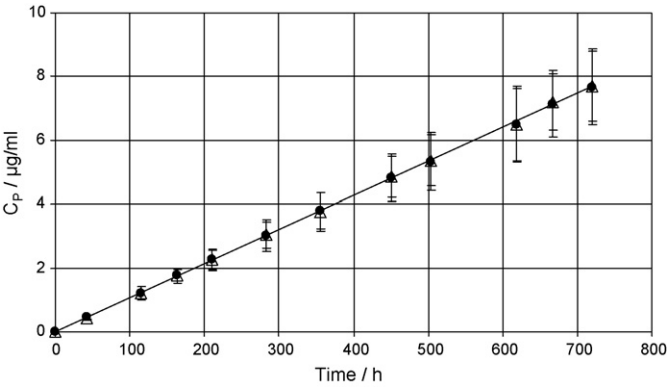

Fig. 8. Concentration of the degradation product phenol, C_P, vs. time in the two-layer System (L) with (●) and System (O) without (△) a monomolecular layer of lecithin on the interface ($n = 3$).

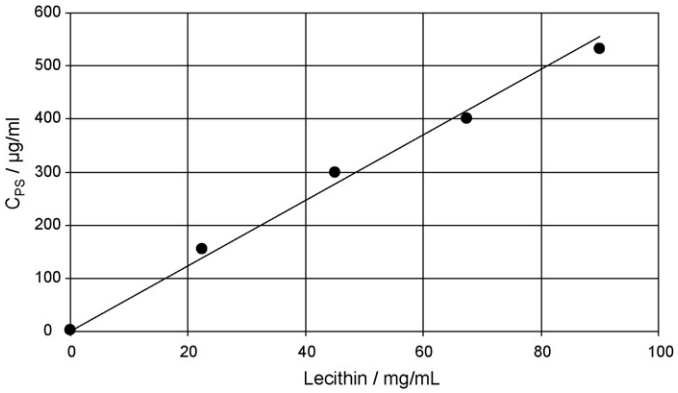

Fig. 9. Concentration of phenyl salicylate, C_{PS}, vs. lecithin concentration in the aqueous phase of the two-layer system.

Table 4
Partition coefficients of phenyl salicylate and phenol between Miglyol and aqueous phase for different lecithin concentrations

Lecithin content (mg/mL)	Phenyl salicylate	Phenol
0	21,628 ± 818	10.2 ± 2.3
22.5	161.1 ± 4.4	3.9 ± 1.2
45	84.0 ± 3.2	2.8 ± 0.3
67.5	62.4 ± 1.1	1.8 ± 0.4
90	46.9 ± 0.8	1.5 ± 0.1

it is noteworthy that the slope, dC_P/dt, is not constant for higher lecithin concentrations but increases with time.

4.3. Emulsions

An even faster degradation of phenyl salicylate was found in O/W emulsions prepared with lecithin contents of 0.6, 1.2 and 2.4%. The plots of the phenol concentrations are given in Fig. 11. Again the increasing slope, dC_P/dt, with time must be noted.

The droplet parameters characterizing the emulsions are summarized in Table 5. The values show clearly a decreasing droplet size with increasing lecithin content.

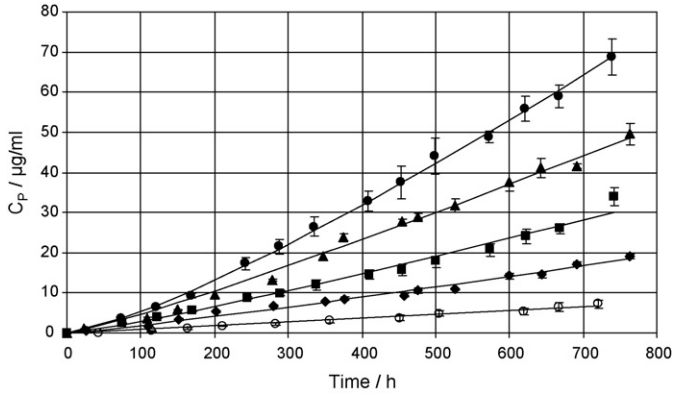

Fig. 10. Concentration of the degradation product phenol, C_P, vs. time in the two-layer system with different liposomal dispersions as aqueous phases. The lecithin concentrations were 0 mg/mL (○), 22.5 mg/mL (♦), 45 mg/mL (■), 67.5 mg/mL (▲) and 90 mg/mL (●) ($n = 3$).

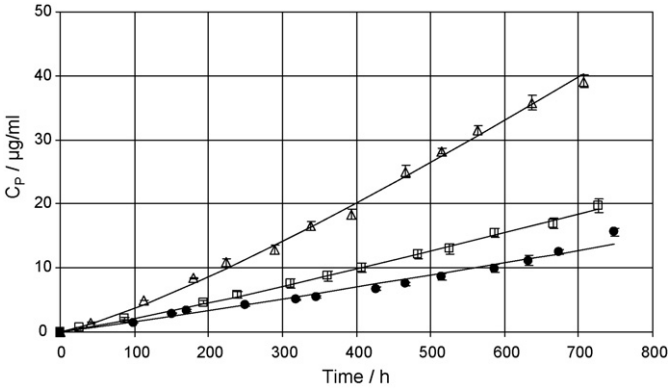

Fig. 11. Concentration of the degradation product phenol, C_P, vs. time in O/W emulsions with different lecithin contents. The lecithin contents were 0.6% (●), 1.2% (□) and 2.4% (△).

5. Discussion

The important initial finding is the constant concentration of phenyl salicylate in the aqueous phase during hydrolysis. That means the material transport across the interface is fast compared to the decomposition rate. Any loss by saponification is immediately compensated by the re-supply from the oil phase. This holds true for even the lowest specific interfacial area of $55.1\,cm^2/600\,cm^3 = 0.092\,cm^{-1}$, and will do so even more for the large specific interfacial areas of at least $4.2 \times 10^6\,cm^2/250\,cm^3 \approx 17{,}000\,cm^{-1}$ in emulsions. For the degradation kinetics studied here the interface does not act as a diffusion barrier.

Neither does a monolayer of lecithin as emulsifier reduce the material transport across the interface to an extent slowing down the degradation. As demonstrated by Fig. 8, systems with and without a lecithin monolayer do not show different kinetics.

The interpretation of the kinetic data produced with excess emulsifier and emulsions may be based on a simple two-phase model. As depicted in Fig. 12, an aqueous phase volume, V, is covered by an oil volume, V_o, giving a total volume of V_t. Phenyl salicylate, PS, and phenol, P, equilibrate between the

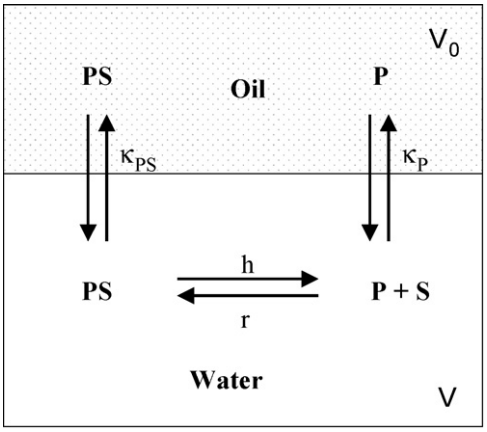

Fig. 12. Two-phase model of the degradation of phenyl salicylate. V: volume of the aqueous phase; V_o: volume of the oil phase; $V_t = V + V_o$: total volume; PS: phenyl salicylate; P: phenole; S: salicylate; κ_{PS} and κ_P: partition coefficients.

Table 5
Particle characterisation of the tested emulsions

Method	Emulsion 0.6% lecithin			Emulsion 1.2% lecithin			Emulsion 2.4% lecithin		
	P1	P2	P3	P1	P2	P3	P1	P2	P3
Start of storage									
PCS									
z-Average (nm)	362	353	355	222	202	212	150	160	154
PI	0.22	0.20	0.33	0.11	0.12	0.08	0.08	0.08	0.96
LD									
D50 (μm)	0.416	0.400	0.394	0.325	0.240	0.314	0.205	0.208	0.201
D99 (μm)	1.638	1.234	1.615	0.732	0.676	0.701	0.536	0.553	0.515
Zeta potential (mV)	−41.7	−43.0	−42.7	−44.7	−43.8	−42.8	−42.9	−44.0	−44.2
4 weeks of storage									
PCS									
z-Average (nm)	354	363	347	226	214	227	158	164	161
PI	0.42	0.29	0.37	0.15	0.17	0.22	0.1	0.12	0.12
LD									
D50 (μm)	0.386	0.445	0.384	0.398	0.276	0.324	0.388	0.259	0.272
D99 (μm)	1.575	1.258	1.412	1.246	0.717	1.057	0.686	0.657	0.629
Zeta potential (mV)	−42.2	−41.4	−44.7	−44.13	−44.9	−45.1	−42.7	−43.5	−45.0

two phases according to their partition coefficients, κ_{PS} and κ_P. Any molecular transport of salicylic acid into the oil phase is neglected.

No degradation occurs in the oil phase (see Fig. 3), whereas in the aqueous phase the reversible reaction pair of saponification and esterification has in principle to be taken into account. The rate constant of the first is h that of the second is r. Denoting the concentrations of phenyl salicylate, phenol and salicylic acid in the aqueous phase [PS], x, and [S], and the total phenol concentration X, the reaction rate is given by Eq. (1)

$$\frac{dX}{dt} = h[PS] - r[S]x \tag{1}$$

Since salicylic acid and phenol are formed in equimolar quantities and the molecular transfer of salicylic acid into the oil phase is negligible, the following mass balances apply:

$$[S]V = XV_t \tag{2}$$

$$XV_t = xV + x\kappa_P V_o \tag{3}$$

which changes Eq. (1) to read

$$\frac{dX}{dt} = h[PS] - r\left(\frac{V_t^2}{V(V+\kappa_P V_o)}\right)X^2 \tag{4}$$

Leuenberger (2002) reported that the saponification of acetylsalicylic acid is catalyzed by its degradation products. This effect might explain the acceleration of the degradation with time, as observed in Figs. 10 and 11, and can be allowed for in Eq. (4) by adding a second-order term for [PS] and x with the rate constant η. Substituting x by X through Eq. (3), Eq. (4) then extends to read:

$$\frac{dX}{dt} = h[PS]+\eta[PS]\left(\frac{V_t}{V+\kappa_P V_o}\right)X - r\left(\frac{V_t^2}{V(V+\kappa_P V_o)}\right)X^2 \tag{5}$$

Since [PS], κ_P, and the volumes are constant, Eq. (5) can be simplified:

$$\frac{dX}{dt} = H_o + HX - RX^2 \tag{6}$$

where

$$H_o = h[PS]$$

$$H = \eta[PS]\left(\frac{V_t}{V+\kappa_P V_o}\right)$$

$$R = r\left(\frac{V_t^2}{V(V+\kappa_P V_o)}\right)$$

A detectable onset of the reverse reaction of esterification would abate the slope of the time plot for phenol. This, however, is not observed in Figs. 10 and 11 and it may therefore be a reasonable approximation to neglect the esterification for the duration of the kinetic observations presented here. Eq. (6) would then simplify:

$$\frac{dX}{dt} = H_o + HX \tag{7}$$

with the solution

$$X = \frac{H_o}{H}(e^{Ht} - 1) \tag{8}$$

The solution for Eq. (6) is somewhat bulkier but still analytically feasible.

Least-square fits for both solutions, Eq. (8) and that for Eq. (6), were applied to the time plots in Figs. 10 and 11. The differences between the two were found to be within the analytical error, which confirms that esterification can be neglected. We may therefore conclude that the degradation kinetics in the time interval covered by the studies are satisfactorily described as saponification, accelerated by the formation of phenol.

Table 6
Rate constants h and η for the two-layer systems and emulsions of different lecithin concentrations, C_L, corrected for the loss to the interface

System	[PS] (mmol/L)	C_L (mg/mL)	A_{IV} (cm^2/mL)	h (h^{-1})	η (L/(mmol h))
Two-layer, no lecithin	0.005	0	0.242	1.8×10^{-2}	1.3×10^{-1}
Two-layer, with lecithin	0.725	22.5	0.242	2.9×10^{-4}	10.8×10^{-4}
Two-layer, with lecithin	1.391	45	0.242	2.1×10^{-4}	10.1×10^{-4}
Two-layer, with lecithin	1.872	67.5	0.242	2.8×10^{-4}	4.0×10^{-4}
Two-layer, with lecithin	2.490	90	0.242	2.8×10^{-4}	3.9×10^{-4}
Emulsion, 0.6% lecithin	0.161	3.2	16,800	8.4×10^{-4}	1.2×10^{-2}
Emulsion, 1.2% lecithin	0.258	7.3	28,300	8.6×10^{-4}	2.7×10^{-3}
Emulsion, 2.4% lecithin	0.579	17.6	38,900	8.3×10^{-4}	1.9×10^{-3}

The catalysis of the hydrolysis by the degradation product phenol is allowed for by a second-order term.

The fits of Eq. (8) to the plots in Figs. 10 and 11 yielded, on using the definitions of H_o and H for Eq. (6), the rate constants h and η compiled in Table 6. This calculation requires the knowledge of the aqueous phenyl salicylate concentration [PS]. In case of the emulsion systems [PS] had to be calculated by using the linear correlation of [PS] and the lecithin concentration (see Fig. 9).

Because of the large specific interfacial areas of emulsions compared with the two-layer systems the loss of lecithin through the formation of the interfacial layer had to be taken into account.

Given the same volume of the oil phase, $V_o = 25$ mL, in all emulsions, the decrease in droplet size means an increase in the interfacial area, A_I, which can be calculated from the number of droplets, N_d:

$$A_I = N_d \pi z_{av}^2 \tag{9}$$

where

$$N_d = \frac{6V_o}{\pi z_{av}^3} \tag{10}$$

Allowing for the total volume V_t of the emulsion and substituting N_d by Eq. (10), the volume-related, or specific, interfacial area

$$A_{IV} = \frac{6V_o}{z_{av} V_t} \tag{11}$$

Assuming for lecithin the molecular surface area, $a = 7.2 \times 10^{-15}$ cm^2 (Wabel, 1998), and the molecular mass, $M = 720$ g/mol, of phosphatidylcholin, the surface concentration of a monomolecular layer,

$$\Gamma_{mono} = \frac{M}{aN_L} \tag{12}$$

where N_L is Avogadro's number, amounts to 1.66×10^{-7} g/cm^2. This surface concentration leads to the masses of lecithin monolayers given in Table 7 for the different emulsions. The droplet diameters of Table 5 were used to calculate, through Eqs. (9) and (10), the interfacial areas, A_I, which were then multiplied with the surface concentration and related to the total volume of 250 mL to obtain the loss in lecithin concentration. The data are in accordance with Férézou et al. (1994a) who separated emulsions containing 10% lipid and 1.2% egg phospholipids (PL) by different ultracentrifugation steps into a lipid cake and a PL-rich aqueous mesophase. For the mesophase he determined a PL concentration of ~5.5 mg/mL. Given a total PL amount of 12 mg/mL, an interfacial PL concentration of 4.7 mg/mL is deemed a sufficient approximation for our calculations (see Table 7).

The total PL amount of the emulsions was corrected by the loss of lecithin through the formation of the interfacial layer. This PL concentration was used to calculate [PS]. The values are shown in Table 6.

5.1. First-order reaction rate constant h without enhancement by phenol catalysis

As pointed out in Section 4.2, the hydrolysis increases with increasing lecithin concentration in the two-layer system. This cannot be attributed, however, to a rise in the first-order reaction rate constant, h, but is due to the higher phenyl salicylate concentration caused by the lecithin forming liposomes in the aqueous phase. The h values for the four different lecithin concentrations roughly remain in the range from 2 to 3×10^{-4} h^{-1} and show no significant tendency with increasing lecithin concentration. In fact, performing the least-square fits with the mean of the four h values, rather than fitting h individually, produces curves that are still covered by the scattering of the measured phenol concentrations. This result confirms that the hydrolysis in emulsions – or two-layer model systems – is a zero-order reaction depending only on the concentration of the degrading compound. Compared to the two-layer system without lecithin, however, it is remarkable that h in the presence of lecithin is lower by more than one order of magnitude. That means lecithin increases the concentration of phenyl salicylate in the aqueous phase, but only a small part of this increase is translated into the acceleration of the zero-order reaction rate of hydrolysis. Probably most of the phenyl salicylate is incorporated into the lecithin liposomes in a way that protects it from hydrolysis. As can be derived from the ratio of h values in the presence and absence of lecithin, only

Table 7
Mass loss of lecithin, m_{mono}, to the monolayers of the different emulsions s

	Lecithin content (%)		
	0.6	1.2	2.4
Mean z_{av} (nm)	357	212	154
A_I (cm^2)	4.2×10^6	7.1×10^6	9.7×10^6
m_{mono} (g)	0.70	1.18	1.62
c_{mono} (mg/mL)	2.8	4.7	6.4

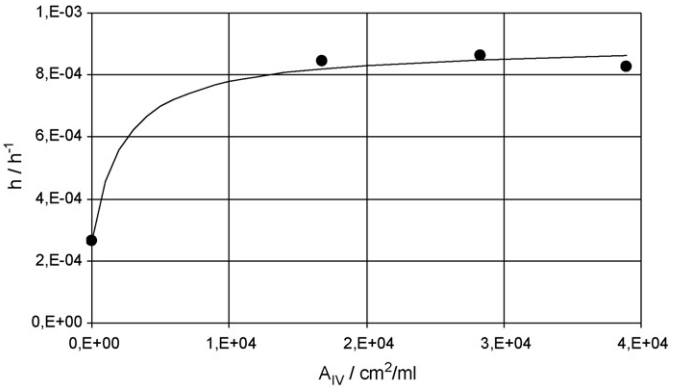

Fig. 13. The basic reaction rate constant, h, without catalytic enhancement by phenol, vs. the specific interfacial area.

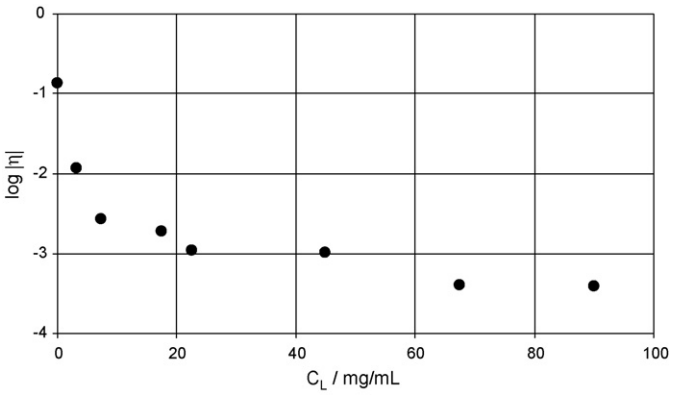

Fig. 14. Rate constant η relating the reaction rate of saponification to the phenol concentration, plotted semi-logarithmically vs. the lecithin content.

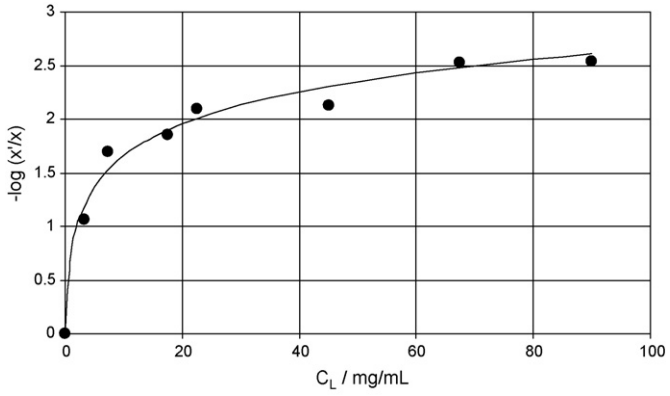

Fig. 15. Portion, x'/x, of the total phenol concentration available for catalysis of the saponification vs. lecithin concentration.

1–1.5% of the phenyl salicylate get in contact with water and are accessible to saponification.

The molar ratio of lecithin over phenyl salicylate is around 30 in the two-layer systems. A value of 32 is also reached for the emulsion containing 2.4% of lecithin after correction for the loss to the interfacial monolayer. Although in this case the protection by incorporation of phenyl salicylate into the liposomes should be the same as for the two-layer system, the h value has more than doubled, an acceleration that might be attributed to the interfacial area as indicated by the plot in Fig. 13. The suggestion is that conditions at the interface favour the hydrolysis by effects such as a different pH microenvironment or special molecular arrangements lowering the activation energy of the saponification. This effect would have fully developed for typical specific interfacial areas prevailing in O/W emulsions as presented in Fig. 13 and would not be enhanced by further increasing the interface. Considering the interface as additional distribution space, the saturation of kinetic enhancement may be explained by the competition of both the drug substance and the lecithin for an interfacial position. The increase of the interfacial area is caused by an increase of the lecithin concentration, which initially shifts the phenyl salicilate distribution towards the interfacial space thus supporting the degradation. Higher lecithin concentrations, however, would not lead to a corresponding increase of interfacial phenyl salicilate as the lecithin molecules would occupy the additional interfacial positions.

5.2. Enhancement by phenol catalysis, second-order rate constant η

As with the first-order reaction rate term it is worth studying the influence of the lecithin content in the systems. Fig. 14 shows the semi-logarithmic plot of η versus lecithin content.

Regardless of the interfacial area a decline of η is observed with increasing lecithin content. As with phenyl salicylate, an incorporation of part of the phenol into the liposomes may be assumed, where it would not be able to act as a catalyst of saponification.

The measured η values in Table 6, based on the total phenol concentration, would then be apparent rate constants, diminished by the reduced phenol concentration available for catalysis.

The portion of the phenol concentration outside the liposomes would be provided by the ratio of any η value of Table 6 in the presence of lecithin over that without lecithin, 1.3×10^{-1} L/(mmol h). Denoting the catalyzing part of the phenol concentration x' whereas its total concentration in the aqueous phase is x, as used in Eq. (5), the catalytic portion of phenol, x'/x, was plotted semi-logarithmically versus lecithin content in Fig. 15. The fitted curve in the diagram is based on an incorporation of phenol into the liposomes following an equation adopted from Langmuir's adsorption isotherm:

$$x - x' = \frac{x_s x'}{K + x'} \tag{13}$$

where x_s is the saturation concentration of phenol in the lecithin liposomes. Following this interpretation, the interface between oil and water has no bearing on the catalytic effect of phenol. The apparent decline of η with increasing lecithin concentration only reflects the removal of part of the phenol into the liposomes where it is no longer available to catalyze the saponification of phenyl salicylate.

6. Conclusion

Saponification of salicylic acid in O/W emulsions follows a zero-order reaction kinetics catalyzed by the degradation

product phenol. Parameters affecting the kinetics are emulsifier concentration and interfacial area between oil droplets and aqueous phase. The interface does not act as a diffusion barrier for the material transfer of salicylic acid from oil to water, but enhances the reaction rate probably because of the pH of the microenvironment or a molecular arrangement favouring the hydrolysis. Lecithin as emulsifier shifts the partition coefficient of salicylic acid towards higher concentrations in the aqueous phase, thus accelerating the hydrolysis. This is counteracted, however, by incorporation of part of the salicylic acid into the liposomes formed by excess lecithin, where the compound is protected from saponification. Likewise, the degradation product phenol is incorporated into the liposomes so that part of it is removed as catalyst of the saponification.

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