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# Rheological properties of phospholipid-stabilized parenteral oil-in-water emulsions—effects of electrolyte concentration and presence of heparin

Mats Silvander<sup>a,\*</sup>, Anders Hellström<sup>a</sup>, Torbjörn Wärnheim<sup>b</sup>, Per Claesson<sup>c</sup>

<sup>a</sup> YKI, Institute for Surface Chemistry, Box 5607, SE-114 86 Stockholm, Sweden

<sup>b</sup> Fresenius-Kabi, Rapsgatan 7, SE-751 74 Uppsala, Sweden

<sup>c</sup> Department of Chemistry, Surface Chemistry, Royal Institute of Technology, Drottning Kristinas väg 51, SE-100 44 Stockholm, Sweden

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## Abstract

The rheological properties of the parenteral oil-in-water emulsion Intralipid<sup>TM</sup> were investigated. The viscosity data at different phase volumes correlated well with that obtained via a theoretical model developed by Yaron and Gal-Or. The model also describes the temperature dependence well. The effects of electrolyte addition were also investigated. Monovalent sodium chloride had practically no influence on viscosity. Calcium chloride, on the other hand, had a large impact on viscosity even at low concentrations. It was shown that the obtained maximum in viscosity coincided with the zeta-potential being close to zero. The resulting increase in viscosity is due to flocculation that leads to an increase in apparent phase volume. A similar behaviour was obtained with magnesium chloride with the difference that the maximum in viscosity was shifted to higher electrolyte concentrations. This is interpreted as that because magnesium binds strongly to the hydration water than does calcium. The addition of the negatively charged anti-coagulant heparin causes flocculation in the presence of small amounts of calcium. The amounts of calcium needed for such bridging flocculation is lower than what is needed in order to create a positive potential at the surfaces of the droplets. A fraction of the flocs is not broken down even by extensive shear.

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**Keywords:** Rheology; Viscosity; Intralipid<sup>TM</sup>; Specific adsorption; Bridging flocculation; Emulsion

## 1. Introduction

Phospholipid stabilised lipid emulsions used for parenteral nutrition have a proven long term physical and chemical stability. However, when used in clinical practice they are often mixed with other macro-

and micronutrients such as amino acids, glucose, vitamins and electrolytes and this could lead to that the emulsion stability is affected (Johnson et al., 1989; Silvers et al., 1998). Because of the potential clinical impact of instability, the effect of such additives have been extensively investigated, in particular the flocculation rates in model emulsions with added electrolyte (Washington, 1990a,b; Washington et al., 1989, 1990). The emulsion droplets carry a substantial negative potential that is important for the emulsion stability and is affected by the unspecific

\* Corresponding author. Present address: BioAgri AB, Box 914, SE-751 09 Uppsala, Sweden. Tel.: +46-18-67-4917; fax: +46-18-67-4901.

E-mail address: mats.silvander@bioagri.se (M. Silvander).

and specific interactions between the droplet surface and electrolytes (Washington, 1990a,b; Washington et al., 1989, 1990). Another additive sometimes used in parenteral nutrition admixtures is heparin, which is added to prevent coagulation and thrombosis (Johnson et al., 1989). This component is also recognised to affect the emulsion stability, in particular used in combination with divalent cations.

While the investigated parenteral fat emulsions in general have contained a limited amount of oil (volume fraction  $\phi < 0.3$ ), rheological investigations of emulsions, with few exceptions, have dealt with more concentrated systems (Pal, 2000b; Richardson, 1953; Tadros, 1994). Rheological characterisation in the less concentrated regimens is important for many applications in order to gain a better understanding of how the emulsions are affected during flow. Since emulsions under such relative dilute conditions display viscosities close to that of water a measuring technique of high sensitivity is needed. The double-gap geometry ensures a large surface area in contact with the dispersion and thus, efficiently increases the sensitivity.

The present study is aimed at using rheological measurements to study any changes in emulsion structure or stability brought about by the presence of selected components of practical relevance. Firstly, the effect of parameters such as phase volume of oil and temperature on the viscosity of the dispersions has been investigated. Secondly, addition of monovalent or divalent electrolytes at different concentrations has been monitored. Finally, the influence of added heparin was examined. In parallel to the rheological measurements, studies of the emulsion droplet size distribution and zeta-potential were carried out to obtain a more comprehensive description of the system.

## 2. Materials and methods

### 2.1. Materials

Intralipid<sup>TM</sup> 10, 20 and 30% was obtained from Fresenius-Kabi, Uppsala. The Intralipid emulsion consists of soybean oil stabilised by purified egg phospholipids. The main phospholipid components are phosphatidylcholine and phosphatidyl-ethanolamine. In addition, a small amount of negatively charged lipids are present causing a negative droplet surface

potential at neutral pH. Heparin was obtained from LEO. Sodium chloride, calcium chloride, and magnesium chloride were of analytical grade and purchased from Merck. Glycerol of analytical grade was obtained from Sigma. Egg-lecithin was obtained from Lipid Products, Nutfield, UK.

### 2.2. Liposome preparation

Liposomes were prepared by evaporating egg-lecithin dissolved in chloroform. The vial with the resulting lipid film was stored under vacuum over night. Medium was added to the lipid film and after eight cycles of freeze-thawing the sample was extruded through a polycarbonate filter of 100 nm pore size at least 10 times.

### 2.3. Rheology

A Bohlin VOR rheometer was used to determine viscosity. A double-gap (DG 24/27) geometry was employed for high accuracy and sensitivity. The distance between the shearing surfaces was 3 mm. The shear rate was, unless otherwise stated, kept below 200 s<sup>-1</sup> since turbulence occurred at higher shear rates. At the conditions used, the dispersions displayed Newtonian behaviour. All presented data points on viscosity represent a mean value of at least four times and the standard deviation was less than 5% in all cases.

### 2.4. Particle size

The size distributions of the emulsions were determined by laser diffraction. For this purpose a Mastersizer 2000 from Malvern Instruments was used. The method uses Mie-theory in order to determine the particle size distribution from the diffraction pattern.

### 2.5. Zeta-potential

An acoustosizer from Matec Applied Sciences was used in order to determine the zeta-potential of the emulsion droplets. The method is described in detail elsewhere (O'Brien et al., 1993). In short, an oscillating electric field is generated between two gold-plated disc electrodes immersed in the emulsion. In turn the droplets will oscillate in the field depending on their effective surface charge. This creates a sound wave that

is detected. The zeta-potential can be obtained when the droplet fraction and the density of the droplets are known. The presented data on zeta-potential are from one single measurement even though the experiments were performed at least twice for control.

## 2.6. Theoretical

Several models for describing the rheological behaviour of particle dispersions are available. A prerequisite for the models to be valid is that the particles are spherical and non-aggregated. The former assumption is reasonable as long as the capillary number  $N_{Ca} = \eta_c \dot{\gamma} / (\sigma / R)$  is small. Here  $\eta_c$  is the viscosity of the continuous phase,  $\dot{\gamma}$  the shear rate,  $R$  the droplet radius and  $\sigma$  the interfacial tension. The conditions in the present study were such that the capillary number was in the order of  $10^{-6}$ . The assumption of a spherical shape of the droplets is thus valid.

One of the simplest models is described by the Einstein equation that relates the relative Newtonian viscosity,  $\eta_r$ , to the particle volume fraction,  $\phi$  (Barnes, 1994; Tadros, 1994).

$$\eta_r = \frac{\eta}{\eta_c} = 1 + 2.5\phi$$

The equation is applicable for a diluted system (no particle interactions) of solid particles (no slip).

The Taylor equation is better suited to describe the effect of dispersed liquid droplets since it allows for tangential stress to cause flow within the droplets. Still, the model does not take particle interactions into consideration and is thus only valid for very dilute systems, i.e.  $\phi < 0.01$  (Barnes, 1994; Tadros, 1994).

$$\eta_r = \frac{\eta}{\eta_c} = 1 + \frac{5\eta_d / (\eta_c + 2)}{2(\eta_d / \eta_c + 1)}\phi$$

A more general theory was early developed (Yaron and Gal-Or, 1972). Their cell model is based on the assumption that the field properties around a droplet attain extreme values at some distance away from the droplet and that the cell describes the statistical average of the ensemble of randomly moving particles. The resulting expression for the relative viscosity reads:

$$\eta_r = 1 + \left\{ \frac{5.5[4\lambda^7 + 10 - (84/11)\lambda^2 + (4\eta_c / \eta_d)(1 - \lambda^7)]}{10(1 - \lambda^{10}) - 25\lambda^3(1 - \lambda^4) + (10\eta_c / \eta_d)(1 - \lambda^3)(1 - \lambda^7)} \right\} \phi$$

Here  $\lambda$  stands for  $\phi^{1/3}$ . Despite that the only parameters that enter the equation are the oil volume fraction and the viscosities of the dispersed and the continuous phases, the model has been shown to describe experimental data in semi-dilute and concentrated regimes fairly well (Pal, 2000a).

## 3. Results and discussion

The particle sizes of Intralipid<sup>TM</sup> emulsions having different concentrations were determined by use of laser diffraction. The size distribution for the 10% Intralipid is shown in Fig. 1. For the more concentrated Intralipid the size distributions were somewhat shifted towards larger droplets even though the measured sizes were in the range 0.04–1 µm. The average particle sizes were 0.24, 0.33 and 0.40 µm, respectively for 10, 20 and 30% Intralipid. Only very small variations (<3%) in average size between batches could be detected. It should be recognised that values of the droplet sizes measured and quoted in the literature will depend on the measuring principle and on the actual instrumentation because of varying sensitivity over the measured size range. The measured droplet sizes contain a substantial contribution from the smaller population of liposomes as discussed below, which according to the compositional difference will overestimate the emulsion droplet size differences between the different products.

The viscosity of the emulsions with different Intralipid concentrations was determined. The effect of phase volume on viscosity is shown in Fig. 2. As expected an increase in viscosity is obtained upon increasing the phase volume of oil. The experimental data are compared with two different theoretical models. As can be seen, the data correlates well with the Yaron/Gal-Or model in the whole interval. In contrast, only for the most diluted samples the data are consistent with the Taylor model. This is as expected (see Section 2.6).

Further, the temperature dependence was investigated within a practical range of 10–60 °C. As expected an increase in temperature leads to reduced

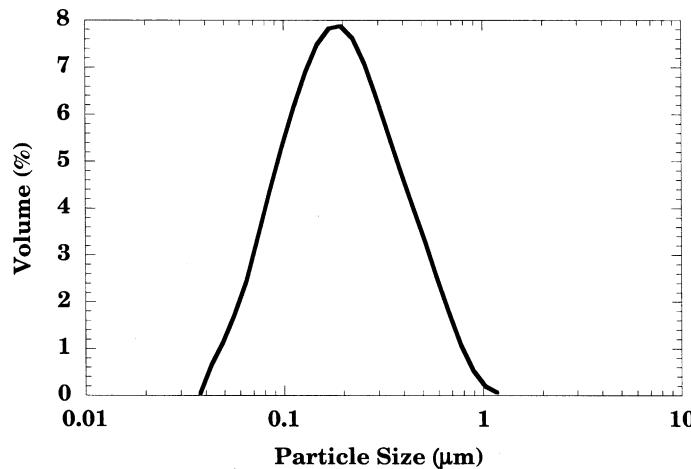


Fig. 1. Size distribution of 10% Intralipid<sup>TM</sup>.

viscosity. The data was compared to the models above using the viscosity of the continuous phase (3% glycerol in water) and the oil phase from literature (Weast, 1988). The result shows that the Yaron/Gal-Or model describes the effect of temperature well (Fig. 3).

The influence of the size distribution of the droplets is worth commenting on since the models used do not consider this parameter at all. A smaller droplet size is expected to increase the viscosity due to shorter mean interparticle distances (Tadros, 1994). However, studies have shown that the actual size of the droplets has no measurable effect (Pal, 2000a; Tadros, 1994) on

the Newtonian viscosity unless the oil fraction is well above 0.5. At higher oil fractions, on the other hand, there is a dependence on particle size, i.e. the smaller the droplets the higher the viscosities (Pal, 2000a). More concentrated emulsions also display non-Newtonian behaviour. We conclude that the applied theoretical Yaron/Gal-Or model describes the main appearance of the emulsions under study even though it cannot be expected to predict effects of subtle changes.

In order to investigate how partial coalescence affected the emulsions, samples containing 30% Intralipid were heated to 80 °C for 10 min and then

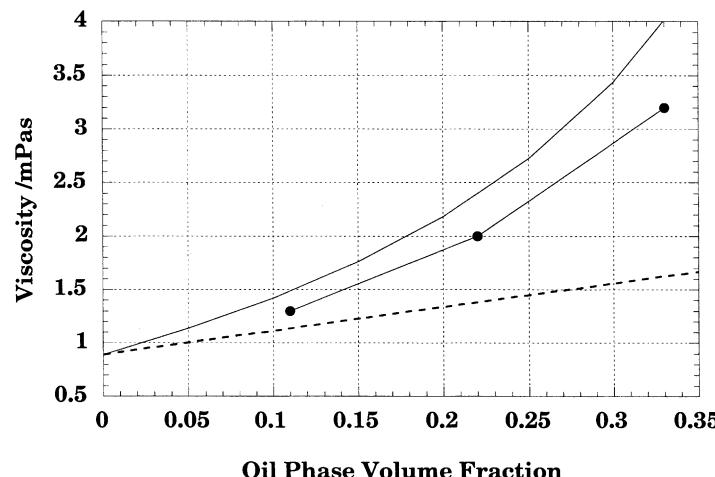


Fig. 2. Viscosity as a function of phase volume:  $T = 25^\circ\text{C}$ ; solid line, Yaron/Gal-Or model; dotted line, Taylor model.

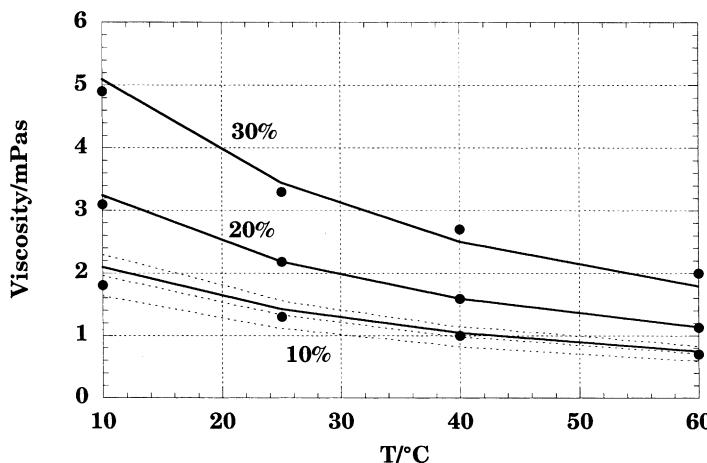


Fig. 3. Viscosity as a function of temperature: solid line, Yaron/Gal-Or model; dotted line, Taylor model; shear rate,  $60\text{ s}^{-1}$ .

cooled to 25 °C again. After heating, the viscosity was reduced by 5–10%. The reduction in viscosity for the heated samples reflects a reduced number of particles, i.e. coalescence has taken place. Thus, the Yaron/Gal-Or model describes the general behaviour of the emulsions well, but it does not account for structural changes that effect the properties on a finer scale. It is important to stress the model cannot either be expected to work for deformed or aggregated droplets.

Parenteral fat emulsions contain an excess of phospholipids that coexist with the emulsion droplets as liposomes (Rotenberg et al., 1991). An estimate gives

that the concentration of lipids in the form of liposomes is in the order of a few mM (Rotenberg et al., 1991). In our study, the viscosity of a pure 2 mM liposome (mean droplet size 100 nm) dispersion was found to be 0.93 mPa s. In addition, Intralipid 10% was diluted 1:1 with either water or a liposome dispersion. The measured viscosities were 1.01 and 1.07 mPa s, respectively. The obtained data, thus, suggest that the influence of the liposomes on the overall viscosity is minimal.

The investigated emulsion droplets carry a potential in the order of  $-65\text{ mV}$  as measured using the electroacoustic technique (Fig. 4). These values are

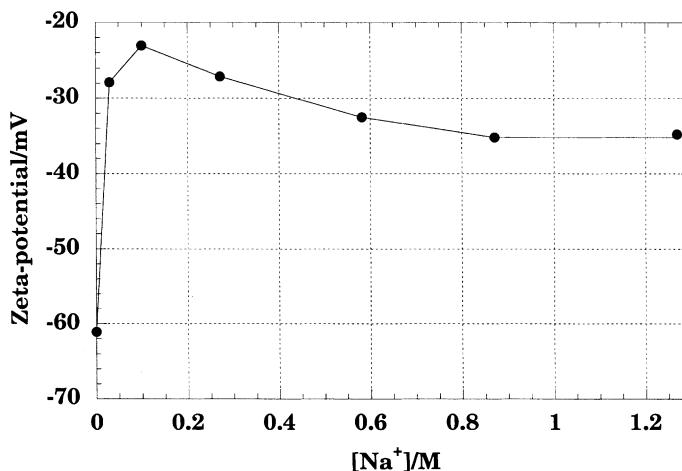


Fig. 4. Zeta-potential for different NaCl concentrations: oil volume fraction, 0.2.

somewhat higher as compared to earlier literature values using the same technique, which is around  $-40$  to  $35$  mV (Carasso et al., 1995). The reason for this discrepancy is not evident even though it is recognised that due to the low ionic strength small differences in ion concentration between different samples will yield large differences in zeta-potential. In contrast to photon correlation techniques (Washington, 1990b) the acoustosizer measurements do not need any dilution of the native concentrated emulsion. Because of this the experimental setup and the interpretation of data is more straight forward compared to when dilution is required, since the zeta-potential is very sensitive to the nature of dilution medium (Washington, 1990b). For diluted systems values of  $-45$  to  $40$  mV has been reported (Washington, 1990a; Washington et al., 1989, 1990).

The data in Fig. 4 deserve some comment. A somewhat surprising maximum, i.e. least negative value, in zeta-potential is seen for concentrations close to  $100$  mM NaCl. Earlier studies have shown that the zeta-potential is dependent on pH (Lindstrom et al., 1999). This is due to the presence of polar lipids that have acid/base functionality. What is also known is that the degree of ionisation depends on the ionic strength of the solution. A high ionic strength decreases the free energy of the electrical double-layer. This admits a larger degree of ionisation of ionisable groups on the emulsion droplet surface and thus, a higher surface charge density is obtained. It can not

also be ruled out that a preferential adsorption of chloride over sodium occurs. This could be rationalised by the higher polarisability for the anion. We suggest that the appearance of Fig. 4 is due to the increased surface charge density. For comparison the calculated surface charge density at the plane of shear is shown in Fig. 5. The shear surface charge density,  $\sigma$  was calculated from the known zeta-potentials,  $\psi_0$  and ionic strengths by use of the Grahame equation:  $\sigma = \sqrt{8\epsilon\epsilon_0 kT} \sinh(e\psi_0/2kT) \sqrt{[\text{NaCl}]} \quad (\text{Israelachvili, 1994})$ . The surface charge density increases linearly above  $0.2$  M. The unexpected decrease in zeta-potential at elevated ionic strength is thus due to an increase in surface charge density that predominates the screening effect.

The viscosity of the emulsions was not affected notably by the addition of practically relevant concentrations of monovalent electrolyte. Concentrations upto  $150$  mM had no measurable effect. At concentrations above  $500$  mM a small increase in viscosity was seen but this increase corresponds well with literature data on the viscosity increase obtained when sodium chloride is dissolved in water (Weast, 1988). The minute effect is thus due to the bulk properties of the brine itself and not due to any impact of the salt on the droplet interactions.

In contrast, addition of calcium chloride results in a substantial increase in viscosity with a maximum in the vicinity of  $3$  mM (Fig. 6). Above this concentration the viscosity again decreases. The originally negative

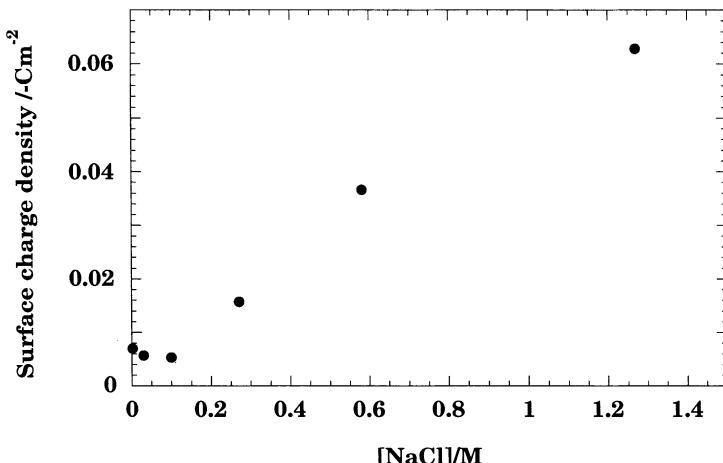


Fig. 5. The dependence of surface charge density on sodium chloride concentration.

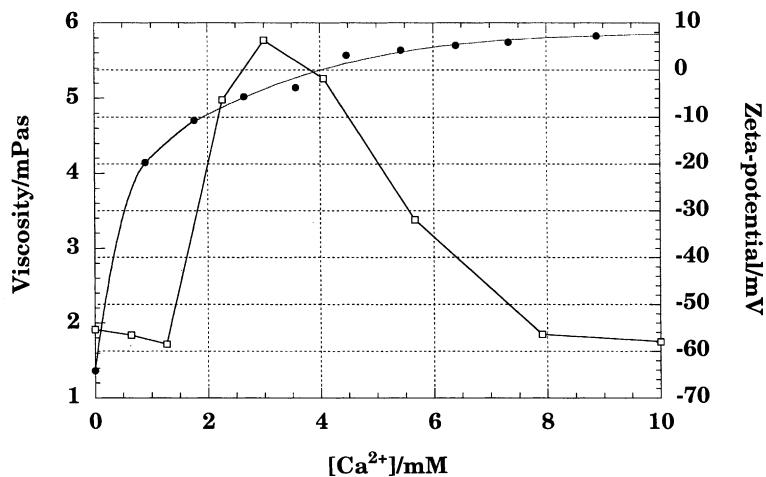


Fig. 6. Viscosity and zeta-potential at different calcium chloride concentrations: (□), viscosity; (●), zeta-potential; oil volume fraction, 0.2; shear rate, 60 s<sup>-1</sup>.

zeta-potential is reduced upon calcium addition and is finally changing sign to positive. This behaviour is by now well established and is considered due to specific adsorption of calcium ions to the droplet surface (Washington, 1990b). It is obvious that the maximum in viscosity coincides with the region where the absolute zeta-potential is very small. This indicates that the origin of the viscosity increase is flocculation. Flocculation increases the apparent phase volume of the dispersed droplets since the void space between the aggregated droplets captures water. Importantly, the

appearance of the maximum viscosity also coincides with the maximum rate of flocculation as determined by turbidimetry (Washington, 1990b).

The flocculation appears, however, to be loose in character. No alteration in size distribution could be determined by the laser diffraction technique. In this context, it is important to recognise that the employed technique subjects the sample to substantial shear within the sample chamber that is why only more tightly bound flocs are expected to last. The obtained maximum in viscosity corresponds to an apparent

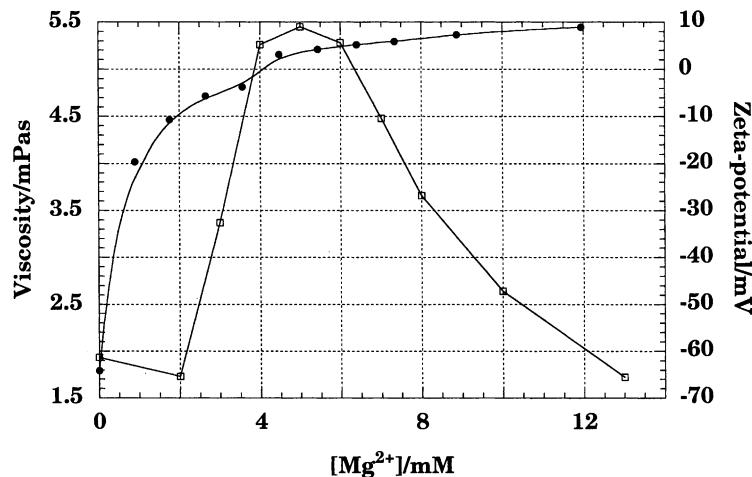


Fig. 7. Viscosity and zeta-potential at different magnesium chloride concentrations: (□), viscosity; (●), zeta-potential; oil volume fraction, 0.2; shear rate, 60 s<sup>-1</sup>.

phase volume of 30%. An additional interesting feature is the decrease in viscosity at concentrations below 1.5 mM. This small but significant effect is due to the decrease in electrostatic screening length and is often referred to as a secondary electroviscous effect (Hiemenz and Rajagopalan, 1997).

Similar experiments were performed exchanging calcium chloride for magnesium chloride as electrolyte (Fig. 7). The data have the same principle appearance but, interestingly, the maximum in viscosity is shifted to higher concentrations for magnesium compared to calcium. This difference cannot be traced to any difference in the amount of ions needed to neutralise the surface. However, the finding is in accordance with other data showing that the binding affinity to phospholipid surfaces is stronger for  $\text{Ca}^{2+}$  than for  $\text{Mg}^{2+}$  (Newton et al., 1978; Nir, 1984; Yermiyahu et al., 1994). The molecular origin of this effect is probably that the hydration water is stronger bound to the magnesium ion than what is the case for the calcium ion, as manifested in e.g. longer exchange rates (Israelachvili, 1994).

The negatively charged polysaccharide heparin was added to Intralipid<sup>TM</sup> at different concentrations. Heparin has previously been reported to induce flocculation in similar systems via a bridging mechanism under certain conditions (Johnson et al., 1989). Neither size, zeta-potential of the droplets, nor viscosity were affected by the presence of heparin in the range 1500–8000 IU/l without addition of divalent ions.

(1 IU  $\approx$  6 mg). This was expected since heparin and the droplets are both negatively charged. In contrast, a small amount of calcium chloride added to the Intralipid<sup>TM</sup>/heparin mixture causes a significant increase in viscosity. Importantly, this occurs even at concentrations where the zeta-potential is still significantly negative. Here it is worth mentioning that the change in zeta-potential upon increasing the calcium concentration was the same as without heparin (data not shown). The data indicate that heparin with bound calcium in turn binds to the negative surfaces of the droplets, which may cause flocculation by the bridging mechanism. In favour of this interpretation is also the observation that no substantial effect on viscosity was obtained upon further addition of calcium chloride from 1 mM up to 22 mM (Fig. 8). At the latter concentration a positive zeta-potential is expected.

Thus, it appears as if the zeta-potential of the emulsion droplets does not govern the bridging flocculation. The flocculation, as determined by an increase in viscosity, is similar at low concentrations where the zeta-potential is negative and at concentrations where the potential is positive.

A variation of the shearing rate (Fig. 9) clearly shows that shearing of the flocculated samples leads to some deflocculation that is detected as a decrease in viscosity. The steady-state viscosity obtained is still much elevated compared to the corresponding phase volume without heparin. If extensive coalescence would appear this would decrease the viscos-

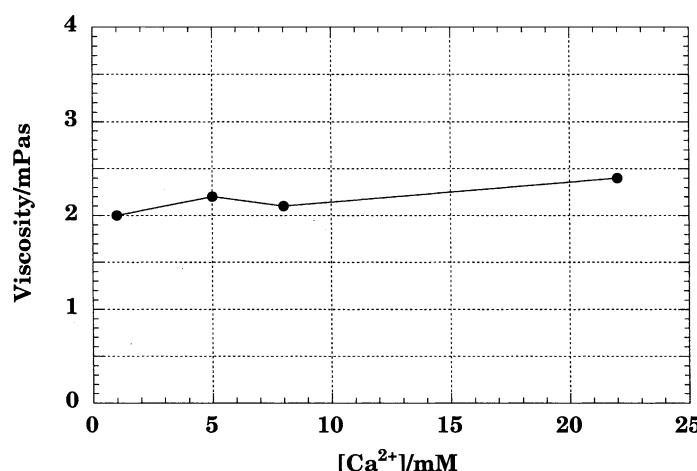


Fig. 8. Viscosity of a 5% Intralipid emulsion containing: 8000 IU/l heparin at different calcium concentrations: shear rate, 50 s<sup>-1</sup>.

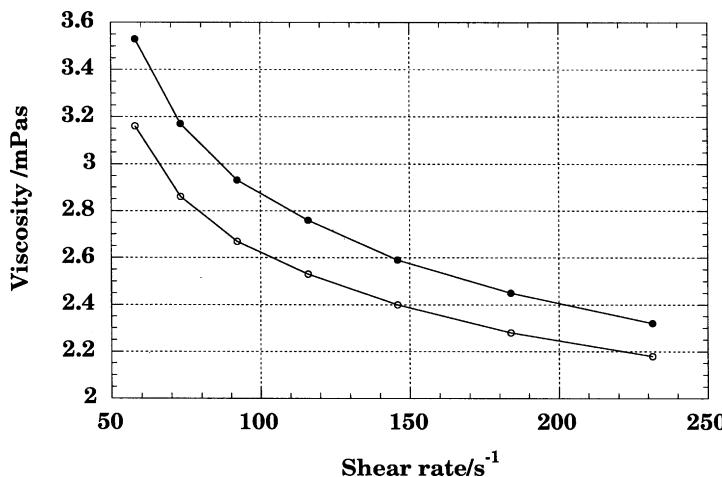


Fig. 9. Viscosity of a 5% Intralipid™ emulsion containing 5 mM CaCl<sub>2</sub> and heparin of two different concentrations. (●), 15,000 IU/l; (○), 12,000 IU/l. Before measuring the samples were pre-sheared at 20 s<sup>-1</sup> for 30 s and left standing without shear for another 30 s.

ity due to the growth in droplet size. Since this is not observed the degree of coalescence is probably minor. The elevated viscosity, even at high shear rates, shows that a significant fraction of the flocs is held together strongly enough not to be deaggregated in the shear field. Another interesting observation was that the actual level of the viscosity was shown to differ between similar preparations. This shows that the mixing procedure and sample history in general is very important for this kind of samples. This can be related to the slow conformational changes of adsorbed polyelectrolytes, in this case heparin, and the slow redistribution of adsorbed heparin between individual emulsion droplets. Thus, the equilibrium situation is reached very slowly. Nevertheless, the same trends were observed for the different samples.

#### 4. Conclusions

The Yaron/Gal-Or equation predicts the viscosity well in the region  $\phi = 0.1\text{--}0.3$  for the investigated Intralipid™ emulsions. This also applies for the temperature dependence where the viscosity of the continuous phase is decisive for the emulsion viscosity.

When sodium chloride is added no effect on the viscosity is obtained except at very high concentrations where a small increase is observed. This is due to the

electroviscous effect of the brine, similar to that obtained for sodium chloride in pure water.

Addition of divalent ions causes a transition from a negative zeta-potential to a positive one due to specific ion adsorption. When the zeta-potential is close to zero an increase in emulsion viscosity by about a factor of three is found. This is due to flocculation. A larger amount of magnesium chloride is needed to obtain the same viscosity effect as for calcium chloride. This is due to the higher binding affinity of the calcium ions.

The presence of heparin induces flocculation in the presence of calcium chloride. Importantly, the amounts of calcium needed for efficient flocculation are not enough to create a positive zeta-potential of the droplet surfaces. We interpret this as a binding of calcium ions to heparin that subsequently binds to the negative emulsion surface. An important fraction of the flocs is not broken down by extensive shear, which indicates a strong binding within the flocs.

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