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International Journal of Pharmaceutics 107 (1994) 121–132

international journal of pharmaceutics

Fat emulsions for parenteral nutrition. IV. Lipofundin MCT/LCT regimens for total parenteral nutrition (TPN) with high electrolyte load

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(Received 20 July 1993; Modified version received 26 November 1993; Accepted 13 December 1993)

Abstract

The stability of TPN regimens with high electrolyte load based on the emulsions Lipofundin MCT/LCT and Intralipid was investigated. The regimens were of identical composition containing 66.7 mmol/l monovalent ions and 6.7 mmol/l divalent ions (3.4 mmol Ca^{2+}, 3.3 mmol other divalent ions such as Mg^{2+}). Stability was monitored by microscopy, Coulter counter, photon correlation spectroscopy (PCS) and laser diffractometry. Zeta potential measurements were performed by laser Doppler anemometry (LDA). The Lipofundin regimen was regarded as being stable for 7 days of storage, the Intralipid regimen for at least 2 days but not 7 days. The storage time was limited due to visible heavy creaming and the increased numbers of particles > 4 μm. Microscopic, Coulter counter and PCS two-time-window analysis proved most suitable for monitoring the stability. Analysis of the top phase of the regimens proved to be distinctly more sensitive than that of the homogeneous mixture of the regimens after shaking. The instabilities observed were mainly attributed to the reduction of the zeta potential to the range of maximum flocculation.

Key words: Fat emulsion stability; Parenteral feeding mixture; Lipofundin MCT/LCT; Intralipid; Medium chain triglyceride (MCT); Total parenteral nutrition

1. Introduction

The physical stability of fat emulsions is limited by the formation of large droplets with the potential to block blood capillaries. In many stability investigations only one or two particle sizing methods are applied. Comparing such studies is difficult if different methods are used, since limited information is available about the comparability of the methods themselves. Previously, a broad range of different sizing methods has been compared with regard to their sensitivity and ability to characterize emulsions (Müller and Heinemann, 1992). The emulsions used in this study were specially prepared model emulsions containing increasing numbers of large particles. The different methods were then applied to assess the physical long-term stability (2 years) of

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Elsevier Science B.V.
SSDI 0378-5173(93)E0378-4

"real" emulsions, i.e., commercial Lipofundin MCT/LCT emulsions (Müller and Heinemann, 1993). MCT/LCT emulsions were chosen because they had been recently introduced to the market and – compared to traditional LCT emulsions – less stability data are available. In this study photon correlation spectroscopy (PCS) proved to be most sensitive in monitoring the extremely small changes which occurred during 2 years of storage. Systems with more distinct changes in particle size distribution are electrolyte containing regimens for parenteral nutrition. Lipofundin MCT/LCT based regimens for TPN containing a low and medium electrolyte load have been investigated recently (regimens I–III; Müller and Heinemann, 1994). These regimens were stable for at least 21 days, microscopic photography and Coulter counter analysis being most suitable to quantify the fraction of large particles. To assess the suitability of the methods to characterize rapidly coalescing systems, Lipofundin MCT/LCT regimens with high electrolyte load were prepared. Regimens were also prepared with a traditional LCT emulsion – Intralipid – to determine which method would be most suitable to detect effects on stability caused by displacement of one commercial product by another.

2. Materials and methods

2.1. Materials

Lipofundin MCT/LCT emulsion 20% and the solutions (trade products) glucose 5, glucose 40, glucose 50 and potassium phosphates were purchased from B. Braun Melsungen (Melsungen, Germany). The mean droplet diameters of the Lipofundin MCT/LCT emulsions were 274 and 326 nm, the zeta potentials measured in distilled water −39.8 and −38.2 mV (10 and 20% emulsion, respectively), the size difference ΔD 14 nm (cf. section 2.2) and the polydispersity index 0.10 for both emulsions (photon correlation spectroscopy data). Intralipid 20% (mean droplet diameter 380 nm, size difference ΔD 26 nm, polydispersity index 0.15, zeta potential in distilled water −37.5 mV), Addamel, Addiphos and Vamin 14 were purchased from KabiVitrum (Uxbridge, U.K.).

Vamin 14 and Aminoplasmal L-10 contained a mixture of amino acids and electrolytes, respectively. Electrolytes in Vamin 14 were as follows: Na, 100.0 mmol/l; K, 50.0 mmol/l; Ca, 5.0 mmol/l; Mg, 8.0 mmol/l; Cl, 100.0 mmol/l; sulfate, 8.0 mmol/l; acetate, 135.0 mmol/l. Electrolytes in Aminoplasmal were: Na, 45.0 mmol/l; K, 25.0 mmol/l; Mg, 2.5 mmol/l; acetate, 59.0 mmol/l; Cl, 62.0 mmol/l; H_2PO_4, 9.0 mmol/l; malate, 7.5 mmol/l. Glucose 5/40/50 contained 55 g/440 g/550 g glucose monohydrate per l. Composition of the electrolyte solutions: potassium phosphates (K, 1.0 mmol/ml; phosphate, 0.6 mmol/ml), potassium chloride, 7.45% (K, 1 mmol/ml; Cl, 1 mmol/ml), sodium chloride, 5.85% (Na, 1 mmol/ml; Cl, 1 mmol/ml), Addamel ($CaCl_2$, 0.50 mmol/ml; $MgCl_2$, 0.15 mmol/ml; $FeCl_3$, 5.0 μmol/ml; $ZnCl_2$, 2.0 μmol/ml; $CuCl_2$, 0.5 μmol/ml; NaF, 5.0 μmol/ml; KI, 0.1 μmol/ml), Addiphos (phosphate, 2 mmol/ml; K, 1.5 mmol/ml; Na, 1.5 mmol/ml).

TPN bags (Nutrimix™) were obtained from B. Braun Melsungen; the bag volume was 3.0 l, the bag material EVA.

2.2. Methods

The TPN mixtures were prepared as described previously (Müller and Heinemann, 1994) and stored at 4°C. Their composition is given in Table 1 (regimen IV contained Lipofundin MCT/LCT 20%, regimen V Intralipid 20%). For reasons of comparison for the discussion, regimen I with low electrolyte load investigated previously (Müller and Heinemann, 1994) is also listed. The electrolyte loads of monovalent and divalent cations were 66.7 and 6.7 mmol/l water in regimens IV and V, respectively, and 3.4 mmol of the divalent cations were Ca^{2+}.

In total 10 bags per regimen were prepared to study the stability. Prior to removing samples, the bags were stored for 24 h at room temperature to simulate the infusion time. For characterization by light scattering techniques two bags per regi-

Table 1
Composition of Lipodundin MCT/LCT and Intralipid regimens for parenteral nutrition (regimen I after Müller and Heinemann, 1994)

Compound	Regimen no.		
	I	IV	V
Aminoplasmal L-10	1000 ml	–	–
Vamin 14 with electrolytes	–	1000 ml	1000 ml
Glucose 40	1000 ml	–	–
Glucose 5	–	1000 ml	1000 ml
Glucose 50	–	500 ml	500 ml
Lipofundin MCT/LCT 10%	500 ml	–	–
Lipofundin MCT/LCT 20%	–	500 ml	–
Intralipid 20%	–	–	500 ml
NaCl 5.85%	60 ml	–	–
KCl 7.45%	40 ml	–	–
Potassium phosphates	20 ml	–	–
Addiphos	–	15 ml	15 ml
Addamel	–	10 ml	10 ml

men were used. Samples were drawn directly after preparation, and after 1, 2, 4, 7, 14, 21 and 119 days from the same bag. The bags were shaken before withdrawing the samples. Before analysis the samples were stored at room temperature to simulate the infusion.

For microscopic assessment of stability, eight bags per batch were used and investigated directly after preparation (0 days), at 2, 7 and 21 days. Before microscopic analysis the bags were stored for 1 day at room temperature. At each time interval, samples were removed from the top phase of the bag to enhance the statistical probability of finding larger droplets (Müller and Heinemann, 1992, 1994). The term 'top phase' signifies that the samples were drawn 5 mm below the surface of the regimen (bags stored in upright position, no shaking of the bags before sample drawing). Then the bags were shaken and samples withdrawn from the homogeneous mixture of the regimen to assess differences between the two methods of sample drawing. Microscopic assessment of emulsion stability: microscopic photographs were taken from the undiluted TPN mixtures at a magnification of ×1250 as described previously (Müller and Heinemann, 1992). The mean diameters of the bulk population and the presence of larger particles were classified according to a specially developed scheme (cf. Table 2) (Müller and Heinemann, 1992).

A Multisizer I from Coulter Counter (Germany) was employed to determine the content of larger particles per volume unit of fat emulsion. The TPN regimens were diluted with 0.9% NaCl solution to a final concentration of 0.5 g fat/l. From this mixture, 80 μl were analyzed, using a 30 μm capillary. Therefore, the 80 μl TPN-NaCl dilution corresponded to a volume of approx. 2.1 μl (regimen I) and to 1.2 μl undiluted regimen (regimens IV and V). The tables list the absolute numbers of particles > 2.04 and > 4.08 μm per 2.1 and 1.2 μl regimen, respectively. A Malvern Laser Diffractometer 2600 (Malvern Instruments, U.K.) was used for particle size analysis in the range above 0.5 μm. The regimens were characterized by the volume diameter 90% ($D_{(90\%)}$), the maximum particle size detected (= upper size limit of the class in which particles were detected) and the percentage of particles below 1.2, 5.0 and 8.0 μm (Müller and Heinemann, 1992).

Photon correlation spectroscopy was used to determine the diameters of the bulk population ($D_{25\ \mu s}$) and the diameter weighted by the fraction of larger particles present ($D_{100\ \mu s}$) as described previously (Müller and Heinemann, 1992, 1993). The diameters were obtained by applying a sample time of 25 and 100 μs, respectively. The size difference $\Delta D = D_{100\ \mu s} - D_{25\ \mu s}$ is a measure of the fraction of particles distinctly larger than the bulk population of about 0.3 μm (so called two-time-window analysis). The ΔD value increases with increasing number of large particles.

The PCS correlation functions were transformed to a size distribution by Fourier transform (Müller and Heinemann, 1992, 1993, 1994). The PCS system consisted of a Malvern Spectrometer RR 102 with a helium-neon laser (4 mW) and a photomultiplier (PM). The PM signal was fed simultaneously to a 4-bit K7025 correlator and a 1-bit loglin correlator (Malvern). The data from the high resolution 4-bit correlator were used to calculate the diameters and a polydispersity index, the logarithmic function from the loglin correlator being used to calculate the size distribution.

Zeta potential measurements were performed with a Malvern Zetasizer II (Malvern Instruments) as described previously (Müller and Heinemann, 1992). Due to the high electrolyte content of the regimen a small bore capillary (1 mm) was used to minimize the current and Joule heating. The regimens were diluted with a medium containing electrolytes identical to the composition in the TPN bags. Electrolyte mixtures were prepared by replacement of the fat emulsion by a water-glycerol mixture.

3. Results and discussion

3.1. Characterization of fat emulsions

The Lipofundin MCT/LCT emulsions have been used previously for the preparation of regimens with low and medium electrolyte load (Müller and Heinemann, 1994). The size difference between the 10 and 20% emulsions (274 and 326 nm diameter) was attributed to differences in the homogenization conditions, e.g., power density distribution and concentration of dispersed phase. The small size differences ΔD and polydispersity indices placed the emulsions in the category "very fine" according to the microscopic assessment scheme. The PCS diameter of the Intralipid emulsion ($D_{25\ \mu s} = 380$ nm) representing the mean size of the bulk population was distinctly larger. Intralipid was also placed in the category "fine" when considering the low values of PI, ΔD and the $D_{(90\%)}$ (Müller and Heinemann, 1993). The maximum particle sizes detected were greater in Intralipid (5.0 μm) than in Lipofundin (2.4 μm). The large values in Intralipid were regarded as batch dependent. In other batches values similar to that of Lipofundin MCT/LCT were obtained (unpublished data).

The zeta potentials (ZP) were similar for all three emulsions (approx. −40 mV) and in the range typically obtained with emulsions after preparation or following a short storage period. Identical zeta potentials were expected because of the similar composition (egg lecithin as emulsifier). The potentials of about −40 mV are above the −31 mV required for sufficient stabilisation

Table 2
Microscopic assessment of droplet sizes in the regimens I, IV and V based on the microscopic scheme (Müller and Heinemann, 1992, 1993)

Regimen	Storage time (days)	Homogeneous mixture				Top phase			
		Size of bulk population (μm)		Frequency of droplets > / = 3 μm		Size of bulk population (μm)		Frequency of droplets > / = 3 μm	
		A	B	A	B	A	B	A	B
I	0	< 1	< 1	+	+	–	–	–	–
	2	< 1	< 1	+	+	< 1	< 1	+	+
	7	< 1	< 1	+	+	< 1	< 1	+	+
	21	< 1	< 1	+	+	< 1	< 1	+	+
IV	0	< 1	< 1	+	+	–	–	–	–
	2	–	–	–	–	–	–	–	–
	7	< 1	< 1	+ +	+	> 1	> 1	+ + +	+ + +
	21	< 1	< 1	+	+	> 1	> 1	+ + +	+ + +
V	0	< 1	< 1	+ +	+ +	–	–	–	–
	2	< 1	< 1	+	+	> 1.5	> 1.5	+ + +	+ + +
	7	< 1	< 1	+ +	+ +	> 1.5	> 1.5	+ + +	+ + +
	21	< 1	< 1	+ +	+ +	> 1.5	> 1.5	+ + +	+ + +

At each time interval two bags (A and B) of each regimen were analysed. Classifications for the size of the bulk population: < 1 μm, > 1 μm (between 1.0 and 1.5 μm), > 1.5 μm. Frequency of droplets > / = 3 μm: a few particles detectable (+), particles detectable (+ +), particles increasingly detectable (+ + +).

by electrostatic repulsion (Ney, 1973). The electrophoretic measurements were performed in a 1 mm capillary, yielding slightly lower values than in a large 4 mm capillary (Müller and Heinemann, 1994).

3.2. Microscopic assessment of the stability of the regimens

After preparation regimens IV and V both possessed a size of the bulk population below 1 μm similar to regimen I with low electrolyte load (Table 2). This was found to be consistent throughout the storage period of 21 days when analysing the homogeneous mixture of regimens (i.e., after shaking). At day 0 in the Lipofundin MCT/LCT regimen (IV) only a few particles larger than 3 μm were detectable, whereas more were found in regimen V (Table 2). In general, a larger number of droplets > 3 μm was observed in regimen V during the 21 days of storage (homogeneous mixture of regimen).

When analysing the top phase a mean diameter of the bulk phase > 1.5 μm was assessed in regimen V compared to > 1 μm in regimen IV. In both regimens an increased number of droplets > 3 μm was observed. This is in contrast to regimen I with low electrolyte load which remained unchanged for 21 days (Table 2). The size of the largest droplet detected by microscopy remained unchanged in regimen I (2.0 μm) for 21 days. In contrast, it increased to 18–20 μm in the high load regimens IV and V. Large droplets of 20 μm were observed in regimen V on day 2 compared to droplets of only 5 μm in size in regimen IV until day 7. The presence of a few very large droplets does not allow one to conclude a difference in stability of the two regimens, which has a relevance for the administration of the system. As discussed previously in more detail (Müller and Heinemann, 1992), the presence of a few large particles in an emulsion should cause no toxic effects. Apart from their low number the droplets are biodegradable by the lipoprotein lipase situated at the luminal surface of capillary endothelial cells (Bryan et al., 1976). Therefore, possible embolization by a capillary blockade is reversible, and emulsion degradation takes place within a few hours. The microscopic photographs obtained after mixing of regimen IV showed a fine emulsion and the absence of large droplets. On day 7, analysis of the bags after shaking (homogeneous mixture) yielded microscopic photographs similar to those obtained after mixing on day 0. A few distinctly larger droplets could only be detected by analysing the top phase. This demonstrates the importance of the sampling procedure. Similar microscopic photographs were obtained for regimen V although the regimen appeared more coarse after mixing. Very large particles (18–20 μm) were detected in the visibly creamed top phase of both regimens after 21 days (Fig. 1, upper). However, after shaking of the bags these particles disappeared and were no longer detectable (Fig. 1, lower). From the photographs one could assess a stability of 21 days or only 7 days depending on whether the homogeneous emulsion or the top phase were analysed.

To summarize, the differences observed in this microscopic analysis appeared too small to distinguish between regimens IV and V in terms of stability. They are both very similar although regimen IV tended to appear slightly more stable. The largest particles detectable were smaller in regimen IV than in regimen V. In addition, there was more visible creaming in regimen V.

3.3. Coulter counter assessment of the stability of regimens

Analysis of the homogeneous regimens directly after mixing yielded about 1300–1500 particles > 2 μm in the Lipofundin MCT/LCT regimen IV (Table 3). These numbers were similar to those obtained in feeding mixtures with low and medium electrolyte load (e.g., regimen I, Table 3, upper) (Müller and Heinemann, 1994). In Intralipid regimen V up to 37 000 particles > 2 μm were counted. This appears to be a large amount. However, the particles > 4 μm are more relevant with regard to possible capillary blockade. The numbers of these particles were similar in both regimens. The number of particles > 2 μm scarcely changed in regimen IV but decreased in regimen V during the storage period. This phe-

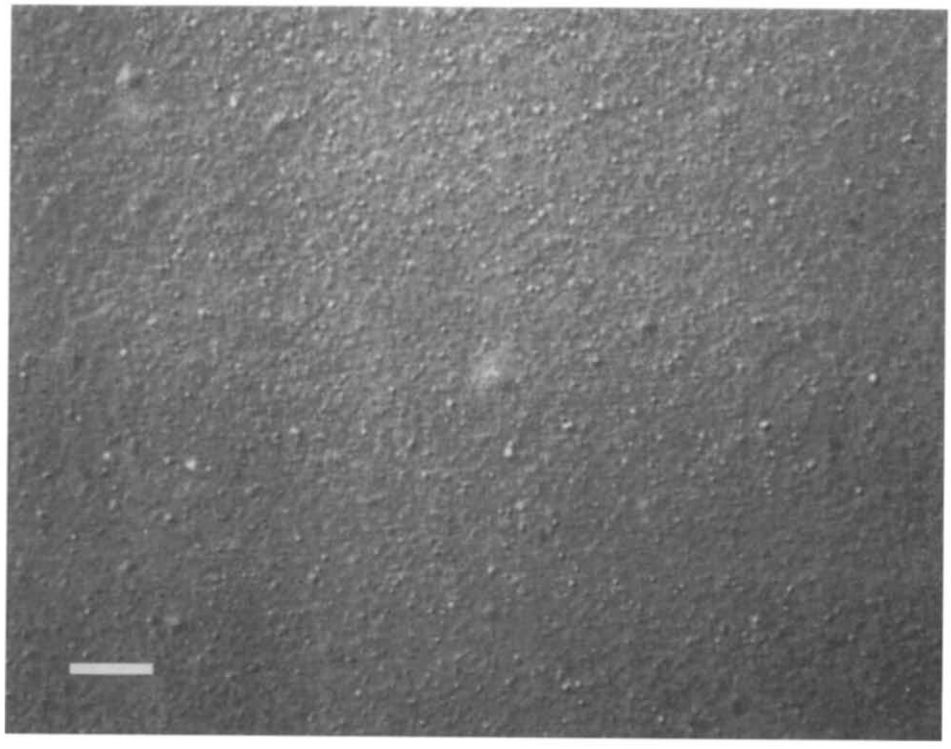

Table 3
Coulter counter results of regimens I, IV and V, number of particles in the top phase and after shaking in the homogeneous mixture of the regimen

Time (days)	Regimen	Number of particles > 2 μm and > 4 μm							
		Homogeneous mixture				Top phase			
		A		B		A		B	
		> 2 μm	> 4 μm	> 2 μm	> 4 μm	> 2 μm	> 4 μm	> 2 μm	> 4 μm
0	I	523	97	275	53	–	–	–	–
2		100	13	60	5	277	24	487	38
7		277	11	189	10	1428	116	1133	116
21		94	13	493	190	529	53	1792	172
0	IV	1370	153	1527	124	–	–	–	–
2		–	–	–	–	–	–	–	–
7		2289	264	906	89	3520	317	12792	1767
21		1365	120	1928	228	55608	20093	24806	3593
0	V	37815	159	15863	79	–	–	–	–
2		3330	385	217	5	– [a]	– [a]	– [a]	– [a]
7		13452	214	17076	223	– [a]	– [a]	– [a]	– [a]
21		30521	253	24050	271	– [a]	– [a]	– [a]	– [a]

[a] Top phase could not be analysed, due to heavy creaming.

nomenon was observed previously in low-electrolyte regimens (Müller and Heinemann, 1994). The decrease in number was attributed to coalescence of the particles to larger droplets. The volume of the large droplets remains constant whereas their number decreases. Therefore, a decrease in particle number is an indication of droplet coalescence. The very low number of particles > 4 μm in the homogeneous mixture of regimen V corresponded with the observed visible heavy creaming. The number decreased because the particles floated to the surface layer. Considering only the number of particles larger than 4 μm in the homogeneous mixture of the regimens one could consider both as being stable for 21 days.

The visible creaming in regimen IV was less distinct than in regimen V and caused no problems when drawing samples from the top phase. At day 21, the increase in the number of particles > 2 and > 4 μm was very distinct in regimen IV (e.g., 55 608 in bag A, Table 3). From these high numbers and a few single large droplets observed during the microscopic analysis, as a precautionary measure the regimen was not considered as being stable at day 21. To be on the safe side, the low particle numbers in the homogeneous mixture of the regimen cannot be the only criterion to limit the storage time.

Analysis of the creamed top phase in regimen V could not be performed on days 2, 7 and 21. The creaming was very heavy and formed a visible thick and firm top layer. On day 7 the regimen was analysed before redispersing the creamed top phase. A sample was drawn from the middle of the bag. The Coulter counter analysis yielded 90 and 112 particles > 2 μm, and two and three particles > 4 μm, for bags A and B, respectively. These extremely low numbers indicated that heavy coalescence must have taken place leading to creaming of the large droplets formed. The large particle numbers found in the homogeneous mixture of the regimen after redis-

Fig. 1. Microscopic photographs of regimen IV after 21 days of storage. Large droplets are increasingly detectable in the sample drawn from the top phase (upper). These particles are no longer detectable in the homogeneous mixture (i.e., after shaking of the regimen, lower), the number of large particles seems to be rather low (bar: 10 μm).

persing of the top phase by shaking confirmed this (approx. 15000 particles >2 μm, approx. 200 >4 μm, Table 3). The heavy creaming leading to a relatively thick top phase might cause problems when infusing the regimen. It is questionable to what extent the creamed layer redisperses after shaking and to what extent flocculates redisperse. Due to safety precautions regimen V is therefore not considered stable at 7 days of storage. However, setting the storage time for regimens IV to at least 7 days appeared justified.

3.4. PCS assessment of the stability of regimens

The PCS diameter remained unchanged for both regimens IV and V throughout the entire storage period of 21 days as observed in low-electrolyte regimens (e.g., I) (Table 4). The width of the size distribution also remained unchanged as indicated by the polydispersity indices (PI) obtained. The higher PI values of regimen V are due to the a priori broad distribution of the emulsion used for the preparation of the bag (PI = 0.15). This confirmed that even at high electrolyte load bulk populations of emulsions do not undergo a shift in size (Müller and Heinemann, 1994). There is no simultaneous coalescence of the total population. Single particles start to coalesce, and the coalescence of these particles continues leading to very large droplets. Eventually they form a second droplet population resulting in a bimodal size distribution of the emulsion. The bulk population, however, remains unchanged as demonstrated by the constant mean diameter even after 119 days of storage (Table 4). The formation of the larger droplets is reflected by the increase in ΔD from 14–26 to 30–50 nm in both feeding mixtures. The reduction to 16 nm in regimen IV after 119 days might be caused by the disappearance of large particles (>5 μm) from the measuring range of PCS (5 nm–5 μm).

Table 4
PCS characterization data determined in two bags (A and B) of each regimen

Storage time (days)	Regimen	Bag A			Bag B		
		$D_{25\,\mu s}$	ΔD	PI	$D_{25\,\mu s}$	ΔD	PI
0	I	274 nm	14 nm	0.11	275 nm	10 nm	0.13
1		273 nm	12 nm	0.12	275 nm	13 nm	0.10
2		277 nm	17 nm	0.12	276 nm	14 nm	0.11
7		272 nm	21 nm	0.12	277 nm	16 nm	0.13
14		276 nm	21 nm	0.11	279 nm	19 nm	0.11
21		271 nm	27 nm	0.10	278 nm	24 nm	0.12
119		265 nm	26 nm	0.13	–	–	–
0	IV	318 nm	29 nm	0.11	316 nm	23 nm	0.12
1		318 nm	32 nm	0.12	318 nm	21 nm	0.12
2		318 nm	35 nm	0.12	318 nm	33 nm	0.11
7		318 nm	38 nm	0.13	320 nm	19 nm	0.13
14		316 nm	29 nm	0.12	319 nm	29 nm	0.13
21		321 nm	25 nm	0.15	308 nm	46 nm	0.12
119		320 nm	16 nm	0.13	–	–	–
0	V	378 nm	24 nm	0.15	364 nm	28 nm	0.14
1		411 nm	53 nm	0.19	386 nm	44 nm	0.17
2		392 nm	32 nm	0.19	401 nm	38 nm	0.15
7		378 nm	24 nm	0.18	380 nm	32 nm	0.17
14		382 nm	43 nm	0.19	379 nm	48 nm	0.18
21		381 nm	50 nm	0.16	380 nm	49 nm	0.19
119		381 nm	45 nm	0.16	–	–	–

Samples were drawn continuously from the same bag ($D_{25\,\mu s}$, mean diameter of bulk population; ΔD, size difference; PI, polydispersity index of bulk population).

3.5. PCS polydispersity analysis

Transform of the PCS correlation functions to size distributions showed a distinct fraction of particles above 1 μm in both regimens (Fig. 2). Analysis after 21 days of storage showed a slight increase in the fraction of these larger particles in regimen IV. In regimen V a bimodal distribution was obtained. A population of larger particles had separated from the bulk population. This is in agreement with the observations that the mean diameters of the bulk populations remain unchanged (Table 4) and coalesced particles continue to grow forming a second population. In low-electrolyte regimens polydispersity analysis was found not to be sensitive enough to follow the increase in the fraction of larger particles during 21 days of storage (Müller and Heinemann, 1994). At high electrolyte load the number of coalesced particles is distinctly greater as seen above (section 3.3). The increase in number is sufficiently high to be reflected in the size distribution obtained from PCS data. However, as discussed previously (Müller and Heinemann, 1992), the polydispersity analysis is not considered as suitable to quantify the distribution in size classes. Single particle counting techniques are required to obtain reliable percentages in the size classes.

3.6. Laser diffractometer analysis of regimens

The LD data show a distinct decrease in the percentage of particles below 1.2 μm after 7 days of storage, accompanied by an increase in the diameter 90% ($D_{(90\%)}$) (Table 5). In contrast, the characterization parameters for the stable low-electrolyte regimen I were constant for a period of 119 days. After 14 days the percentage below 1.2 μm dropped to 40% in both regimens IV and V. In the previously described regimen III with medium-electrolyte load (Müller and Heinemann, 1994), it took 119 days to reach such a low percentage. No difference in stability between regimens IV and V could be concluded from the LD data. A distinct reduction in physical stability was found at day 7. The limitation of storage for both regimens to 7 days or less based on Coulter counter and microscopy data appeared justified.

Surprisingly, at 119 days of storage, the percentages below 1.2 μm had increased and $D_{(90\%)}$ had decreased close to their values in the regimens after mixing at day 0 (Table 5). The quality of the emulsions apparently seemed to have improved. It should be noted that the LD used provided only relative proportions of the particle fractions above 0.5 μm. A strong increase in the fraction 0.5–1.2 μm might take place due to heavy coalescence of droplets from the bulk population. This leads to a relative increase of the fraction below 1.2 μm, thus simulating a 'better' emulsion. In fact it reflects continuing coalescence. Very large particles continue to coalesce which reduces their number below the detection limit. In addition, they might leave the measuring range of the instrument (particles > 118 μm). Large droplets were clearly seen on the surface by the naked eye but were not detectable by the

Table 5
Laser diffractometer data of regimens I, IV and V

Storage time (days)	Regimen I		Regimen IV		Regimen V	
	< 1.2 μm	$D_{(90\%)}$	< 1.2 μm	$D_{(90\%)}$	< 1.2 μm	$D_{(90\%)}$
0	97.5%	1.15 μm	81.0%	2.02 μm	88.4%	1.28 μm
1	97.4%	1.15 μm	84.3%	1.48 μm	82.1%	1.55 μm
7	96.8%	1.16 μm	64.2%	4.63 μm	69.1%	6.26 μm
14	97.4%	1.15 μm	40.7%	5.10 μm	38.8%	9.86 μm
21	97.0%	1.16 μm	–	–	60.3%	7.48 μm
119	97.3%	1.15 μm	89.4%	1.25 μm	88.4%	1.32 μm

< 1.2 μm, percentage of droplets below 1.2 μm, i.e., percentage of particles between 0.5 and 1.2 μm of the total population of particles > 0.5 μm; $D_{(90\%)}$, diameter 90%, i.e., 90% of the particles (volume distribution) are below this size.

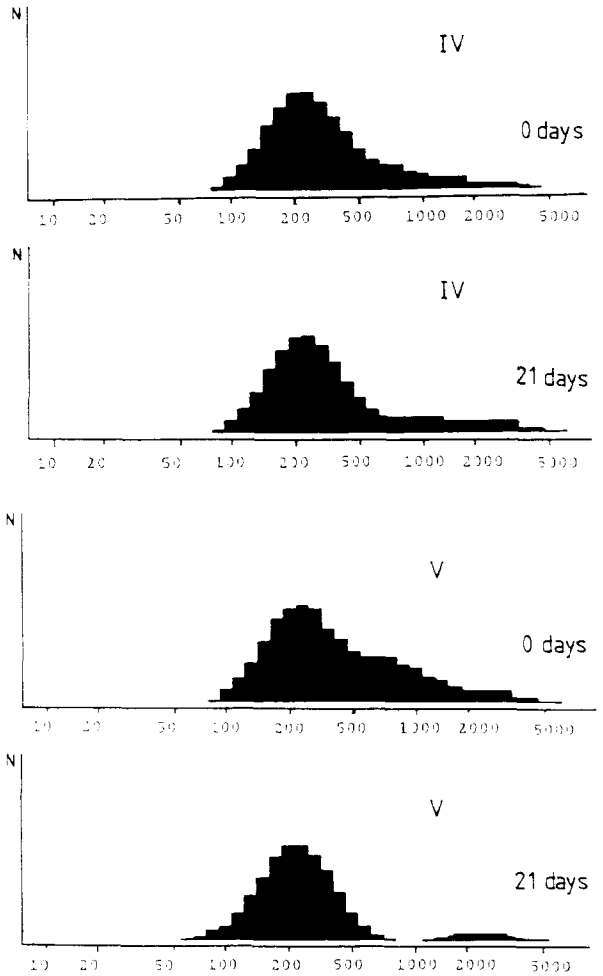

Fig. 2. Size distribution of regimens IV and V obtained by Fourier transform of the correlation function after mixing (day 0) and after 21 days of storage (fitting of four delta functions at 80, 251, 793 and 2500 nm) (details of method: cf. Müller and Heinemann, 1992).

LD. Such effects will also increase the relative proportion of particles below 1.2 μm and reduce the $D_{(90\%)}$. Care should therefore be taken when monitoring systems with low physical stability only by PCS and LD. The systems might appear falsely stable when the time intervals of the analysis are too large to monitor interim reductions of the percentage below 1.2 μm (e.g., day 14, Table 5).

The size distributions obtained by LD are similar for both regimens IV and V. They do not quantify the amount of particles present per volume unit. The absorption measured in the measuring cell of the LD can, however, be used as an estimate of the absolute concentration of larger droplets. Regimen V seemed to contain a slightly higher concentration of larger particles as indicated by the absorption value of 1.935 compared to 1.230 in regimen IV (arbitrary units of the LD, 50 μl emulsion in 20 ml distilled water after 119 days of storage). Readings obtained previously with low and medium electrolyte load regimens (Müller and Heinemann 1994) were in the range of 0.5–1.2.

3.7. Zeta potential measurements

Due to the high load of monovalent and divalent ions (66.7 and 6.7 mmol/l water, respectively) the zeta potentials in regimen IV and V were in the range of -5 to -7 mV. They were distinctly lower than in the low electrolyte system I or medium electrolyte regimens II and III (-14.7, -13.1 and -11.3 mV, respectively) (Müller and Heinemann, 1994). Regimen I, II and III contained similar loads of monovalent ions (73.9, 63.1 and 62.9 mmol/l water) but a reduced amount of divalent ions (1.0, 3.0 and 4.7 mmol/l water). The potentials in regimens IV and V were not sufficient for stabilisation by electrostatic repulsion. They were even close to the potential range of maximum flocculation (-5 to $+3$ mV (Ney, 1973)). The load of monovalent ions was similar to that of regimen I, however, the concentrations of divalent ions were distinctly greater. The divalent ions cause a more rapid decay of the potential in the diffuse layer. Regimens IV and V contained 3.4 mmol/l Ca^{2+} ions. Concentrations as low as 0.5 mmol/l lead to a reduction of the potential from about -40 to about -20 mV (Fig. 3). Calcium concentrations used in the regimens cause a reduction to about -5 to -10 mV. No difference was found between various divalent ions with regard to their zeta potential reducing effect. Calcium and magnesium behaved identically. The point of zero charge was found to be at about 5 mmol/l; further addition of calcium leads to a charge reversal. The calcium adsorbs onto the droplets, leading to a positive zeta potential in the range of $+10$

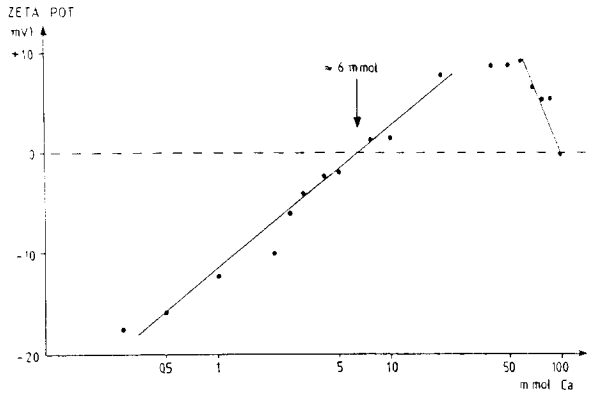

Fig. 3. Zeta potential of fat emulsion droplets as a function of the calcium concentration (addition of $CaCl_2$; measurements performed in a 1 mm capillary of the Zetasizer II).

mV. Lowest stability can be observed at the point of zero charge. The charge reversal leads again to a slightly more stable emulsion compared to uncharged emulsions at the point of zero charge. However, a potential of 10 mV is not high enough for sufficient stabilization. In addition, the fusion promoting effect of calcium ions on phospholipid layers (Düzgünes et al., 1981) will increase the coalescence of lecithin stabilized droplets. Addition of very high calcium concentration reduces the potential again to zero leading to maximum coalescence. The destabilizing effect of divalent ions could be clearly seen when determining the maximum droplet size in emulsions directly after addition of calcium. At low calcium concentrations (0.5 mmol/l) no increase in the maximum size could be detected. The maximum size increased with increasing calcium concentration, being largest close to the point of zero charge (ZP −1.8 mV) (Fig. 4). Further increase reversed the charge and slightly stabilized the emulsion again, indicated by the minor drop in maximum size in the positive potential range. Further calcium addition led again to stronger coalescence with the largest droplets at 100 mmol/l calcium (Fig. 4).

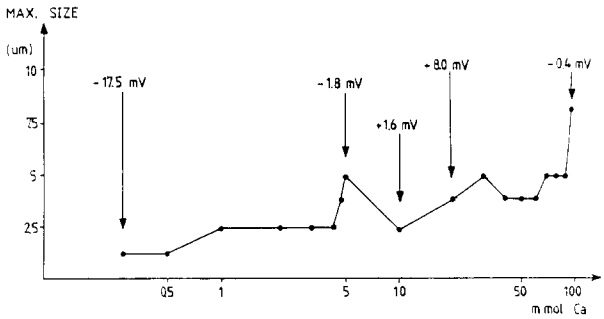

Fig. 4. Maximum particle size (μm) detected in Lipofundin fat emulsion as a function of the calcium concentration (mmol/l); zeta potentials (mV) are given for several calcium concentrations (arrows). Sizing was performed using a laser diffractometer immediately after addition of calcium chloride solution (mixing of fat 10% emulsion and double concentrated calcium chloride solution 1:1).

4. Conclusions

The instability of the regimens can be mainly attributed to reduced electrostatic repulsion. The electrolytes will also interfere with the hydration of the emulsifier film. In addition, the divalent ions promote fusion of phospholipid layers. Both effects could not be directly measured in the emulsions but are likely to contribute to the observed coalescence. The divalent ions are similar in their zeta potential reducing effect (Stampa et al., 1991). Therefore, the reduction in electrostatic repulsion cannot be attributed to one species (e.g., calcium) but is a function of the total concentration of divalent ions. However, the acceleration of fusion seems to be species specific. Calcium is more efficient than magnesium; in combination magnesium acts synergistically on the fusion capacity of calcium.

Microscopy, Coulter counter analysis and PCS were considered as most suitable for monitoring the stability. PCS provided information about the bulk population, which was not accessible to the other methods. The Coulter counter had the clear advantage of quantifying the number of larger particles per volume regimen. The laser diffractometer (LD) provides only relative percentages of the particles above 0.5 μm. In the investigated mixtures of low stability the interpretation of the LD data was rather difficult. LD data were suitable when monitoring relatively stable regimens with low and medium electrolyte load, e.g., regi-

mens I–III (Müller and Heinemann, 1994). Microscopic photography proved valuable in assessing emulsion stability by analysis of the top phase and in measuring the maximum droplet size.

Most information was obtained by analysis of the top phase. No distinct increases in the number of large droplets were detected in the homogeneous mixtures of regimens (i.e., after shaking) – either by Coulter counter or by microscopic analysis. Considering only these data a stability of 21 days would have been assessed. The numbers of droplets accumulating in the top phase were distinctly larger than in the previously investigated low-electrolyte regimens I–III. The Intralipid regimen showed visible heavy creaming forming a thick, creamed top layer. The redispersibility of this layer was difficult; the high numbers of large droplets found after day 2 in the regimens by Coulter counter measurements (Table 3) might partially have been flocculated smaller droplets. The redispersibility of such flocculates after i.v. administration appears uncertain. The storage time of the Intralipid containing regimen was therefore considered as being 2 days. Due to precautionary reasons the storage time of the Lipofundin MCT/LCT was limited to only 7 days – despite the results from the homogeneous regimens, showing the absence of large droplets or droplet flocculates. The observed differences in the stability between Intralipid and Lipofundin MCT/LCT containing regimens might partially be due to differences in the composition of the emulsifier. A stability determining role has been attributed to minor components present in the emulsifier film (Davis and Hansrani, 1979). In addition, the quality of the emulsion used for preparing the regimen is considered to be important. The Intralipid emulsion in this study was a batch which was relatively coarse. An a priori present large fraction of large particles is expected to shorten the storage time of high-electrolyte regimens. This leads to the question of whether the storage time of regimens with critical stability does not solely depend on which commercial fat emulsion has been used. The storage time might also be affected by the quality of the respective emulsion batch.

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