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Fat emulsions for parenteral nutrition. III: Lipofundin MCT/LCT regimens for total parenteral nutrition (TPN) with low electrolyte load

R.H. Müller and S. Heinemann

Department of Pharmaceutics and Biopharmaceutics, The University of Kiel, Gutenbergstr. 76–78, D-2300 Kiel 1 (Germany)

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Summary

The physical stability of three regimens for total parenteral nutrition (TPN) based on the MCT/LCT (50:50) emulsion Lipofundin MCT/LCT [1] was investigated. A range of different methods was employed for complete characterization of the feeding mixtures (sizing methods: photon correlation spectroscopy, microscopic photography, Coulter counter, laser diffractometer; zeta potential determination: laser Doppler anemometry). The regimens contained about 70 mmol/l 1:1 electrolytes and increasing concentrations of divalent cations (1.0–4.7 mmol/l, e.g., calcium, magnesium). During the storage period of 21 days the mean diameter and the width of the bulk population remained unchanged. No increase in the number of particles larger than the bulk population could be detected in the homogeneous mixtures of the regimens (after shaking). Analysis of the top phase (surface layer) revealed a slight increase in the number of large particles in the two regimens with higher content of divalent cations. This indicates the importance of the sampling procedure. Microscopic and Coulter counter analysis proved to be most sensitive in detecting these larger droplets. All three regimens were considered as physically stable for at least 21 days.

Introduction

Mixed systems for total parenteral nutrition (TPN) containing electrolytes, carbohydrates, amino acids, fats, vitamins and trace elements, possess a limited stability depending on their composition. The addition of most carbohydrates does not give rise to stability problems. A reduced stability was observed in the case of pH shifts caused by the carbohydrate. Dextrose has been reported to reduce the pH of Intralipid to 3.5 (Black and Popovich, 1981). This reduced stability was explained by the low zeta potential of −15 mV at pH 3.5, compared to −30 mV and more above pH 5 (Davis, 1982). In contrast, other carbohydrates were found to even enhance the stability of emulsions against added electrolytes such as calcium (Washington et al., 1990). A protective effect has also been attributed to amino acids (Boberg and Hakansson, 1964; Kawilarang

Correspondence to: R.H. Müller, Department of Pharmaceutics and Biopharmaceutics, The University of Kiel, Gutenbergstr. 76–78, D-2300 Kiel 1, Germany.

[1] In Germany: Lipofundin™ MCT, B. Braun Melsungen AG.

et al., 1980; Black and Popovich, 1981; Davis and Galloway, 1986). They can interact with free calcium, reducing its interaction with the emulsifier film. Vitamins did not affect the emulsion (Kleinberger and Pamperl, 1983). Electrolytes reduce the zeta potential and subsequently the stabilizing electrostatic repulsion. Divalent cations are more damaging than the monovalent species. Recently, increased attention has been focused on another stabilizing force, hydration repulsion (Washington, 1990). Hydration repulsion is a short-range force, which can prevent coalescence when the emulsion droplets flocculate in the primary minimum. Another factor, which has attracted attention primarily in the area of liposomes, is the fluidity of the phospholipid layers (Ribier and Handjani-Vila, 1984). Rigid emulsifier films reduce the likelihood of coalescence when two droplets hit each other or when droplets approach closely in the flocculated state. The fluidity, or its reciprocal term microviscosity, depend very much on the composition of the layer. Increasing cholesterol content reduces the fluidity, that means it increases the microviscosity and subsequently the stability of the phospholipid layer (Shinitzky and Inbar, 1976). The microviscosity is additionally influenced by the external environment (e.g., addition of NaCl, urea) (Miyagishi et al., 1987). However, the internal environment of o/w emulsions (oil phase) also interacts with the emulsifier film. It influences its structure and structure related properties (e.g., microviscosity and subsequently stability against coalescence). Most of the published TPN stability studies deal with emulsions based on soybean oil (Frank, 1973; Kawilarang et al., 1980; Burnham et al., 1983; Allwood, 1984; Davis and Galloway, 1986), or others such as safflower oil (Sayeed et al., 1987). Some time ago attention was focused on the replacement of long-chain triglycerides (LCT) in soybean oil partially by medium-chain triglycerides (MCT) (Sailer and Müller, 1981). The MCT appeared as rapidly effective calorie donors, since they are rapidly oxidized and not stored in the body.

Meanwhile, products containing a mixture of MCT/LCT (50:50) are on the market (Lipofundin MCT/LCT™; B. Braun Melsungen, Germany). Change of the internal environment might alter the properties of the emulsifier film and subsequently emulsion stability. The microviscosity of the emulsifier film decreased by about 20% compared to egg lecithin stabilized LCT emulsions (Diederichs et al., 1991). The reduction in microviscosity (rigidity) of the egg lecithin film did not reduce the long-term stability of Lipofundin MCT/LCT (Müller and Heinemann, 1993). The emulsion proved to be physically stable for a period of 2 years. However, the forces stabilizing emulsions are diminished in TPN regimens. Therefore, the effect of low and medium electrolyte concentration in TPN regimens, containing Lipofundin MCT/LCT, was investigated. The physical stability was monitored using a range of methods as described previously (Müller and Heinemann, 1992).

Materials and Methods

Materials

Lipofundin MCT/LCT emulsions 10% (batch 604181A) and 20% (batch 615182B), and the solutions (trade products) glucose 10, glucose 40, glucose 25 with electrolytes, potassium phosphates, calcium gluconate, potassium chloride 7.45%, sodium chloride 5.85% were purchased from B. Braun Melsungen (Melsungen, Germany). Addamel was obtained from KabiVitrum (Uxbridge, U.K.).

Aminoplasmal L-10 contained a mixture of amino acids and electrolytes (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 10/40 contained 110 g/440 g glucose monohydrate per l, glucose 25 with electrolytes 275 g glucose monohydrate with added electrolytes (Na, 70.0 mmol/l; K, 25.0 mmol/l; Mg, 2.5 mmol/l; Zn, 0.08 mmol/l; H_2PO_4, 6.0 mmol/l; Cl, 94.0 mmol/l; acetate, 0.15 mmol/l). Composition of the electrolyte solutions: potassium phosphates (K, 1.0 mmol/ml; phosphate 0.6 mmol/ml), calcium Braun (calcium gluconate, 94 mg/ml; calcium D-saccharate, 5 mg/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). TPN bags (Nutrimix™) were obtained from B. Braun Melsungen; the bag volume was 3.0 l and the bag material EVA.

Methods

The TPN mixtures were prepared in a laminar air flow unit; the glucose and electrolyte solutions were mixed first, then the fat emulsions were added to the bag (addition under gravity). The regimens were stored at 4°C. Their composition is given in Table 1.

In total 10 bags per regimen were prepared to study the stability. Prior to sample withdrawal the bags were stored for 24 h at room temperature to imitate the infusion time. For characterization by light scattering techniques two bags per regimen were used. Samples were withdrawn directly after preparation, and after 1, 2, 4, 7, 14 and 21 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 1 day at room temperature. At each time interval, samples were withdrawn from the top phase of the bag to enhance the statistical probability of finding larger droplets (Müller and Heinemann, 1992). Top phase denotes that the samples were withdrawn 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 withdrawal.

TABLE 1

Composition of Lipofundin MCT / LCT regimens for parenteral nutrition

Compound	Regimen no.		
	I	II	III
Aminoplasmal L-10	1000 ml	1000 ml	1000 ml
Glucose 40	1000 ml	–	–
Glucose 25 + electrolytes	–	1000 ml	1000 ml
Glucose 10	–	500 ml	500 ml
Lipofundin MCT/LCT 10%	500 ml	–	–
Lipofundin MCT/LCT 20%	–	500 ml	500 ml
NaCl 5.85%	60 ml	–	–
KCl 7.45%	40 ml	–	–
Potassium phosphates	20 ml	20 ml	20 ml
Calcium gluconate	–	10 ml	10 ml
Addamel	–	–	10 ml

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 (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 fat emulsion. The instrument is based on the Coulter Counter principle. 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 II and III). The tables contain the absolute numbers of particles > 2.04 and > 4.08 μm per 2.1 and 1.2 μl regimen, respectively. The mean PCS droplet diameters were determined in parallel using a Malvern Autosizer (Malvern Instruments, U.K.).

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 weight 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 de-

scribed 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 (for the correlation between ΔD, laser diffractometer and Coulter counter data, and microscopic assessment of emulsion quality, see Müller and Heinemann (1992)). The PCS correlation functions were transformed to a size distribution by Fourier transform as described previously (Müller and Heinemann, 1992, 1993). 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 (PI), 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 the replacement of Lipofundin MCT/LCT by a water-glycerol mixture.

Results and Discussion

Characterization of fat emulsions used for preparation of regimens

The Lipofundin MCT/LCT emulsions used for the preparation were characterized by light scattering (Table 3). The mean PCS diameters were in the range reported previously (Müller and Heinemann, 1993). The mean diameter of the 10% emulsion was below the values found for the 20% emulsion. The size difference was attributed to different homogenisation conditions (Müller and Heinemann, 1992). The width of the size distribution was remarkably narrow for the 20% emulsion (PI = 0.10, ΔD = 14 nm). In general, values between 0.14 and 0.20 (PI) and 15–25 nm (ΔD) were obtained previously (Müller and Heinemann, 1993). No particles larger than 2.4 μm were detected by laser diffractometry (LD), indicating high quality emulsions with regard to the absence of larger droplets. Based on these

TABLE 2

Scheme for microscopic assessment of emulsion stability

Size of bulk population /frequency of particles > / = 3 μm	Homogeneous mixture		Top phase	
	Regimen A	Regimen B	Regimen A	Regimen B
Size of bulk population				
< or = 1 μm	o	o	o	o
> 1 μm	o	o	o	o
> 1.5 μm	o	o	o	o
Droplets > / = 3 μm are				
Few but detectable [a]	o	o	o	o
Detectable [b]	o	o	o	o
Increasingly present [c]	o	o	o	o
Size of largest detectable droplet (μm)	....	....	....	....

The mean droplet size of the bulk population is measured and the frequency of particles > / = 3 μm determined (frequency of droplets: [a] 0–0.5 droplets per micrograph; [b] 1–2 droplets/micrograph; [c] > 2 droplets/micrograph). Samples are withdrawn from the top phase (5 mm below the surface) and after shaking from the homogeneous mixture

TABLE 3

Laser light scattering characterization data of Lipofundin MCT/LCT 10 and 20% emulsions used for the preparation of the TPN regimens

MCT	PCS data				LD data			Zeta potential (mV)
	$D_{25\,\mu s}$	$D_{100\,\mu s}$	ΔD	PI	< 1.2 μm	$D_{(90\%)}$	Maximum size	
10%	274 nm	288 nm	14 nm	0.10	97.2%	1.15 μm	2.4 μm	−39.8 (−48.0[a])
20%	326 nm	340 nm	14 nm	0.10	92.1%	1.20 μm	2.4 μm	−38.2 (−49.3[a])

PCS, photon correlation spectroscopy; LD, laser diffractometer. The zeta potential measurements were performed in distilled water using a 1 mm capillary ([a] zeta potential in 4 mm capillary).

characterization data, the Lipofundin MCT/LCT 10 and 20% emulsions could be classified in the category 'very fine' (Müller and Heinemann, 1992, 1993).

Zeta potential (ZP) measurements using a large bore 4 mm capillary yielded values of −48 to −50 mV. These were in the range of zeta potentials measured before. However, the 4 mm capillary is not suitable for measurements in solutions with higher conductivity. To reduce the current and subsequent Joule heating, a capillary with 1 mm diameter needed to be employed for the zeta potential measurements in the regimens. The ZPs of the emulsions in distilled water were also determined using the 1 mm capillary to allow a better comparison. The ZPs in the two capillaries were found not to be identical. The small capillary yielded values about −10 mV lower (Table 3). This phenomenon was attributed to the different design of the cells. In the 4 mm capillary measurements are performed at the stationary layer. The 1 mm capillary is coated with methylcellulose to avoid electro-osmosis, and measurements are performed in the centre of the capillary. The dependence of the measured zeta potential on the capillary design was also observed

TABLE 4

Microscopic assessment of droplet sizes in the regimens I, II and III based on the scheme given in Table 2

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	+	+
II	0	< 1	< 1	+	+	−	−	−	−
	2	< 1	< 1	+	+	< 1	< 1	++	+
	7	< 1	< 1	+	+	< 1	< 1	++	+
	21	< 1	< 1	+	+	< 1	< 1	+++	+++
III	0	< 1	< 1	+	+	−	−	−	−
	2	< 1	< 1	+	+	< 1	< 1	+++	++
	7	< 1	< 1	+	+	< 1	< 1	++	+
	21	< 1	< 1	+	+	< 1	< 1	+++	+++

At each time interval two bags (A and B) of each regimen were analyzed. Frequency of droplets >/= 3 μm: a few particles detectable (+), particles detectable (++), particles increasingly detectable (+++) (cf. Table 2).

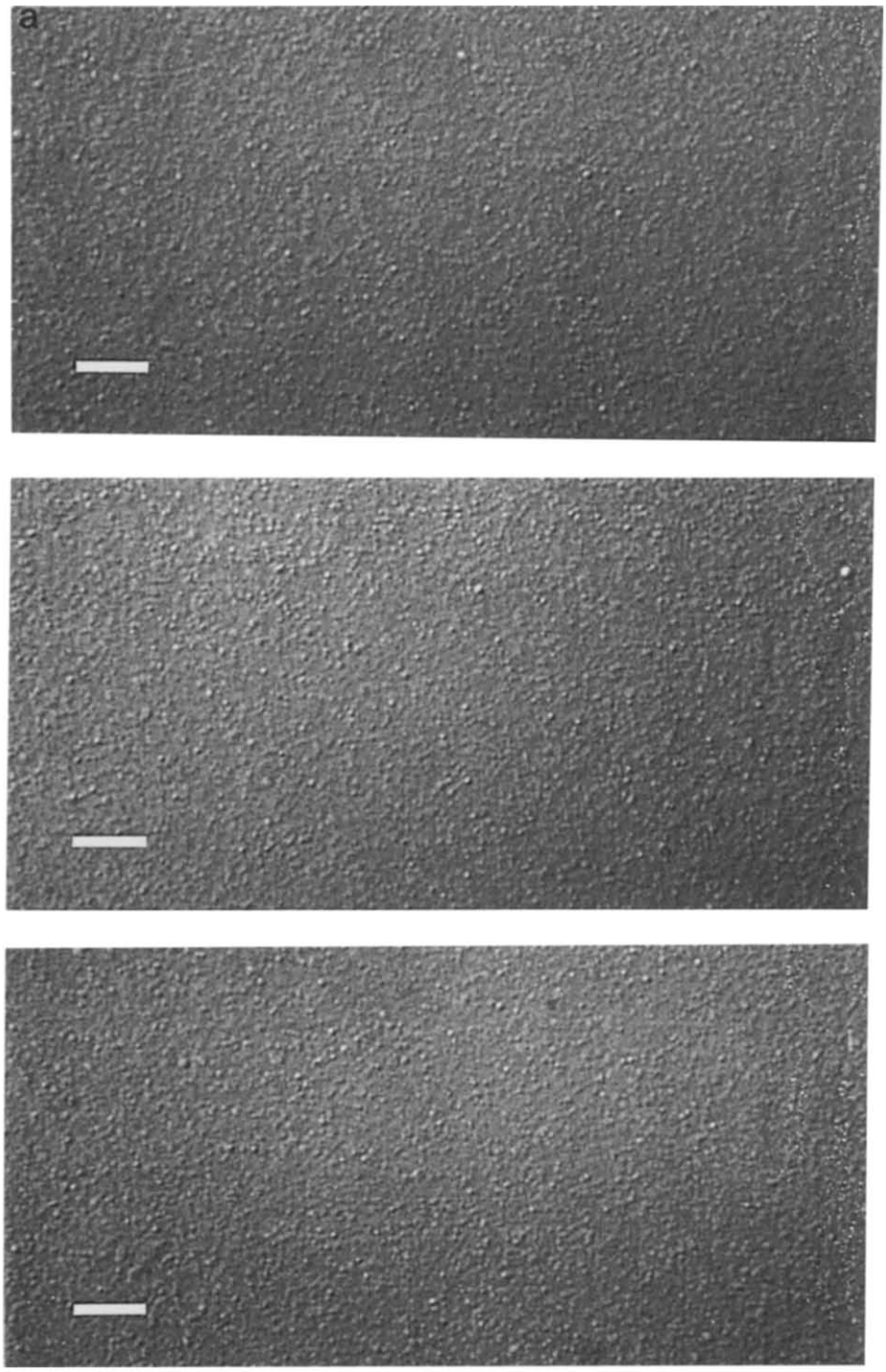

Fig. 1. (a) Microscopic photographs of regimen I (upper), regimen II (middle) and regimen III (lower), homogeneous samples taken directly after preparation of the regimens (bar: 10 μm). (b–) Microscopic photographs of (b) regimen I, (c) regimen II and (d) regimen III after 21 days of storage: sample from the homogeneous mixture after shaking of the regimen (upper) and sample withdrawn from the top phase of the regimen without preceding shaking (lower). For explanation see text (bar: 10 μm).

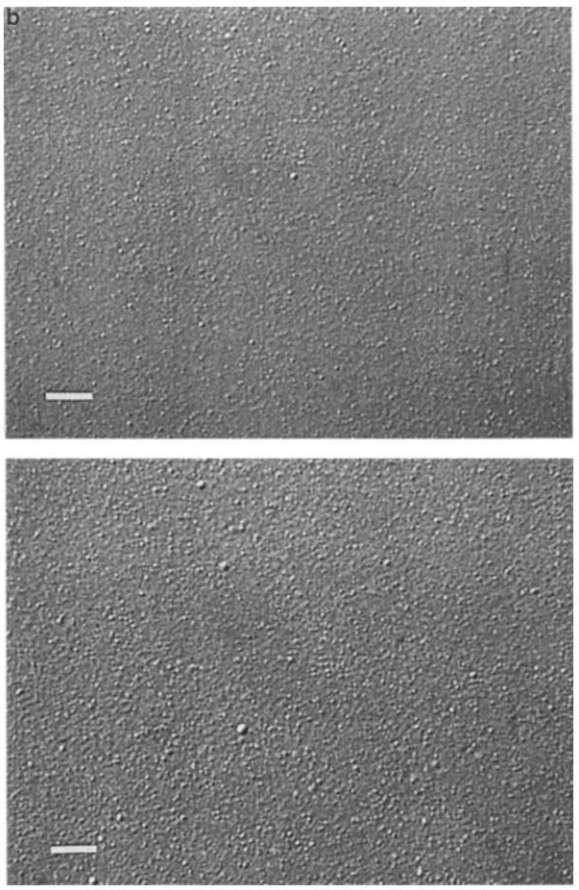

Fig. 1 (b).

with other instruments (AWPS – amplitude weighted phase structure function; Zetasizer III). To assess the effect of electrolytes, the ZPs in the regimens were compared with those of the fat emulsions determined in the 1 mm capillary (−39 mV). Meanwhile, Malvern Instruments provides a software routine (duty cycling) allowing the use of the large 4 mm capillary at high electrolyte concentrations. The switching on and off cycles of the electric field eliminate the distortions by Joule heating.

Microscopic assessment of the stability of regimens

Immediately after preparation all three regimens possessed a mean droplet diameter below 1 μm, only a few particles larger than 3 μm being detectable. These results were found to be consistent during the storage period of 21 days when examining the homogeneous mixtures of the regimens (= regimens after shaking) (Table 4). However, analysis of samples withdrawn from the top phase allowed us to differentiate between the regimens in terms of physical stability (Table 4). The size of the bulk population was still below 1 μm in all systems but the frequency of droplets > 3 μm increased in regimens II and III. In contrast, regimen I showed no increase in the number of droplets > 3 μm during 21 days and was assessed as the most stable one. On day 2 a slight increase was observed in regimen II, and a more distinct increase in regimen III. After 21 days in both regimens an increased number of droplets > 3 μm was detectable in the top phase. The results demonstrate the influence of the sampling procedure on the stability data obtained. Little difference existed between the microscopic photographs of the homogeneous emulsions after mixing at day 0 (Fig. 1a) and photographs taken from the homogeneous mixtures of the three regimens after 21 days storage (Fig. 1b–d, upper). However, distinct differences could be seen on examination of the photographs from the top phase after 21 days of storage (Fig. 1b–d, lower). Claims of regimen stability based on the sizing data presented therefore must include a description of the sampling procedure.

The maximum sizes of oil droplets measured by microscopy are given in Table 5. No increase was found for the most stable regimen I, increases up to 4 and 10 μm being detected in regimens II and III, respectively. The data for regimen III again demonstrate the importance of the sampling procedure. An increase in the size of the largest droplet detected was only observed in samples withdrawn from the top phases without shaking the bags. The microscopy data showed a decrease in stability from regimen I to III.

Coulter counter assessment of the stability of regimens

The samples withdrawn from the homogeneous mixtures of the regimens after shaking showed distinctly lower numbers of larger particles for regimen I on the day of preparation (day O). The numbers of particles > 2 and > 4 μm

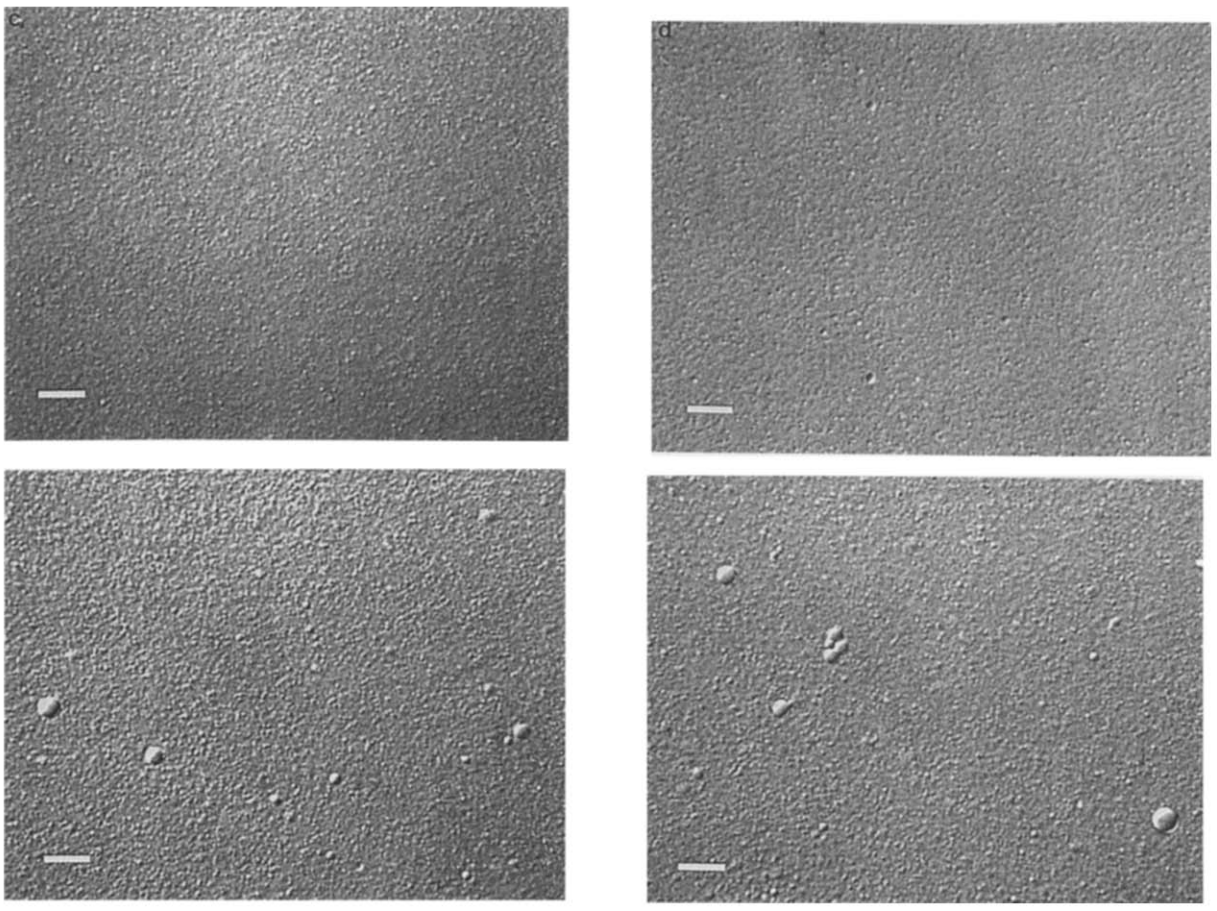

Fig. 1 (c,d).

were about 2–4-times higher in regimens II and III (Table 6). The differences became smaller with increasing storage time. The regimens appeared to be similar on day 21. There is a reduction in the number of larger droplets with increasing storage time in regimens II and III. This was explained by the progressive coalescence of the large droplets which diminishes their number,

TABLE 5

Size of largest droplet (μm) detected by microscopy analysing the microscopic photographs

Time (days)	Regimen I				Regimen II				Regimen III			
	Homogeneous mixture		Top phase		Homogeneous mixture		Top phase		Homogeneous mixture		Top phase	
	A	B	A	B	A	B	A	B	A	B	A	B
0	2.0	2.0	–	–	2.0	2.0	–	–	2.0	2.0	–	–
2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	6.0	2.0
7	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	3.0	2.0
21	2.0	2.0	2.0	2.0	2.0	2.0	4.0	4.0	2.0	2.0	7.0	10.0

e.g., two 4 μm droplets coalesce to one 5 μm droplet. However, there is little formation of new large droplets due to coalescence of small 300 nm droplets from the bulk population which could compensate for this reduction in number. Applying a stress to an emulsion leads to the formation of a bimodal distribution but not to a shift of the unimodal population (Müller, 1991). The emulsion droplets which are 'damaged' coalesce and continue to coalesce forming larger and larger droplets. The droplets which 'survived' the stress undamaged will show no coalescence and retain their original size (Fig. 2). In TPN regimens the maximum stress is applied on commencing the addition of the emulsion to the electrolyte mixture (= highest electrolyte concentration). This phase will be most damaging to the emulsion and is regarded as the major cause of droplet coalescence in the investigated systems. The electrolyte concentration and therefore stress is lowest after addition of the complete emulsion volume. The electrolyte concentration after complete mixing appeared to cause little coalescence in regimens with low or medium electrolyte load, as indicated by the reduction in the number of droplets larger than 2 μm during the storage period.

The number of larger droplets in the top phase showed no tendency to decrease during the storage time (Table 6). This could be explained by the accumulation of droplets in the top phase due to continuing flotation. However, the data varied considerably between the bags within one regimen. The numbers of droplets > 2 μm were found to be between 2822 and 14884 in the six bags investigated of regimen II. Despite these variations a clear difference could be seen between the most stable regimen I and the less stable II and III. The data from the top phase were more suitable to differentiate than those from the homogeneous mixtures of the regimens.

PCS assessment of the stability of regimens

The mean diameters of the bulk populations were unchanged for all regimens during the storage period (Table 7). This confirmed that the bulk population was stable as discussed above and did not undergo a shift towards larger sizes. The width of the bulk population (PI) showed no significant change during the first 21 days in all regimens. PCS measurements were also performed after 119 days of storage (17 weeks). The mean diameter of the bulk population was still

TABLE 6

Coulter counter results of regimens I–III

Time (days)	Regimen	Number of particles > 2 and > 4 μm							
		Homogeneous phase				Creamed 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	II	1929	113	1390	109	–	–	–	–
2		906	34	1001	27	14534	820	2822	135
7		521	9	583	17	11843	682	5670	324
21		1090	49	595	22	14884	938	7960	450
0	III	1292	84	1806	141	–	–	–	–
2		1214	50	577	27	18769	1211	1615	102
7		793	31	1064	52	11564	1175	2254	156
21		846	22	532	29	13123	1207	7685	540

The numbers of particles > 2.04 and > 4.08 μm were determined in samples withdrawn from the top phase and from the homogeneous mixture after squeezing of the bag.

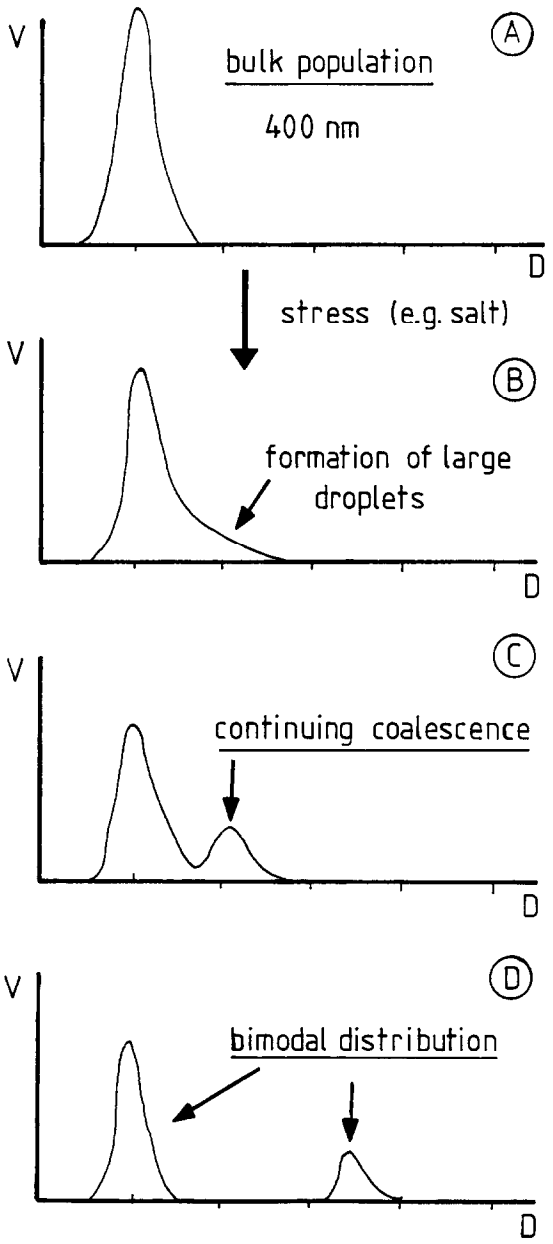

Fig. 2. Application of stress to an emulsion (A) leads to droplet coalescence and the formation of an inclined distribution (B). The larger droplets continue to coalesce and grow in size (C) leading finally to a separated population of large droplets (D). The volume of the large droplets stays unchanged (area under curve in volume distribution – V) but their number decreases (Müller, 1991).

constant in all regimens. The PI of regimens I and II was unchanged whereas that for regimen III had increased from 0.11 to 0.19 (Table 7). From these data regimen III was found to be the least stable one.

The formation of larger particles was monitored by the increase in ΔD compared to the values of the fat emulsions ($\Delta D = 14$ nm for 10 and 20% emulsion). In regimen I an increase from 14 nm to about 26 nm was observed over 119 days (Table 7). Such a ΔD still corresponds to an emulsion of the category very fine (Müller and Heinemann, 1992, 1993). In regimens II and III ΔD increased within 1 day after mixing from 14 to about 20 nm. During 119 days ΔD showed little change for regimen II but increased for regimen III to about 40 nm after 3 weeks. Despite this increase the emulsion mixture still belongs to the category 'fine'. The ΔD values could place the emulsions in order of decreasing stability: I, II and III.

PCS polydispersity analysis

The correlation functions were transferred to size distributions by Fourier transform of the correlation function (Müller and Heinemann, 1992) (Fig. 3). The distributions obtained were in agreement with the measured ΔD values. The size distributions showed a larger fraction of particles above 1 μm for regimens II and III. However, as discussed previously (Müller and Heinemann, 1992), the method does not appear to be sensitive enough to detect very small increases in the percentage of larger particles. Therefore, polydispersity analysis could differentiate between regimen I and the other two mixtures but was found to be insufficiently sensitive to monitor small changes during storage. The size distributions determined by polydispersity analysis after 3 weeks of storage did not reveal differences from those obtained directly after mixing of the regimens.

Diffractometer analysis of regimens

The LD data showed no change for regimen I during 119 days. Slight decreases in the percentage of particles below 1.2 μm and increases in $D_{(90\%)}$ were obtained in regimens II and III (Ta-

ble 8). A clear disadvantage of the LD used was its limitation of giving only a relative distribution of particles. The data provide no information about the absolute number of particles larger than 0.5 μm. Therefore, a system with different numbers of larger particles but identical relative fractions will be characterized by identical distributions and $D_{(90\%)}$ values. The measurement of relative fractions within the measuring range 0.5–120 μm might explain the fluctuations in the percentage below 1.2 μm and the $D_{(90\%)}$ in regimens II and III. They reflect shifts in the relative weight fractions above 0.5 μm due to coalescence. The relative proportion of classes changes due to coalescence of droplets > 0.5 μm to larger droplets, which reduces the percentage below 1.2 μm. Simultaneously droplets < 0.5 μm coalesce to droplets > 0.5 μm increasing the fraction below 1.2 μm. These fluctuations, due to the two overlapping processes, make it more difficult to differentiate between regimens II and III at 21 days of storage. After 119 days of storage regimen III is distinctly less stable than regimen II. The percentage of particles below 1.2 μm fell to 46% (Table 8). This supports the considerations about the formation of a population of larger droplets, leading to a bimodal size distribution (cf. Fig. 2).

However, the LD also measures the turbidity in the measuring cell, which provides an indication of the concentration of larger particles. The turbidity values at identical weight concentration of fat were: 0.995 (I), 1.160 (II) and 1.295 (III). Considering the increased turbidity value of regimen III, it could be identified as the least stable regimen at day 21 – despite the similar size distribution data in Table 8 (percentage of droplets < 1.2 μm, $D_{(90\%)}$).

TABLE 7

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	II	325 nm	22 nm	0.11	324 nm	21 nm	0.11
1		325 nm	22 nm	0.11	325 nm	21 nm	0.11
2		328 nm	19 nm	0.11	330 nm	23 nm	0.12
7		318 nm	25 nm	0.12	322 nm	20 nm	0.11
14		325 nm	28 nm	0.12	323 nm	26 nm	0.12
21		328 nm	28 nm	0.14	327 nm	24 nm	0.15
119		321 nm	19 nm	0.11	–	–	–
0	III	322 nm	15 nm	0.11	321 nm	15 nm	0.12
1		312 nm	20 nm	0.12	325 nm	21 nm	0.14
2		323 nm	21 nm	0.13	326 nm	20 nm	0.12
7		327 nm	26 nm	0.12	325 nm	19 nm	0.11
14		327 nm	26 nm	0.12	331 nm	23 nm	0.13
21		326 nm	45 nm	0.13	334 nm	34 nm	0.13
119		330 nm	42 nm	0.19	–	–	–

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

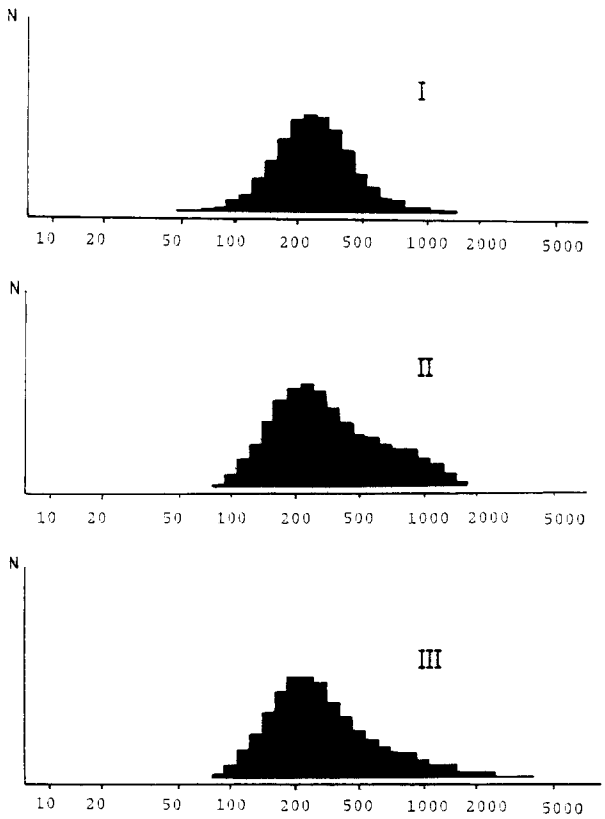

Fig. 3. Size distribution obtained by Fourier transform of the correlation function (y-axis, probability; x-axis, size (nm); regimen I: fit of six delta functions at 10, 30, 91, 274, 828, 2500 nm; regimen II and III: fit of four delta functions at 80, 251, 793, 2500 nm).

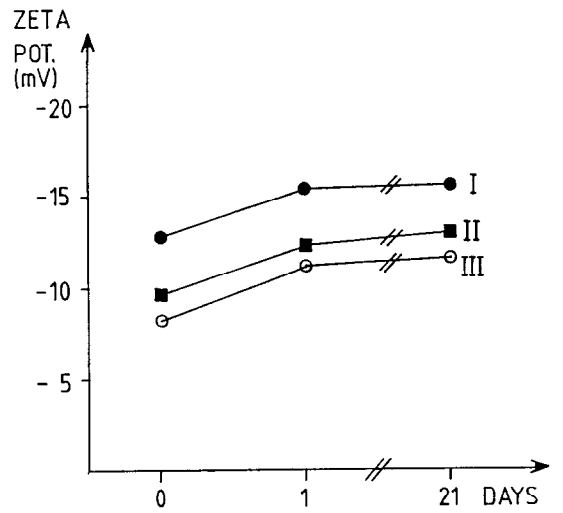

Fig. 4. Zeta potential in TPN regimens I, II and III vs storage time (days) measured at electrolyte concentrations present in the regimens.

Zeta potential measurements in regimens

The zeta potentials of the emulsions were in the range of -39 mV providing sufficient electrostatic stabilization. The potentials dropped in the regimens to about -15 mV (I) and about -10 mV (II and III) (Fig. 4). The potential in the diffuse layer decays more rapidly at higher electrolyte concentrations. The oil droplets can approach each other much closer before the diffuse layers overlap to an extent which causes electrostatic repulsion (Fig. 5). If they are able to approach sufficiently close for the van der Waals attractive forces to outweigh those of electrostatic repulsion, the droplets will coalesce. According to Ney (1973), a zeta potential > -31 mV is required to provide electrostatic stabilization in inorganic suspensions. Potentials in the range of

TABLE 8

Laser diffractometer data of regimens I–III (< 1.2 μm, percentage of droplets below 1.2 μm, = percentage of particles between 0.5 and 1.2 μm of the total particles > 0.5 μm; $D_{(90\%)}$, diameter 90%)

Storage time (days)	Regimen I		Regimen II		Regimen III	
	< 1.2 μm	$D_{(90\%)}$	< 1.2 μm	$D_{(90\%)}$	< 1.2 μm	$D_{(90\%)}$
0	97.5%	1.15 μm	89.9%	1.24 μm	89.2%	1.25 μm
1	97.4%	1.15 μm	82.9%	1.52 μm	89.5%	1.24 μm
4	97.7%	1.15 μm	80.7%	1.86 μm	69.6%	4.06 μm
7	96.8%	1.16 μm	74.2%	3.02 μm	82.6%	3.91 μm
21	97.0%	1.16 μm	80.9%	1.89 μm	75.9%	2.00 μm
119	97.3%	1.15 μm	95.5%	1.20 μm	46.6%	7.94 μm

−5 to −15 mV will lead to flocculation, maximum flocculation occurring in the range −5 to +3 mV. The attractive forces between emulsion droplets are regarded as being weaker than between inorganic particles (e.g., reduced hydrophobic interaction due to hydrophilic droplet surface; (Müller and Heinemann, 1993)). This might explain the absence of flocculation/coalescence in regimen I and the minor coalescence in regimens II and III despite the low zeta potentials.

Lagaly (1984) calculated that 20 nm particles with a surface potential of −51.4 mV are still stable in 900 mmol/l electrolyte solution (1:1). A potential of −25.7 mV is still sufficient for stabilization in 100 mmol/l.

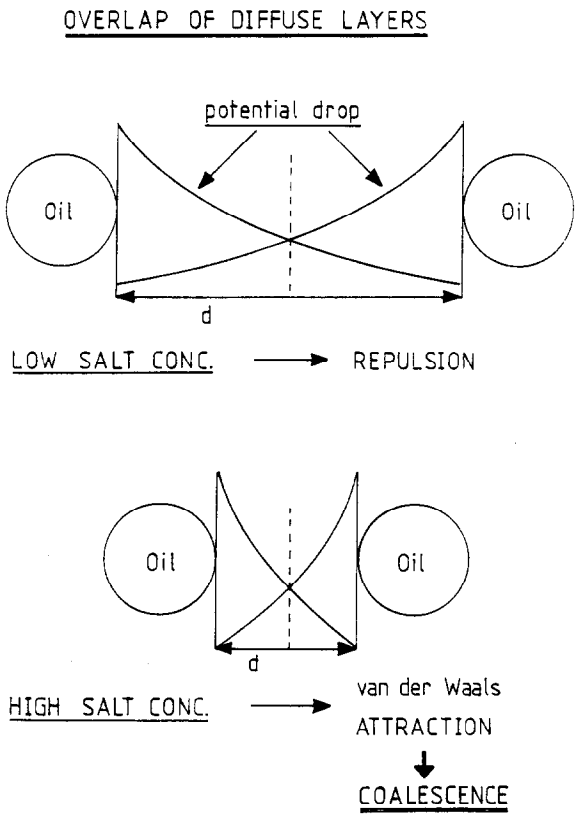

Fig. 5. Potential decay in diffuse layer around oil droplets at low (upper) and high electrolyte concentration (lower). The droplets can approach each other closer at high electrolyte concentration before the potentials in the diffuse layers overlap to an identical extent.

TABLE 9

Electrolyte load in the TPN regimens calculated in mmol/l water phase (= total regimen volume − oil volume)

TPN regimen	Cation concentration (mmol/l water phase)		Ca^{2+} (mmol/l water)
	Monovalent ions	Divalent ions	
I	73.9	1.0	–
II	63.1	3.0	0.8
III	62.9	4.7	2.5

The energy barrier V_m due to electrostatic repulsion in both cases is about 0.5 erg/particle. The particles can approach at a distance of 10 and 5 Å, respectively (maximum of energy barrier, V_m). The zeta potentials of the fat emulsions in water correspond to the surface potentials described by Lagaly. They are about −40 mV, thus being above the discussed −25.7 mV. The maximum electrolyte concentration in the regimens is about 70 mmol/l (1:1) and 5 mmol/l (divalent ions) (Table 9). Therefore, the total effect of the ions in the regimens will be similar to that caused by 100 mmol/l 1:1 electrolyte. At electrolyte concentrations of > 100 mmol/l small (20 nm) and large particles (400 nm) result in identical electrostatic repulsion (Lagaly, 1984). From these considerations, the emulsion droplets in all three investigated regimens should be sufficiently stabilized by electrostatic repulsion.

Flocculation of the droplets does not necessarily lead to coalescence. The rigidity of the emulsifier film can prevent the merging of droplets. However, large flocculates can also block capillaries and must be avoided. Flocculates would be less of a problem if they could be redispersed by shaking or would redisperse themselves when entering the bloodstream during infusion. However, the ability to redisperse in the blood is questionable, especially when they are flocculated in the primary minimum (Washington, 1990).

Regimen III contained a high concentration of calcium ions (2.5 mmol/l). Calcium and magnesium are known to promote the fusion of phospholipid bilayers (Düzgünes et al., 1981). The presence of both ions will not lead only to an additive effect. Magnesium has a synergistic ef-

fect on the membrane fusing capacity of calcium (Düzgünes et al., 1981). Regimen III contains both ions, calcium being present at a relatively high concentration. This might explain the instability found at 119 days of storage (Table 8) despite the calculated electrostatic stability. The stabilization of colloidal droplets composed of membrane-like surfaces (egg lecithin film) appears more complex compared to solid inorganic or organic dispersions. Compounds added to TPN regimens might reduce not only the electrostatic repulsion, but also the hydration of the lecithin film and subsequently the hydration repulsion.

Conclusions

The PCS analysis indicated a constant mean diameter and width of the bulk population. Coulter counter results revealed a constant number of larger particles in the homogeneous mixture of regimen I, and a decrease in number of larger particles in regimens II and III during the 21 days of storage. This supports the assumption that emulsion droplets destabilized during the mixing procedure continue to grow (coalesce). The droplets which were not destabilized remain stable as expected from the electrostatic repulsion calculations. The growth of the larger droplets obviously cannot be inhibited by the electrostatic repulsive force present in the mixed regimens. The continuous growth finally leads to a bimodal distribution of very large particles limiting the shelf life of the mixtures.

Microscopic photography of undiluted emulsions was suitable to differentiate between the regimens in terms of physical stability. This cost-effective microscopic technique therefore appears to be sufficient to assess the stability of regimens in hospitals. Most important is the sampling procedure. Sampling from the top phase is most sensitive. Mixing of the bags and withdrawal of samples from the homogeneous mixture of the regimens reduce the sensitivity. Regimens might appear falsely stable.

In contrast to microscopy, Coulter counter analysis allowed us to quantify the number of larger particles per volume unit of regimen. The total number of particles > 2 and > 4 μm and their increase with time were minor. Both methods could differentiate between the regimens in terms of stability. The stability decreased from regimen I to II and III. This corresponds to the increasing electrolyte load. Last but not least, it should be pointed out that the macroscopic appearance of the bags is also an important stability criterion although a creaming is not necessarily visible. From the data obtained with the various methods all three regimens were considered to be stable for at least 21 days.

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