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Stability assessment of o/w parenteral nutrition emulsions in the presence of high glucose and calcium concentrations

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ABSTRACT

The purpose of the present study was to examine how the colloid stability features of o/w parenteral nutrition emulsions made with SMOFlipid (lipid emulsion based on soybean oil, medium chain triglycerides, olive oil and fish oil) will change in the presence of high concentration of calcium and glucose if usual micronutrients are also present, according to the needs of the clinical nutrition patient. Particle size analysis, zeta potential, dynamic surface tension measurements and light microscopic screening were carried out to evaluate the possible changes in the kinetic stability of the emulsions. Our results indicate that the higher glucose concentration of 15 or 20% could not compensate the emulsion-destabilizing effect of higher (5 mM) calcium concentration even in the presence of a modern fat emulsion. Therefore calcium demand of undernourished patient requiring 5 mM or higher final Ca^{2+} content in nutrient solution should be supplemented in another way.

1. Introduction

Authors examined in their previous studies the kinetic stability of long chain triglycerides (LCT) vs. long and medium chain triglyceride (LCT–MCT) mixtures, later on the features of different forms of LCT–MCT mixtures (random lipids vs. structured lipids) in standard composition [1,2].

A new lipid emulsion based on soybean oil, medium chain triglycerides, olive oil and fish oil (SMOFlipid) was also tested for safety, tolerance, metabolic and clinical efficacy in surgical patients [3]. It was found that the SMOFlipid is clinically safe and well tolerated in most clinically nourished patients. There are observations that SMOFlipid may be associated with a better liver tolerance and a shorter length of hospitalization [4].

During the last some years the use of SMOFlipid as fat-component in fixed composition TNA for children and infants are also applied because of the beneficial physiological, therapeutic and pharmaceutical properties [5]. The use of some additives is still a question mark, especially in higher concentrates. As known from previous studies, bivalent cations, like Ca^{2+} and Mg^{2+}, worsen the stability of parenteral nutrition admixtures containing lipid emulsion by destruction of fat droplets with negative surface charge [6]. The higher the electrolyte concentration is, the higher the instability of the emulsion-system to a certain point. In contrast, high amino acid and glucose concentrations in TNAs have stabilizing effect due to buffering capacity, increasing the viscosity and by worsening the electrical conductivity of the continuous phase [7,8]. The electrostatic repulsive forces counteract the mechanical forces resulting in coalescence of oil droplets under certain circumstances. On the other hand, in the clinical use calcium-enrichment of the nutrition fluid is a usual demand, especially on the neonatal/pediatric wards. Due to the aggressive incompatibility features of Ca^{2+} in the presence of phosphates (incl. organic phosphates, too) and/or lipid droplets, the supplementation of clinical nutrition solutions with calcium is a living problem, especially if patients' requirement is quite high. The purpose of the present study was to examine the influence of various concentrations of calcium and glucose, as independent variables, on the droplet size and distribution of TNAs, thus their kinetic stability as a function of storage time.

2. Materials and methods

Mixtures were made on basis of the prescription "No F37″ regularly used at the Semmelweis University, Budapest and used in our previous studies [2]. Nine variations of all-in-one mix-

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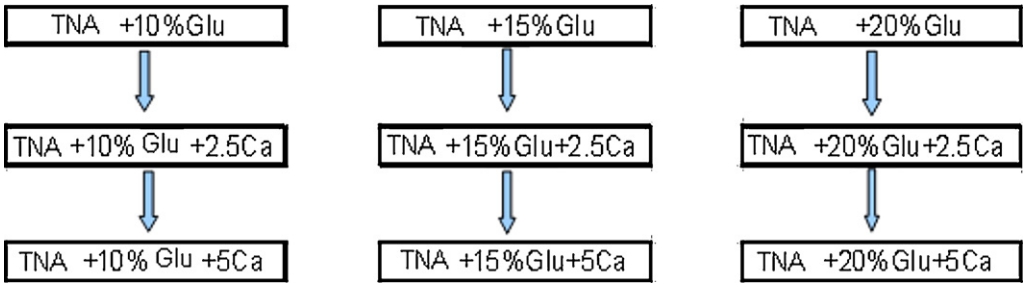

Fig. 1. The arrangement of model TNA mixtures of different component concentrations.

tures (variations of pediatric prescription F37) were prepared for the experiment: TNA plus calcium and glucose enrichment in 3 concentrations each (Fig. 1). Each type of AIO (all-in-one)-mixtures were prepared with SMOFLipid® (Fresenius-Kabi) as fat component and added additional glucose in form of 40% glucose solution (University Pharmacy Department of Pharmacy Administration, Semmelweis University, Budapest) to the aqueous phase and calcium gluconate, respectively. During the mixing process we followed the clinical practice and therefore calcium gluconate (Calcimusc® Gedeon Richter) was added to the ready made solutions in order to obtain clinically correct o/w parenteral nutrition emulsions. As a result of this procedure the concentrations were changed in every regime as demonstrated in Table 1. The enrichment of TNA with calcium resulted solutions containing 28 and 50 mM Ca^{2+} in form of organic compound.

2.1. Preparation and storage of AIO mixtures

The compounding of experimental TNAs was made according to the "SOP for aseptic procedures" of the Semmelweis University Budapest, under laminar airflow box. Mixtures were prepared in a vacuum-chamber under computer-assisted volume regulated process in a closed filling system. Components were mixed as follows: amino acid was added to 50% of the calculated Glucose 10% (or 15% or 20%, according to the experimental design and labeled as sCa10, sCa15 and sCa20, respectively), the remaining Glucose was mixed with the calculated amount of electrolyte and added to amino acid–glucose mixture, finally lipid component was added to the blend of nutrients. The ready made sCa10, sCa15 and sCa20 mixtures were filled into 100 ml bottles, closed and stored according to the study protocol on room (24–28 °C) temperatures. This temperature was chosen because of the imitation of hospital ward conditions. Calcium enrichment was made after completing the basic mixing procedure, just after filling into 100 ml bottles. Calculated amount of calcium gluconate 10% (Calcimusc® 10%, Gedeon Richter, Budapest) was added to the AIO mixtures by syringe. Table 1 summarizes the final compositions of various experimental o/w emulsions.

2.2. Sampling

2 ml samples were taken out of the bottles on the 1st, 4th, 7th, 10th, 14th day of storage and the following three measurements were done with all samples: particle size measurement, zeta-potential measurement, dynamic surface tension measurement plus visual (light microscopic) checking of the mixture. Dilution of samples – if needed – was made just before measurements by the identical fat free mixture. Measurements were always repeated 3 times after each other in one setting and results have been evaluated by statistical computation.

2.3. Dynamic surface tension measurements

Measurement of surface tension is a valuable method for the control of homogeneous dispersity of oil droplets in emulsions today as well [9]. The examinations were carried out on the day of preparation and after 1, 4, 7, 10 and 14 days. The surface tension of the emulsions was determined by dynamic method, applying Wilhelmy plate operations of a computer-controlled KSV Sigma 70 tensiometer (KSV Sigma 70, RBM-R. Braumann GmbH, Germany) at 25 °C ± 0.5 °C. The method determines the maximum mass of liquid pulled from the surface by lifting the specified solid device (plate). The force (f) measured on the electric balance is necessary for lifting out and pushing down the solid measuring device from the surface of the liquid. Figures are expressed in mN/m.

The cohesion work (W_{coh}) was calculated with the following equation:

$$W_{coh} = 2\gamma \quad (1)$$

where γ is the interfacial tension at oil/water interface.

2.4. Zeta-potential measurements

Laser Doppler-electrophoresis (LDE) was used for investigating the surface-electric properties of the emulsion droplets. Measurements were carried out with the freshly prepared emulsions and also, with the samples stored for 1, 4, 7, 10 and 14 days. For electrically charged particles moving in response to an applied electric

Table 1
Composition of experimental o/w parenteral nutrition emulsions (figures are in g/l).

Ingredients	sCa10	sCa15	sCa20	2.8Ca10	2.8Ca15	2.8Ca20	5Ca10	5Ca15	5Ca20
Aminoacids	48	38	28	41	34	24	37	30	21
SMOFlipids	48	38	28	41	34	24	37	30	21
Electrolytes	48	38	28	41	34	24	37	30	21
Glucose	96	152	222	84	136	195	74	120	173
Calcium gluconate	0	0	0	28[a]	28[a]	28[a]	50[a]	50[a]	50[a]
Surfactant	2.84	2.28	1.7	2.5	2.0	1.4	2.2	1.78	1.34
o/w Ratio	4.8/100	3.8/100	2.8/100	4.1/100	3.4/100	2.3/100	3.7/100	3/100	2.1/100

Schematic solution of abbreviations: sCa10 = emulsion without Ca^{2+} and glucose solution used for mixing is 10%; 5Ca20 = emulsion with 5 mM/100 ml Ca^{2+} and glucose solution used for mixing is 20%.

[a] In mM.

Table 2
Dynamic surface tension values of different TPN solutions (mN/m).

Days	sCa10	sCa15	sCa20	2.8Ca10	2.8Ca15	2.8Ca20	5Ca10	5Ca15	5Ca20
0	28.07	27.83	27.49	28.5	28.69	27.66	21.85	25.42	22.3
1	29	31.22	30.67	27.43	25.86	29.41	20.56	22.48	21.28
4	29.66	29	36.22	26.7	26.84	20.09	17	18.02	18.26
7	28.93	30.6	31.73	26.35	28.35	20.48	13.6	13.95	17.9
10	28.25	30.75	35.43	10.35	9.55	27.59			
14	14.34	16	17.63						

Schematic solution of abbreviations: sCa10 = TNA without Ca^{2+} and glucose solution used for mixing is 10%; 5Ca20 = TNA with 5 mM/100 ml Ca^{2+} and glucose solution used for mixing is 20%.

field, a correlation function of laser Doppler-shift was measured with Malvern Zetasizer 4 apparatus at 25 ± 1 °C, and the resulting frequency spectrum was translated to electrophoretic mobility. Using an AZ 104 type cell, 3 mobility measurements were ordinarily done on each emulsion (six samples, different in the glucose and Ca^{2+} content used for their preparation) in cross beam mode. The zeta potential (ζ) of the emulsion droplets was calculated from the mobility measurements, using the Smoluchowsky formula and expressed in mV.

2.5. Particle size measurements

Mean droplet size (MDS), size distribution and polydispersity of the emulsion droplets were measured at 25 °C by an advanced technique of photon correlation spectroscopy (PCS) using a Malvern Zetasizer 4 apparatus (Malvern Instruments, UK) with autosizing mode and auto sample time. Analysis of the fluctuations in the intensity of light scattered from particles undergoing random Brownian motion enables the determination of an autocorrelation function $G(\tau)$ that, in effect, is measure of the probability of a particle moving a given distance in a τ time (τ is the correlation delay time).

$$G_i(\tau)\alpha \sum k_i \exp\left[-\frac{\tau}{t_{c,i}(a_i)}\right] \quad (2)$$

The relaxation time (t_c) of fluctuations is related to the diffusion coefficient (D) of particles:

$$t_c = \frac{1}{DK^2}$$

from which the particle size can be calculated via the Stokes–Einstein equation, K is the wave vector. By determining the autocorrelation function for the dispersions stored at 2428 °C temperature for various times, the diffusion coefficient and the hydrodynamic radii (a_i) of emulsion droplets have been evaluated.

2.6. Visual inspection and microscopy screening

Visual inspection was made before blackboard in daylight once daily at the 1st, 4th, 7th, 10th and 14th day. Normal light microscope (Carl Zeiss, Jena, Germany) was used for the detailed visual observation of the droplets in the different, undiluted test-mixtures. Magnification: 200× and 400×. In every experimental TNA-mixture versions 10 fields each were observed and particles over the size of 500 nm and number of the over-limit droplets were documented manually and by photos.

3. Results

3.1. Surface free energy

Lipids in polar–apolar interfaces form monomolecular films that reduce the interfacial tension therefore surface tension can be one of the indicators of the stability of o/w emulsions like TNAs. Surface tension measurements expressed positive relation between Ca^{2+}-content and the stability, as shown in Table 2. Figures demonstrate drop in lifting force at 14th day without Ca^{2+}, further worsening of cohesion work at the 10th day when TNA-mixture contains 2.8 mmol/100 ml Ca^{2+}, and just 1-day stability at 5 mmol/100 ml independently of the glucose concentrations. The moderate stabilizing effect of glucose could be seen in the medium Ca^{2+} level; in 10 and 15% glucose concentrations instability occurs, but it could not be observed at 20% glucose concentration.

3.2. Electrokinetics

Zeta-potential values of the oil droplet (Table 3) show an overall weakening in surface repulsive force. As basic electric charge of lipid-droplet surface in single lipid-emulsions is ca. −40 mV without electrolytes and dilution. Dilution made during the production process of TNA-mixtures containing <5% lipids resulted in an elevation of surface electric charge, in our previous study the measured values were between −4.1 and −1.7 mV in ready-for-use TNAs [1]. In the present study we obtained values very close to zero or even slightly positive values that mean that the stability of the colloidal emulsions is critical. According to the matching of measured values and the compositions we can state that dramatic differences or tendencies in the zeta potential of the droplets could not be detected.

3.3. Particle size measurements

In case of TNAs droplet size is an essential parameter due to the size limits from physiological point of view. An average globule size

Table 3
Zeta potential values of lipid components in TNA under different calcium and glucose concentrations (all figures are in mV).

Days	sCa10	sCa15	sCa20	2.8Ca10	2.8Ca15	2.8Ca20	5Ca10	5Ca15	5Ca20
0	−0.334	−0.633	−0.466	−0.207	−2.25	0.0426	−0.00066	−0.3	−0.302
1	−0.217	0.0324	0.0925	−0.0875	−0.229	−0.245	−0.607	−0.126	−0.131
4	−1.74	−0.531	−0.175	−0.374	−2.4	−0.518	−1.21	−1.11	−0.374
7	−0.688	−1.22	−1.6	0.056	−0.032	−0.037	0.012	−0.556	−0.158
10	−0.476	−0.216	−0.782	−0.028	−0.027	−0.026	0.021	−0.165	−0.362
14	−0.053	−0.383	−0.041	−0.065	−0.125	−0.432	0.045	0.014	−0.711

Schematic solution of abbreviations: sCa10 = TNA without Ca^{2+} and glucose solution used for mixing is 10%; 5Ca20 = TNA with 5 mM/100 ml Ca^{2+} and glucose solution used for mixing is 20%.

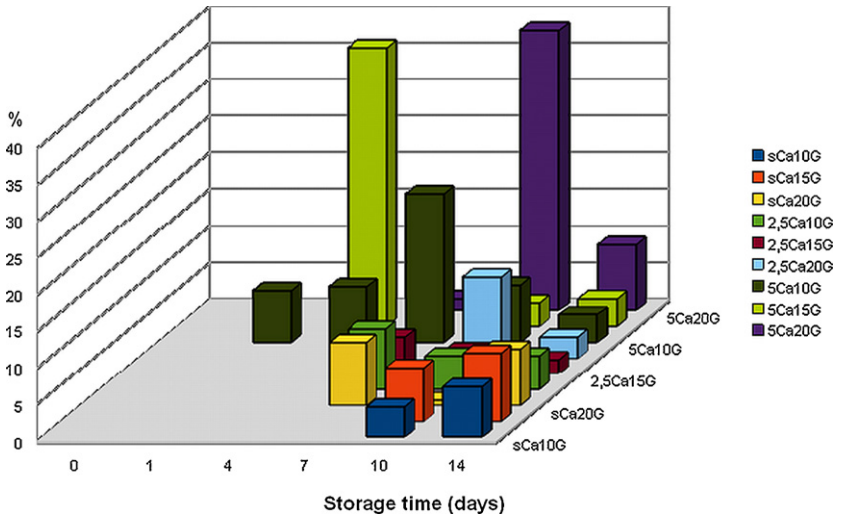

Fig. 2. Percentage of particles of higher than 500 nm average particle sizes in differently supplemented emulsions during the aging process.

of commercially available lipids is around the 300 nm [10]. The USP 30 (2nd suppl., 729) limits the fat-droplets in 500 nm and Japanese Pharmacopoeia has a limit of 700 nm of globules exceeding this size [11]. However, it is clear that from physiological point of view, fat droplets of higher diameters than capillaries (400–900 nm) are dangerous and therefore they have to be avoided in parenteral emulsions. The methods we used in this study allow us to draw conclusion just to the proportion of droplets under 500 nm. Our data show that the quality features of the mixtures just after production agree with the above mentioned limits. Droplets of higher than 500 nm sizes appear during the aging process were not followed. Exact amounts and distribution of the dangerous size droplets were not measured, we just recognized the appearance by light microscope and rough calculation was made on this basis. Correct data can be determined by use of more accurate and appropriate methods for this droplet-size as described by Müller, Driscoll and their coauthors [12,13].

According to the literature, Ca^{2+} facilitates and glucose slows down the formation of big droplets, however so high concentrations with clinical nutrition mixtures were not used in previous studies. The observation that without calcium, the stability is poor at low glucose-concentration is probably due to the low osmolarity as described by Driscoll [14], and this situation remains in the presence of calcium ions, as well.

3.4. Visual inspection and microscopic evaluation

The visual observation confirmed the gradual development of creaming as a consequence of time-dependent oiling out process. The observation of droplets above 500 nm size was in the focus of microscopic evaluation. In this study, during the light microscopic evaluation we did not measured exactly the droplet size above the 500 nm limit but the percentage of droplets above this limit in comparison to the smaller droplets was determined. Results are depicted in Fig. 2.

4. Discussion

Based on clinical demand, TNAs of extreme high calcium concentrations were prepared. As demonstrated in earlier studies, in the presence of 3–3.5 mM Ca^{2+} concentration in final mixture, zeta-potential should reach zero and the colloid stability of the system disappeared [15,16]. Higher amino acid and glucose concentration could stabilize the system in a certain extent, however the composition of the mixture we made according to the macronutrient requirements of the patients, was not enough to express this stabilizing effect. To find the correct balance among the components, theoretical approach is usually not enough. The combination of quantities of different components make so wide range of variations that stability prediction is sometimes totally impossible. Laboratory control of the specific preparation or admixture could be of impact to determine safe nutrition mixtures for a patient requiring extreme concentrates of ingredients. The three-dimensional surface-fit response helps to visualize the stability tendencies (Figs. 3, 4, 5a and b) in our case. High Ca^{2+}-concentrate cracked the emulsion in certain concentrations. Cohesion work study indicated a sudden crack after 10-day storage time if solution did not contain Ca^{2+} (tension fall to the half of mN/m) and a shorter life-time in case of higher Ca^{2+} contents. Fig. 3 shows relative low surface free energy level even in case of fresh samples of 5 mmol/100 ml Ca^{2+} concentration. The three-dimensional surface plot shows the definitive tendency to cracking in presence of Ca^{2+} and no influence of glucose concentration could be demonstrated.

Fig. 4 depicts the instability of the system from the beginning, since zeta-potential stands at near zero level, however it is

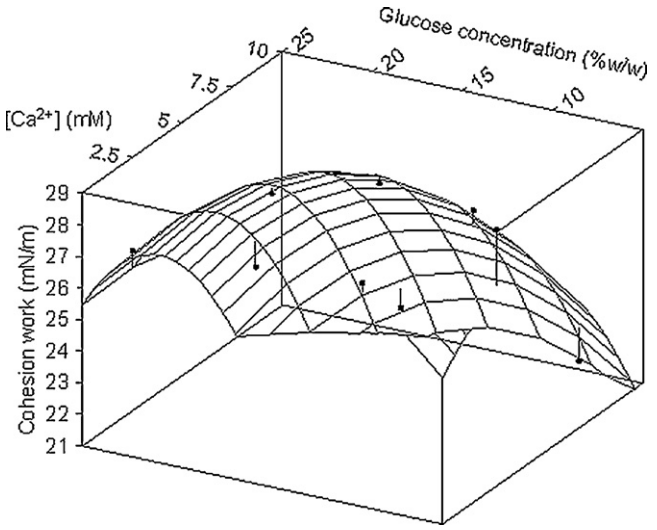

Fig. 3. The effect of independent variables on the cohesion work of emulsions. Cohesion work : $y_1 = 21.77 + 0.93x_1 + 0.94x_2 - 0.03x_1^2 - 0.13x_2^2 - 0.02x_1x_2$; correlation coefficient: 0.9458.

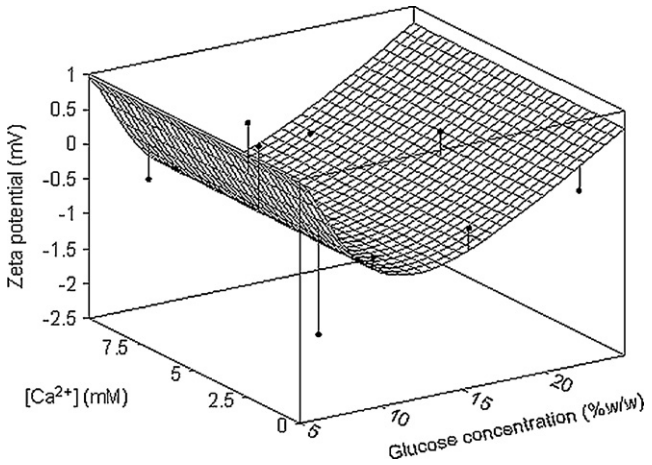

Fig. 4. The effect of independent variables on the zeta potential of emulsions after 4-day storage.

known due to Washington's works [15,16] that in stable emulsions this value should be below −10 mV. In this study we could not demonstrate the beneficial effect of higher glucose concentrate, probably because of the very high divalent cation level. In our third study regarding the particle size distribution, the surface plot of Fig. 5a shows a stable emulsion-format just after mixture production. After storage stability disappeared. Droplets of higher than 500 nm size appeared and after cracking of the system in the oiled out phase (supernatant) a major part of former droplets in form of oil accumulated and in the continuous phase rest of small droplets

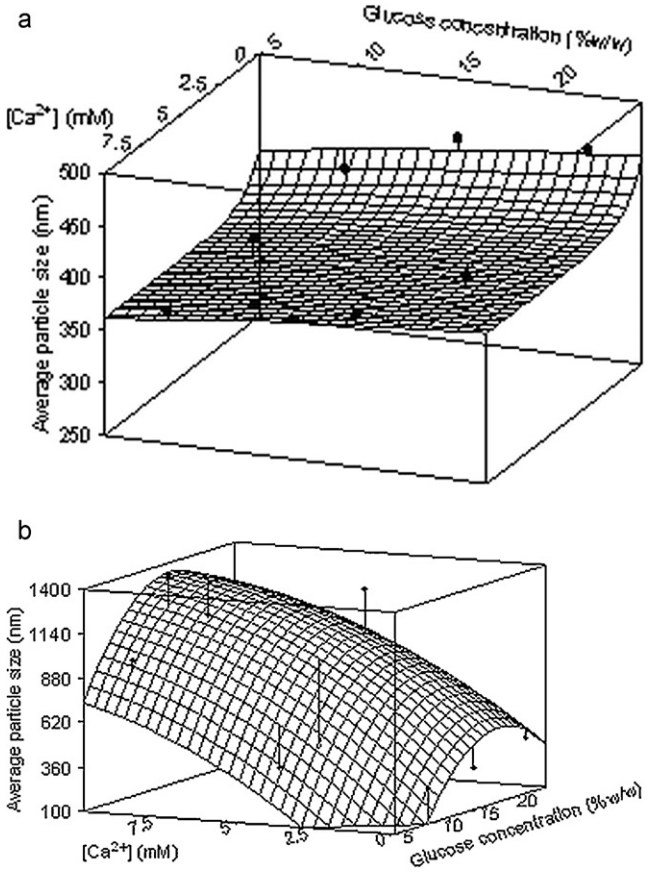

Fig. 5. (a) Average particle sizes just after production of TNA-mixture. (b) Measured, calculated and extrapolated distribution of droplet sizes on the 10th day of storage.

can be detected (Fig. 5b). It seems as if the droplet size distribution would be normalized however this is just the situation after oiling out. This process can be followed by microscopic examination as well. Peaks in huge amounts of oversize globules appeared in higher Ca-concentrations at the 4th and 10th day and for the 14th day proportion of detectable single big fat droplets reduced (Fig. 2). The process of "oiling out" starts with aggregation of small droplets and afterwards it is creaming. In this step dense layer on the top of emulsion can be easily mixed by gentle shaking. In the next step large droplets developed due to the diminished repulsive force between the individual small droplets in the emulsion. Finally, these big droplets coalesced and free oil is released from the droplets because the emulsifier cannot keep interglobular distance any more and the emulsion system cracks, as described by several authors [17,18].

Our measurements demonstrate the importance of the use of different control methods because the sensitivity is different in the prediction of emulsion stability. However, visual inspection of whole mixture and the microscopic view of the samples are still the most valuable methods in verification of stability of the preparation.

5. Conclusions

The high calcium-load of 5 mmol/100 ml in the experimental TNA increased the instability of the mixture. We demonstrated that surface tension, zeta potential and droplet-size measurements are indicators of different sensitivity in verification of colloid stability of the test solutions. With dispersity measurements it can be concluded that at the time of production droplet-size limits of the mixtures meet the requirements, but afterwards destabilization appears.

The higher glucose concentration could not have an influence on interfacial tension, because the concentrated glucose solutions show higher surface tension as a pure water. This study presents a good example for the need of critical evaluation and effective testing of benevolent demands and prescriptions in extreme clinical situations, especially in newborns and small infants.

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