Reviewer's report

Title: Influence of the relative composition of trace elements and vitamins in physicochemical stability of total parenteral nutrition formulations for neonatal use

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Reviewer: Judith Kunutsche

Reviewer’s report:

Lobo et al. studied the influence of additives on the physicochemical stability of formulations for total parenteral nutrition (TPN) particularly concerning the influence of additives on the emulsion droplet size. Size determinations of colloidal fat emulsions are not an easy task and more than one method is often applied to obtain reliable information about size distribution and about the number of larger droplets (> 5 µm) that needs to be limited to ensure safety of TPN. The authors applied 3 different particle sizing methods (dynamic light scattering, optical microscopy and single particle counting) to characterize size and size distribution in addition to determinations of zeta potential, osmolality etc. The manuscript presents a valuable contribution in the field also providing useful information on stability of TPN for the clinical practice. Unfortunately, the manuscript lacks accuracy in many parts.

Major Compulsory Revision

1. Please check correctness and accuracy of information about dynamic light scattering throughout the manuscript. For example:
   a. Methods/Dynamic light scattering: What do you mean with “Z medium”? Assuming that the z-average is mentioned here, note that this size value (together with the polydispersity index) is not a result of CONTIN analysis.
   b. Results/Globule size determination…/2nd paragraph: “… Polydispersity index was less than 0.7 …” Such a high value indicates distinct inhomogeneities in the samples. Please specify the polydispersity indices for the different measurements (e.g. in table 3). The reasons for unusual high values (above 0.3) should be discussed.
   c. Results/Globule size determination…/2nd paragraph: Please correct: “… DLS, are capable of detecting … globule diameters of (0.010 – 500 µm)…”. The upper size limit for DLS measurements is about 1-5 µm. Check the cited references. Please check also the 1st sentence of the 4th paragraph (“The great limitation …”) for accuracy.
   d. Results/Globule size determination…/last paragraph: “… and Laser Diffraction Methods, as DLS, …” Laser “diffraction” (static light scattering) should not be mixed up with dynamic light scattering. Although based on light scattering, the measurement principles (and thus measurement size range, advantages and
limitations, size information) are different. For information about principle of static light scattering see for example H.G. Merkus, Particle Size Measurements: Fundamentals, Practice, Quality (Springer/2009); C. Washington: Particle size analytics in pharmaceutics and other industries (Ellis Hoorwood/1992); J. Kuntsche et al., J.Biomed.Nanotechnol. 5 (2009) 384.

2. Interpretation of the size results obtained by optical microscopy should be done with more care. Light microscopy is a valuable tool for the detection of larger droplets in µm-size range which are not detectable, for example, by dynamic light scattering. However, its usefulness for establishing size distributions or a mean droplet size in colloidal formulations appears a bit questionable. Results shown in table 3 clearly show limitations of light microscopy for this approach: The average diameters vary considerably (i.e. from 400 to 870 nm for formulation NP1) and also the SD values indicate large variations between the single determinations of the same sample. Please add information about repeatability and accuracy of this method for size determinations of colloidal fat emulsions. Information about the largest detected droplet size in the formulations after different storage time/temperature would be desirable.

3. Results/Visual inspection.../2nd paragraph: "... cream layer is visible as a translucent band separated from the remaining TPN ..." Check for correctness! A translucent layer indicates presence of oil on the top on the emulsion (phase separation/cracking, see i.e. reference 26). This is an irreversible process and one should expect an alteration in the fraction of larger emulsion droplets (# 5 µm) in this case. Detailed information about the sampling procedure needs to be added to the manuscript. How it was assured that a homogeneous sample (representative for the whole) has been withdrawn from the infusion bags?

Minor Essential Revisions

4. Introduction/4th paragraph: Please check for accuracy/correctness: “... individual lipid particles must be between 0.4 µm to 1 µm in size ...”. The mean droplet diameter of colloidal fat emulsions is usually even smaller than 400 nm (see manuscript/results/globule size determination.../3rd paragraph and cited references). Furthermore, "... where GSD (globule size distribution) cannot exceed 500 nm ..." should be "... where the mean droplet size should not exceed 500 nm ...".

5. There are discrepancies in the composition of the formulations between the manuscript (Material and Methods, Composition of the admixtures) and Table 1: zinc acetate/sulfate, sodium chloride/hydrochloride, magnesium sulfide/sulfate. Please check and clarify!

6. Methods/DLS: Check correctness of cited reference "... according to the equipment manual (26)...". Improve expression of the sentence "... the measurements taken were specified by international standards ..."? What stands "[MP1]" for?
7. In particle size determinations of colloidal formulations, potential contamination with dust particles may cause problems. This is particularly important in single particle counting. Have required precautions (e.g. filtration of all diluents) been taken for the size determinations in this study? Please add this information in the method section.

8. Results/Zeta potential: The second paragraph is a bit confusing to read and should be improved. Furthermore, the influence of the zeta potential on the stability of parenteral fat emulsions as well as destabilization by additives has already been studied intensively. The authors may consider the literature data, for example: C. Washington, Int.J.Pharm. 66 (1990) 1-21; C. Washington et al., Int.J.Pharm. 54 (1989) 191-197; C. Washington, Int.J.Pharm. 87 (1992) 167-174). Please check for accuracy (2nd paragraph): “… this parameter (zeta potential) reveals the distance between the emulsifier’s charge and the lipid droplets…”

9. There were significant differences in the PFAT between formulations NP1/NP3 and NP2 (table 3). The reasons for this result should be discussed in the manuscript.

10. Results/Globule size determination.../end of 7th paragraph: What do you mean with "... electronic microscopy ..."?

11. Results/Globule size determination.../last paragraph: "... while European Pharmacopoeia indicates only OM for sub-visible particulate matter ..." The actual edition of the European Pharmacopoeia includes light obscuration particle counting for the evaluation of sub-visible particulate matter in injections and infusions even as the preferred method over optical microscopy (see Ph.Eur. 2.9.19. Particulate contamination: Sub-visible particles; Method 1). Please correct!

12. Figure 1: Eliminate the asterisks indicating significant differences in this figure as the differences are likely not significant (as stated in the manuscript, results/Zeta potential determination/1st paragraph).

13. Table 3: Please specify what size value is given in the table for optical microscopy; median (D50) obtained from a size distribution as stated in the manuscript (Globule size determination.../7th paragraph)? Preferably, the z-averages and polydispersity indices obtained by DLS should be added to the table.

Discretionary Revisions

14. Organization of table 1 is a bit confusing and should be improved. Preferably the added volumes of stock formulations should be specified together with the concentration of the active ingredients in the final formulation. All concentrations should refer to the same unit (e.g. amount active ingredient [g or Eq] per mass or volume of final formulation) and should not be given as a dose. Does the value given for the vitamins refer to the added volume of stock solution (MVI 12
opoplex)?

15. Results/pH evaluation/1st paragraph: “... NP3 admixture, which displayed incompatibility due to ...”. Was this incompatibility detected during the study? Otherwise “… which displayed …” should be replaced by “… which may be prone to …” or similar.

16. Results/Globule size determination.../end of 12th paragraph: What do you mean with "... storage conditions ... recommended for technical procedures and political policy, ..."?

17. Tables 1 and 3 contain essential information and should be added to the manuscript and not given as supplementary information.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.