Physical Stability of an All-in-One Parenteral Nutrition Admixture for Preterm Infants upon Mixing with Micronutrients and Drugs

Vigdis Staven[1, 2, 3], Siri Wang[4], Ingrid Grønlie [5, 6, 7], Ingunn Tho[2, 3] *

[1]Hospital Pharmacy of North Norway Trust, Tromsø, Norway

[2]Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

[3]School of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo, Norway

[4]Norwegian Medicines Agency, Oslo, Norway

[5]Norwegian Medicines for Children Network, Bergen, Norway

[6]Hospital Pharmacy at Haukeland University Hospital, Bergen, Norway

[7]Department of Pediatrics, Haukeland University Hospital, Bergen, Norway

*** Correspondence to**

Prof. I. Tho, School of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo, Norway; ingunn.tho@farmasi.uio.no

ABSTRACT

Objectives: The main objective was to investigate Y-site compatibility of intravenous drugs with one standard TPN admixture for preterm infants. Since micro-precipitation was observed in the water phase after addition of trace elements, the concentration effect on micro-precipitation formation developed as a sub goal.

Methods: Seven drugs (ampicillin, ceftazidime, fluconazole, fosphenytoin, furosemide, metronidazole and paracetamol) were mixed in three mixing ratios with one preterm TPN admixture. Samples were investigated within one hour and again after four hours. Precipitation was studied in a lipid-free version called TPN_{aq} by light obscuration, turbidimetry and visual examination. Emulsion stability data was assessed by light obscuration and laser diffraction. pH was measured to assess theoretical risk of precipitation and emulsion destabilization. The influence of different concentrations of trace elements on precipitation was investigated by visual examination, turbidimetry and light obscuration.

Results: Ampicillin, ceftazidime, fosphenytoin and furosemide lead to precipitation after mixing with TPN_{aq}. In some samples of TPN and fluconazole, metronidazole and paracetamol, the emulsion droplet size was above the acceptance limit, although this might also be inherent to the TPN admixture. An unexpected formation of micro-precipitate correlating to increasing amounts of added trace elements, might be caused by an interaction of cysteine and copper, and complicated the compatibility assessment with drugs.

Conclusions: The micro-precipitate resulting from addition of trace element should be investigated further. This study did not provide sufficient evidence to recommend Y-site

infusion of the tested drugs and the preterm admixture; however it might offer some additional support to other compatibility data.

Key words: y-site compatibility, TPN, total nutrition admixture, copper, cysteine, emulsion stability, precipitation, trace elements..

KEY MESSAGES

What is already known on this subject

- TPN admixtures are complex blends and Y-site infusion of incompatible combinations of drugs and TPN might cause precipitation of particles or destabilization of the lipid emulsion, both presenting risk of emboli if infused into the blood circulation.
- There is a lack of documented compatibility data for many drugs and TPN combinations, especially for doses, products and infusion regimes relevant for infants and children, and extrapolation of data generated for the adult population should be done with great care.

What this study adds

- Preliminary compatibility data adopted for preterm infants for seven drugs (ampicillin, ceftazidime, fluconazole, fosphenytoin, furosemide, metronidazole and paracetamol) with a preterm infant TPN formulation.

- The complexity of parallel infusion of drugs and TPN is emphasized by an unforeseen micro-precipitate generated by addition of increasing amounts of micronutrients, yet within recommended range, to the TPN.

INTRODUCTION

Infants and children require varying amounts of nutrients at different stages due to their continuous growth and development[1-2]. There has been an increased focus on standardized total parenteral nutrition (TPN) formulas, hospital-compounded and commercial admixtures, as they have been shown to be well-tolerated, easy to use and reduce the risk of serious mistakes[3-4]. Several benefits have been demonstrated also for preterm infants; recommended nutrition intake and weight gain can be obtained using standardized AIO formulas[5].

Neonates in intensive care units often receive complex therapy with many drugs in addition to TPN, so Y-site administration can be desirable. However, TPN admixtures contain more than 50 different components, and physicochemical interactions leading to formation of precipitates and/or emulsion destabilization are quite possible if mixed with drugs. In the worst-case scenario particles and large oil droplets might cause blockage of blood vessels and even death if infused[6-7]. Documented compatibility data for TPN and drugs in Y-site is important in order to provide safe care for the patients. Extrapolation of existing compatibility data of drugs and TPN admixtures for older children and adults should be done with care because of differences in TPN composition, drug concentrations etc. The aim of this study was to obtain Y-site compatibility data for drugs and one standard TPN admixture used in preterm infants in Norway. Due to the observation of micro-precipitates in the admixture after addition

of trace elements, investigation of the effect of different trace element concentrations on the risk of precipitation in TPN developed as a sub goal.

MATERIALS AND METHODS

Materials

The TPN admixture was intended for peripheral or central administration to preterm infants from four days of age. This admixture can be ordered from Fresenius Kabi or compounded locally in the hospital pharmacy. Table 1 shows an overview of the ingredients of this admixture prepared in a local pharmacy in an ethyl vinyl acetate (EVA) monolayer bag (FrekaMix®, Fresenius Kabi). Drugs and concentrations tested are also shown in Table 1. Ceftazidime and fosphenytoin were reconstituted in glucose 50 mg/ml, and ampicillin and furosemide in NaCl 9 mg/ml. Fluconazole, metronidazole and paracetamol were used undiluted.

Table 1: Overview of the ingredients constituting the TPN admixture prepared at the local hospital pharmacy, and drugs and concentration tested in simulated Y-site

Product type	Name	Manufacturer	Lot No.
3-in-1 TPN admixture for peripheral or central admini-stration	*Preterm regimen from 4 days of age, containing:		-
	Vaminolac®	Fresenius Kabi	16HK0133; 16HB0237
	Glucose 500 mg/ml	Fresenius Kabi	121AH31; 12HKH17
	Water for injection	Local pharmacy	14L08BD; 15B24BH
	Glycophos®	Fresenius Kabi	12HKL28; 12HFL27
	Magnesium sulphate 1 mmol/ml	B.Braun	15035012; 14377012
	Potassium chloride 1 mmol/ml	B.Braun	144118091; 14423012; 14251013
	Calcium chloride 1 mmol/ml	B.Braun	15155036; 14412035; 13503035
	Smoflipid®*	Fresenius Kabi	16HK0062
Trace elements	Peditrace®	Fresenius Kabi	12HFL07, 12HLL97
Vitamins water soluble	Soluvit®*	Fresenius Kabi	10IB6649, 10HM4571
Vitamins lipid soluble	Vitalipid® Infant*	Fresenius Kabi	10HA2297; 10HK2215
Drugs	Ampicillin sodium 50 mg/ml	Bristol-Myers Squibb	3C02634, 4L02584, 5C03610, 3F02259, 3J01732
	Ceftazidime pentahydrate 40 mg/ml	Fresenius Kabi	18H3210
	Fluconazole 2 mg/ml	B.Braun	13212418, 14384404
	Fosphenytoin sodium 10 mg/ml (given in phenytoin sodium equivalents)	Pfizer	J76024, H74522, L58188
	Furosemide 2 mg/ml	Nycomed, Takeda	10820264 L1057442, 10992853
	Metronidazole 5 mg/ml	B.Braun	143448131, 131218131
	Paracetamol 10 mg/ml	B.Braun	14382407
		Fresenius Kabi	16GL0200

* For precipitation testing the lipid emulsion was substituted with water for injection and vitamins were omitted.

Methods

The full composition of two versions of the TPN admixtures used can be viewed in Table 2. For the assessment of potential precipitation the lipid emulsion was substituted with water for injection, and no vitamins were added to the bag[8], in order to avoid camouflage of particles by the white emulsion and strongly colored vitamins. This version was referred to as TPN_{aq}. For investigation of emulsion stability the admixture including lipid and vitamins was compounded[8], and this version is referred to as TPN. Additions of micronutrients were made

in the highest recommended concentrations informed by Fresenius Kabi. However, in TPN_{aq} used in drug compatibility assessments only 8 ml Peditrace per L was added (see result section).

Table 2: Composition of the two versions of TPN admixture: TPN_{aq}, where the lipids are replaced by water for injections (contains no vitamins) and TPN containing all additives.

Ingredients	Per liter TPN_{aq}	Per liter TPN
Lipids *(g)*	-	23.6
Olive oil	-	25%
Soybean oil	-	30%
MCT	-	30 %
Fish oil	-	15 %
Glucose anhydrous *(g)*	56.4	54.2
Amino acids total *(g)*	27.5	26.4
Alanine *(g)*	2.7	2.6
Arginine *(g)*	1.7	1.7
Aspartic acid *(g)*	1.7	1.7
Cysteine *(g)*	0.4	0.4
Glutamic acid *(g)*	3.0	2.9
Glycine *(g)*	0.9	0.9
Histidine *(g)*	0.9	0.9
Isoleucine *(g)*	1.3	1.3
Leucine *(g)*	2.9	2.8
Lysine *(g)*	2.4	2.3
Methionine *(g)*	0.5	0.5
Phenylalanine *(g)*	1.1	1.1
Proline *(g)*	2.4	2.3
Serine *(g)*	1.6	1.5
Taurine *(g)*	0.1	0.1
Threonine *(g)*	1.5	1.5
Tryptophan *(g)*	0.6	0.6
Tyrosine *(g)*	0.2	0.2
Valine *(g)*	1.5	1.5
Sodium *(mmol)*	16.0	16.0
Potassium *(mmol)*	16.0	15.4
Magnesium *(mmol)*	2.0	1.9
Calcium[a] *(mmol)*	4.6	4.5
Phosphate[b] *(mmol)*	8.0	10.3
Chloride *(mmol)*	25.3	24.3
Sulphate *(mmol)*	2.0	1.9
Peditrace®[c] *(ml)*	8[d]	14.5[d]
Zink chloride *(mg)*	4.1	7.4
Copper chloride ($2H_2O$) *(mg)*	0.4[e]	0.8[f]
Manganese chloride ($4H_2O$) *(mg)*	0.03	0.1
Sodium selenite anhydrous *(mg)*	0.03	0.1
Sodium fluoride *(mg)*	1.0	1.8
Potassium iodide *(mg)*	0.01	0.02
Soluvit®[c] *(vials)*	-	2.9
Vitalipid® infant[c] *(ml)*	-	33.4

a: calcium chloride as calcium source;
b: from glycerophosphate, the emulsion and Vitalipid® infant;
c: micronutrient additives
d: corresponds to 0.8 and 1.5 ml trace elements per. 100 ml respectively
e: corresponds to 160 µg/L of Cu^{2+}
f: corresponds to 290 µg/L of Cu^{2+}

Some of the same drugs was previously studied in combination with TPN admixtures for neonates and older children in our set-up[9]. A range of relevant mixing ratios of drug+TPN were calculated in the same way as described earlier[9] to mimic different mixing ratios in the infusion line. Doses of drugs and TPN for preterm infants (weight 200 g - 2 kg) were used in the calculations. ESPEN/ESPGHAN and national guidelines were consulted in order to identify a relevant volume of TPN[1,10]. An infusion time of 8 and 24 hours were used to calculate the infusion rate of TPN. Eight hours are probably too fast for most preterm infants, but was included to constitute an extreme. The BNF for children, national guidelines, the Norwegian Medicines for Children network's reconstitution tables[10-13] and SmPC were used to identify appropriate doses and infusion times of the drugs. Drug concentrations were chosen based on suggestions by clinicians and reconstitution tables[13]. Finally, the infusion rate of the drug was divided by the infusion rate of TPN to obtain the mixing ratio. Mixing ratio 1+1 plus the two most extremes (high drug:low TPN and low drug:high TPN) were chosen to best cover the full range of relevant mixing ratios. If no mixing ratio with excess drug was identified this way, two mixing ratios with excess of TPN were chosen as an alternative[9].

Samples of drug and TPN were mixed in a laminar airflow cabinet by addition of TPN to the drug in sterile 50 ml polypropylene centrifuge tubes (Corning Incorporated, New York, USA). For visual examinations clean and sterilized glass tubes were used (Scherf Präzision Europa GmbH, Meiningen, Germany). Drugs and TPN_{aq} were filtered 0.22 µm before mixing. TPN (with lipids) was not filtered. The samples were tested as soon as possible (within one hour) and again four hours after mixing. The visual examinations were in addition performed 24 hours after mixing.

The possible influence of adding trace elements on precipitation in pure TPN_{aq} was investigated by adding an increasing amount of trace elements (zero to maximum amount stated by manufacturer).

A panel of test methods for assessment of precipitation and emulsion stability was employed (Table 3)[8]. Before mixing with drug, characterization of the drug-free TPN_{aq} and TPN was performed to obtain base line values. The experiments were conducted under ambient laboratory conditions.

Table 3: Overview of test methods for assessment of physical compatibility between TPN and parenteral drugs and the acceptance criteria applied[8]. PFAT5 = volume weighted percentage of fat droplets above 5 µm. FNU = formazin nephelometry units. V.W. MDD = volume weighted mean droplet size.

Methods for detection of potential precipitates in mixed samples (drug+ TPN_{aq})	**Acceptance criteria / points to consider**
Sub-visual particle counting by light obscuration[a]	Particle counts < 1000-2000/ml ≥ 0.5 µm[8], and large particles not exceeding Ph.Eur. limits for large volume parenterals[14].
Turbidity measured by turbidimeter[b]	Turbidity < 0.20-0.30 FNU (taking into consideration background turbidity of unmixed samples)[8]
Visual examination against black background with Tyndall beams[c]	No signs of visible particles or Tyndall effect[8, 15].
pH measured by pH-meter[d]	Evaluation of risk of precipitation of drug and/or calcium phosphate.
Methods for assessment of emulsion stability in mixed samples (drug+ TPN)	**Acceptance criteria / points to consider**
MDD measurements; laser diffraction[e]	V.W. MDD should be <500 nm. Size fraction (%) > 5 µm should be zero[16].
PFAT5 calculated based on droplet size measurements from light obscuration[a]	PFAT5 < 0.40 %[16, 17]
pH measured by pH-meter[d]	pH < 5.5 might be an indication of increased risk of emulsion destabilization[17]

a: Accusizer 780 Optical Particle Sizer, Nicomp PSS, Santa Barbara, USA;
b: 2100Qis Turbidimeter, Hach Lange GmbH, Düsseldorf, Germany;
c: fiber optic light source (Schott KL 1600 LED, Mainz, Germany) and red pocket laser pointer (630-650 nm, max output <1 mW);
d: Metrohm 744 pH Meter, Metrohm AG, Herisau, Switzerland;
e: Mastersizer 2000 and Hydro 2000G sample dispersion unit, Malvern Instruments, Worcestershire, UK

Sub-visual particles were counted using light obscuration (Accusizer 780 Optical Particle Sizer, Nicomp PSS, Santa Barbara, USA). The sensor type was LE-400-05 set in summation mode, measuring particles from 0.5 to 400 µm in 15 ml of undiluted sample[8]. The total particle count/ml ≥ 0.5 µm and the amount of particles ≥ 10 and 25 µm per ml were

determined[8,14]. The background count of the centrifugation tubes was below 100 particles/ml ≥ 0.5 μm[8].

The turbidity of the samples was measured in Formazin nephelometry units (FNU) using a Turbidimeter (2100Qis, Hach Lange GmbH, Düsseldorf, Germany). The sample was gently inverted a few times before measurements[8].

The samples were studied visually against a black background with two light sources, a fiber optic light source (Schott KL 1600 LED, Mainz, Germany) and a red pocket laser pointer (630-650 nm, max output <1 mW). The samples were gently inverted to set possible particles in motion[8,15].

The pH of samples was measured with a pH meter (Metrohm AG, Herisau, Switzerland) calibrated with buffers of pH 4.00, 7.00 and 10.00. Compatibility was theoretically evaluated based on pH-values[8].

The volume weighted mean droplet diameter and volume weighted percent of particles below 500 nm and 1 μm were estimated using laser diffraction (Mastersizer 2000 and Hydro 2000G sample dispersion unit, Malvern Instruments, Worcestershire, UK). The dispersion unit was filled with Milli-Q-water and the samples (≈ 2 ml aliquot) were added to this. The sonication was turned off to avoid breaking up large droplets. The absorbance was set to 0.001 and the refractive index to 1.46[8].

Light obscuration was used to estimate the PFAT5% of the fat emulsion, that is the percent of fat droplets above 5 microns in the large diameter tail[16,17]. The sensor was set in extinction mode and the detection threshold at 1.80 μm. A 40 ml glass beaker was used to dilute the samples, and Milli-Q-water as the dilution medium. Samples were collected with a

micropipette and diluted to concentrations below the instrument's coincidence limit of 9000 particles/ml, using dilution factors of 1:300–1200 (sample:water). The samples were stirred for 60 seconds prior to measurements and during measurements with a magnetic stirrer embedded in the instrument. The sample withdrawal from the diluted emulsions was 15 ml. The counts were distributed over 128 channels, and the equivalent spherical volumes of the oil droplets were calculated. The density of oil used in calculations was 0.92 g/ml and the final fat composition 0.027 g/ml (including fat from Vitalipid® Infant)[8]. The following equation was used to calculate PFAT5[17]:

$$\mathbf{PFAT5} = \frac{[\mathbf{TSV\ (cm3)\ x\ Density\ (g/ml)\ x\ Dilution\ factor}]}{[\mathbf{Sample\ volume\ (cm3)x\ Final\ fat\ composition\ g/ml}]}$$

TSV= total spherical volume, number of particles counted x ESV (equivalent spherical volume;

$$\text{ESV (equivalent spherical volume)} = \frac{\pi \times D^3}{6}$$

Density = density of oil used in the emulsion

Sample volume = the amount of diluted sample measured, here 15 ml

Final fat composition = the amount of lipid in grams/ml in the TPN admixture

Statistical evaluations: calculation of means and standard deviations were performed. Compatibility was evaluated theoretically (pH/physico-chemical properties of drugs/TPN)

and according to stated acceptance criteria and negative controls (base line). An overall assessment of these factors was considered more appropriate than isolated statistical analysis.

RESULTS AND DISCUSSIONS

Characterization of TPN_{aq} without added drug, and investigation of the effect of added trace elements on precipitation

When the highest recommended addition of trace elements (1.5 ml Peditrace®/100 ml) was added to TPN_{aq}, fine powdery particles were seen using Tyndall light, and both the sub-visual particle counts and the turbidity indicated ongoing precipitation in TPN_{aq} (Figure 1). Immediately after filtration of the TPN_{aq} samples into the test tubes, the sub-visual particle counts were ≈1000 particles/ml, but they increased dramatically in number (≈14.000 particles/ml) over the observation time of four hours. Particle sizes were mostly < 1 µm and the particle concentration of 10 and 25 µm particles were well below the Ph.Eur limits[14]. A correlation was observed between the amount of added trace elements and the extent of precipitation (Figure 1). This was also the case for the turbidity measurements, although the FNU values were above the acceptance limit also right after filtration (Figure 1). In visual examination small amounts of haze could be identified, increasing over four hours. After about 24 hours most of the haze seemed to have disappeared in the sample tubes. Furthermore, a brownish color was noticed on the syringe filters used to filter the samples (Figure 2), also

disappearing over time. During the course of the shelf life of the mixture, the precipitation in the TPN_{aq} bag seemed to gradually decrease. In an attempt to avoid precipitation, lower amounts of trace elements (1 ml/100 ml and 0.8 ml/100 ml) were added. 0.8 ml Peditrace®/100 ml corresponds to “normal” use instead of the maximum limits (Table 2). The particle counts were much lower compared to the 1.5 ml/100 ml samples, however the turbidity was not acceptable and haze could still be seen in Tyndall light (Figure 1).

Detection of brown precipitates on in-line-filters used during administration of TPN admixtures have been reported, possibly caused by an interaction between copper and cysteine[18-20]. The preterm admixture contained cysteine, which is typically added as a semi essential amino acid in pediatric TPN[1], and copper was introduced with the trace elements. Thibault suggests a limit of 157 µg copper per litre when using low pH, cysteine containing amino acid solutions[19] which is in the same order of magnitude as in the current study. However, no similar precipitate was detected in our previous study with a TPN admixture containing higher concentrations of trace elements and a similar concentration of cysteine[9]. Foinard et al. observed a stronger color on the filters after filtration of the complete TPN admixture compared to filters used for filtration of a solution containing only amino acids and trace elements, even though the latter mix contained a higher concentration of cysteine and trace elements[20]. This suggests that the concentration of trace elements and cysteine are not the only influencing factors. Additional factors such as pH, redox conditions, ion concentration, combination of metal ions, mixing order, temperature, glucose, derivate of cysteine, packaging (multilayer versus monolayer), light, presence of vitamins etc. have been discussed[18, 21-23].

Fresenius Kabi performed a retest on this particular admixture in a multilayer bag, without finding any precipitate (personal e-mail correspondence, Hege Børringbo, Fresenius

Kabi). The use of different packaging might have prevented the precipitation. On the other hand, Allwood and co-workers found the copper cysteinate (or copper sulphide) precipitate to occur more easily in multilayer bags[23]. However, consulting other authors describing this precipitation we learned that, Foinard and colleagues[20] used a multilayer bag (personal e-mail correspondence, Dr. Aurélie Foinard), and Thibault[19] used a monolayer EVA bag (personal e-mail correspondence, Dr. Maxime Thibault), and both found this precipitate. Another aspect to consider is that studies have shown that TPN ingredients might be contaminated to different extent by trace elements[24], which could have influenced the outcomes in our study as well. Clearly, this is a complex matter and elucidating all influencing factors needs further research. Unfortunately, the nature of the precipitate and the actual copper content of the raw materials and final admixtures were not analyzed. It should be noted that the admixture used in this study is not identical to the one delivered by Fresenius Kabi. The concentrations are the same, however raw materials and bag used are different.

The possible clinical significance of the observed precipitate is not known. It has been discussed that such a precipitate might affect the availability of copper and cysteine and lead to symptoms of deficiency over time[20]. It is also possible that infusion of the particles formed could have a harmful effect. The SmPC of the cysteine containing amino acid solution Primene® (Baxter) includes an instruction to use a final filter during administration of Primene® and trace elements in order to remove particles that may form with e.g. copper, and further recommends to perform blood levels of copper (when medically relevant) if discoloration of filters are noted[25].

Trace elements in the concentration 0.8ml/100 ml, corresponding to “normal” amount of trace elements, was chosen for the compatibility testing with drugs. Base line values for the TPN_{aq} and TPN compositions outlined in Table 2, can be viewed in Table 4. Base line values

for the drugs in the same reconstituted concentrations were reported in a previous work[9]. As can be seen in Table 4 the sub-visual particle counts were low, but high turbidity and small amounts of visual micro-precipitates were still present in TPN_{aq}. Since the test results after mixing with drug would be affected, this has to be kept in mind for the interpretation of the results. For tests on the emulsion stability, the maximum amounts of trace elements were added (Table 2). It is not known whether the precipitate was present in the admixture containing lipid, since this version was not filtered and precipitates would be hidden by the white color.

Table 4: Results from the investigation of possible precipitation and emulsion stability following the mixing of drug and TPN (n≥ 3), V.W. MDD (volume weighted mean droplet diameter) and % size fractions: n=1 with multiple runs. Mix ratios denotes drug+TPN_{aq} or drug+TPN, respectively. Shaded areas highlight values that might indicate an incompatible mix.

Drug	Mix ratio Drug+ TPN/ TPN_{aq}	Investigation of possible formation of precipitation with TPN_{aq}									Testing of emulsion stability with TPN							
		**Particles/ml ≥ 0.5 µm		Turbidity (FNU)		Visible particles and/or Tyndall effect (+/-)			pH		Light obscuration		Laser diffraction			pH		
											PFAT5		% < 500 nm	% < 1 µm	V.W MDD			
		0h	4h	0h	4h	0h	4h	24h	0h	4h	0h	4h	4h	4h	4h	0h	4h	
Baseline (TPN_{aq}/TPN)	-	17 ± 15	136 ± 40	0.32 ± 0.17	0.86 ± 0.50	+/-	+/-	-	5.89	5.89	0.11 ± 0.01	0.18 ± 0.02	82	100	375	5.89	5.89	
Ampicillin 50mg/ml[a]	**1+10**	336 ± 219	113 ± 98	0.28 ± 0.04	0.23 ± 0.06	+	+	+	6.91	6.81	0.07 ± 0.00	0.10 ± 0.01	82	100	374	6.92	6.77	
	1+1	100 ± 36	1932 ± 200	0.45 ± 0.17	0.67 ± 0.06	+	+	+	7.95	7.92	0.08 ± 0.01	0.04 ± 0.01	83	100	373	8.03	7.92	
	2+1	86 ± 4	2287 ± 591	0.96 ± 0.10	1.03 ± 0.16	+	+	+	8.16	8.19	0.07 ± 0.00	0.04 ± 0.01	85	100	370	8.23	8.16	
Ceftazidime 40 mg/ml[b]	**1+10**	55 ± 16	12 ± 6	0.10 ± 0.02	0.20 ± 0.02	-	-	+	6.04	6.00	0.09 ± 0.01	0.23 ± 0.02	83	100	369	6.00	6.04	
	1+1	19 ± 5	18 ± 2	0.12 ± 0.02	0.12 ± 0.03	-	-	-	6.48	6.42	0.12 ± 0.07	0.04 ± 0.03	87	100	360	6.63	6.71	
	1+2	27 ± 12	13 ±6	0.10 ± 0.01	0.10 ± 0.01	-	-	+/-	6.33	6.28	0.09 ± 0.02	0.14 ± 0.02	83	100	370	6.36	6.45	
Fluconazole 2 mg/ml[c]	**1+10**	381 ± 190	180 ± 12	0.16 ± 0.04	0.12 ± 0.01	+	+/-	-	5.85	5.86	0.14 ± 0.02	0.30 ± 0.06	82	100	378	5.84	5.85	
	1+1	360 ± 165	85 ± 19	0.14 ± 0.02	0.09 ± 0.00	+	+/-	-	5.86	5.87	0.12 ± 0.01	0.29 ± 0.04	81	100	382	5.85	5.89	
	9+1	92 ± 4	77 ± 26	0.08 ± 0.02	0.07 ± 0.01	-	-	-	5.85	5.87	0.10 ± 0.01	0.32 ± 0.28	80	100	385	5.88	5.90	
Fosphenytoin 10 mg/ml[b]	**1+50**	135 ± 19	33 ± 4	0.10 ± 0.01	0.10 ± 0.01	-	-	-	5.94	5.96	0.11 ± 0.01	0.15 ± 0.04	82	100	374	5.91	5.92	
	1+1	132 ± 14	27 ± 10	0.09 ± 0.02	0.18 ± 0.08	+/-	+/-	+	7.47	7.44	0.09 ± 0.00	0.04 ± 0.00	83	100	375	7.34	7.23	
	5+1	54 ± 20	52 ± 25	0.08 ± 0.01	0.11 ± 0.01	-	-	-	8.21	8.25	0.09 ± 0.01	0.08 ± 0.01	82	100	378	8.14	8.07	
Furosemide 2 mg/ml[a]	**1+100**	436 ± 215	105 ± 31	0.14 ± 0.05	0.10 ± 0.01	-	-	-	5.87	5.90	0.13 ± 0.03	0.24 ± 0.02	83	100	373	5.84	5.85	
	1+1	561 ± 319	39 ± 16	0.13 ± 0.04	0.08 ± 0.00	+/-	+/-	+/-	5.94	5.98	0.10 ± 0.01	0.04 ± 0.01	83	100	372	5.90	5.93	
	2+1	518 ± 284	51 ± 26	0.12 ± 0.02	0.09 ± 0.01	+/-	+/-	+/-	5.99	6.02	0.09 ± 0.01	0.04 ± 0.02	83	100	374	5.97	5.98	
Metronidazole 5 mg/ml[c]	**1+10**	312 ± 82	218 ± 26	0.26 ± 0.14	0.27 ± 0.16	+/-	+/-	-	5.84	5.85	0.17 ± 0.03	0.35 ± 0.08	82	100	374	5.81	5.83	
	1+1	302 ± 81	118 ± 28	0.18 ± 0.09	0.10 ± 0.02	+/-	-	-	5.63	5.65	0.16 ± 0.01	0.29 ± 0.05	82	100	377	5.60	5.62	
	5+1	252 ± 25	109 ± 85	0.10 ± 0.01	0.10 ± 0.01	-	-	-	5.29	5.28	0.15 ± 0.05	0.27 ± 0.10	82	100	377	5.27	5.29	
Paracetamol* 10 mg/ml[c]	**1+10**	40 ± 1	42 ± 9	0.14 ± 0.01	0.13 ± 0.02	-	-	-	5.70	5.71	0.08 ± 0.00	0.20 ± 0.01	80	100	379	5.75	5.76	
	1+1	13 ± 1	12 ± 4	0.35 ± 0.01	0.36 ± 0.02	+	+	+	5.30	5.31	0.09 ± 0.01	0.15 ± 0.01	83	100	374	5.33	5.33	
	1+2	25 ± 6	28 ± 12	0.26 ± 0.01	0.26 ± 0.01	+	+	+	5.39	5.38	0.12 ± 0.02	0.36 ± 0.15	83	100	374	5.40	5.42	

***** All tests were performed with paracetamol from B.Braun**,** except from laser diffraction measurements, where the paracetamol was from Fresenius Kabi. **Particle counts above 10 and 25 µm are not shown, as the Ph.Eur limits were not exceeded in any of the samples. a: diluted in 9 mg/ml NaCl, b: diluted in 50 mg/ml glucose, c: undiluted

Characterization of TPN (with lipid) without added drug

The lipid droplet size was as expected within the acceptance limits (Table 3 and 4). The PFAT5 was below 0.40 % and the V.W. MDD was well below 500 nm. Even though the admixture was judged to be stable, some creaming and/or flocculation was visible in the bag. Creaming can be reversed as opposed to coalescence, and the admixture might still be safe for infusion provided prior thorough mixing.

Physical Y-site compatibility of drugs and TPN_{aq} (without lipids and vitamins)

All sub-visual particle counts were low after mixing with the different drugs, except for ampicillin where the particle count had increased considerably after four hours (Table 4). This is also described in previous studies[8-9], and is probably caused by calcium phosphate precipitation occurring when the pH-values increases above pK_{a2} of phosphoric acid at pH 7.2[26]. Ampicillin has been found incompatible in some studies[27-28], and compatible in others[29-30]. Based on the current investigations ampicillin and the Preterm mix should be regarded as incompatible.

The turbidity was above the acceptance limit (>0.20-0.30 FNU) for some mixing ratios of samples with ceftazidime, fosphenytoin, metronidazole and paracetamol (Table 4). Ceftazidime had a slightly increased turbidity after four hours in the mixing ratio 1+10, which might be due to the background noise of TPN_{aq}. However, a clear precipitation and color darkening was observed in samples that were re-examined visually after 24 hours, suggesting

317 that the increased turbidity also might be an initial warning of precipitation in progress due to
318 the mixing of drug with high volume of TPN_{aq}. Co-administration might, therefore, be
319 discouraged, however, ceftazidime has been reported to be compatible in studies with other
320 TPN admixtures[9, 28-30].

321 For fosphenytoin a somewhat high, but variable turbidity (high standard deviation) was
322 measured four hours after mixing in mixing ratio 1+1. Although this in isolation could be
323 explained by the background noise, particles were also detected by visual examination in some
324 of the samples immediately and four hours after mixing. After 24 hours, a precipitate was
325 obvious. Since fosphenytoin is formulated with an alkaline pH (8.6)[9], and buffered with
326 trometamol (SmPC), the pH value was quite high (7.5) also after mixing with the Preterm mix.
327 The precipitate might be calcium phosphate due to alkaline pH and/or degradation of the
328 prodrug to the less soluble phenytoin[31]. In mixing ratio 5+1 there were no signs of
329 precipitation, although the pH was 8.2. An explanation might be the lower concentration of
330 TPN and therefore more dilution of calcium phosphate causing less chance of precipitation.

331 The high turbidity observed in mixtures with metronidazole can presumably be
332 explained by the background noise of the TPN_{aq}. In visual examination the haze was very
333 similar to the trace element-induced precipitate, and it seemed to diminish over time like the
334 turbidity of the pure TPN_{aq} stored in sample tubes. The paracetamol samples also showed
335 increased turbidity and Tyndall effect in mixing ratio 1+1 and 1+2, but not in 1+10. In contrast
336 to the above, these findings did not change over time and were also observed in the pure drug.
337 Therefore, the opacity could be attributed to the drug itself and not a sign of incompatibility[8-
338 9]. Fluconazole showed some signs of particles/Tyndall effect during visual examination after
339 mixing with TPN_{aq}, but no other signs of precipitation was detected (Table 4). The haze in
340 fluconazole:TPN_{aq} was similar to the background noise of TPN_{aq}, and decreased over time, and

was not detectable after 24 hours. Therefore, disregarding the trace element-induced precipitations and background noise of pure drug, metronidazole, paracetamol and fluconazole were probably compatible with the TPN_{aq} admixture. This is supported by studies with other admixtures[9, 28-30, 32-33].

The appearance of the particles observed in TPN_{aq} mixed with furosemide was different. Traces of particle formation were occasionally encountered during visual examination, especially in samples examined 4 and 24 hours after mixing. The pH after mixing was close to that of TPN_{aq}, and since furosemide might precipitate in acidic solution it is probably safest to avoid mixing with TPN. This is in correspondence with the findings with one TPN admixtures for children (Numeta G16E) previous tested in our set-up[9], and also with one of Trissel and colleagues' publications[29]. Other reports have concluded with compatibility[28,30,33], including the results for the other TPN admixture for older children (OlimelN5E) tested in our previous mentioned report[9]. The different conclusions might be explained by differences in pH of the TPN products. The more acidic pH of the admixtures for the smallest children could result in an increased risk of precipitating furosemide.

Physical Y-site compatibility of drugs and TPN (with lipid)

Regarding emulsion stability there were only a few occasions where the PFAT5 values of drug+TPN mixtures were above the acceptance criteria of < 0.40 % , that is if the standard deviations are included (Table 4). After mixing with fluconazole, metronidazole and paracetamol the PFAT5 limit was sometimes crossed. As mentioned, some creaming was observed in the bag right after compounding. Therefore, the occasional high PFAT5 values

might be intrinsic to the admixture itself. Scrutinizing the different mixing ratios of drug+TPN for all drugs, the PFAT5 was high also in mixing ratios containing high volume of TPN and low volume of drug. It is less likely that such a small amount of drug would destabilize the emulsion. Nevertheless, based on the current results, we cannot recommend co-administration of the Preterm mix with fluconazole, metronidazole or paracetamol.

ACKNOWLEDGEMENTS

We would like to thank the Northern Norway Regional Health Authority (Helse Nord RHF, grant number SFP1055-12) and the Norwegian Medicines for Children Network, Bergen, Norway for funding the project. We would also like to express our gratitude to clinicians at the pediatric wards at University Hospital Northern Norway/Tromsø and Haukeland/Bergen, Frank Sundby at the Institute of Animal and Aqua-cultural Sciences, The Norwegian University of Life Sciences, Ås, Norway, to the Hospital Pharmacy of Oslo, Rikshospitalet, Oslo, Norway, School of Pharmacy, University of Oslo, Hege Børringbo at Fresenius Kabi and Margaret Aarag Antonsen, Hospital Pharmacy of North Norway Trust and the employees of the Hospital Pharmacy of Tromsø, Norway.

REFERENCES

1. Koletzko B, Goulet O, Hunt J, et al. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenerology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). *J Pediatr Gastroenterol Nutr.*

2005;41:1-87.

2. Fusch C, Bauer K, Böhles HJ, et al., Working group for developing the guidelines for parenteral nutrition of The German Society for Nutritional Medicine. Neonatology/Paediatrics – Guidelines on Parenteral Nutrition, Chapter 13. *Germ Med Sci.* 2009;7: doi: 10.3205/000074.
3. Colomb V, Marlowe ML, Bonnot D, et al. Practical use of a new three-chamber bag for parenteral nutrition in pediatric patients. *ESPEN J.* 2012;7:e93-e99.
4. Meyer R, Timmermann M, Schulzke S, et al. Developing and Implementing All-in-One Standard Paediatric Parenteral Nutrition. *Nutrients.* 2013;5:2006-2018.
5. Rigo J, Marlowe ML, Bonnot D, et al. Benefits of a New Pediatric Triple-Chamber Bag for Parenteral Nutrition in Preterm Infants. *JPGN.* 2012;54:210-217.
6. Levene MI, Wigglesworth JS and Desai R. Pulmonary Fat Accumulation after Intralipid Infusion in the Preterm Infant. *Lancet.* 1980;316:815-819.
7. Bradley JS, Wassel RT, Lee L, et al. Intravenous Ceftriaxone and Calcium in the Neonate: Assessing the Risk for Cardiopulmonary Adverse Events. *Pediatrics.* 2009;123:e609-e613.
8. Staven V, Wang S, Grønlie I, et al. Development and evaluation of a test program for Y-site compatibility testing of total parenteral nutrition and intravenous drugs. *Nutrition Journal.* 2016;15:29.
9. Staven V, Iqbal H, Wang S, et al. Physical compatibility of total parenteral nutrition and drugs in Y-site administration to children from neonates to adolescents. *JPP Journal of Pharmacy And Pharmacology.* 2016;69:448-462.
10. Klingenberg C. Metodebok i Nyfødtmedisin. 4th ed. 2012. Available: http://www.unn.no/metodebok-nyfodtmedisin/category21153.html. Accessed: 01. 2015.

11. BNF for children. London: BMJ Group, The Royal Pharmaceutical Society of Great Britain, and RCPCH Publications. Available: http://www.medicinescomplete.com. Accessed: 10.2015.
12. Akuttveileder i pediatri. Status epilepticus. Norsk barnelegeforening, Den Norske Legeforening. Revised in 2015. Available:<https://www.helsebiblioteket.no/retningslinjer/akuttveileder-i-pediatri/nevrologi/status-epilepticus/konvulsiv-status-epilepticus> Accessed: 01.2015.
13. Norwegian Medicines for Children Network. Reconstitution tables. Available: https://www.legemidlertilbarn.no/helsepersonell/blandekort/Sider/Blandekortliste.aspx. Accessed: 10.2015.
14. European Pharmacopoeia. 2.9.19. Particulate Contamination: Sub-visible Particles. In European Pharmacopoeia, 8th ed.; Supplement 8.5, 2015. Available: http://online6.edqm.eu/ep805/. Accessed: 08.2015
15. Staven V, Waaseth M, Wang S, et al. Utilization of the Tyndall Effect for Enhanced Visual Detection of Particles in Compatibility Testing of Intravenous Fluids: Validity and Reliability. *PDA J Pharm Sci Technol.* 2015;69:270-283.
16. United States Pharmacopeia. General chapters: <729> Globule Size Distribution in Lipid Injectable Emulsions. In USP 38–NF 33. Available: www.uspnf.com Accessed: 08.2015.
17. Driscoll DF, Bhargva, HN, Li L et al. Physicochemical stability of total nutrient admixtures. *Am J Health-Syst Pharm.* 1995;52:623-634.
18. Yamaoka K, Yamaoka H, Nakajima Y, et al. Coloring and Blocking of In-line Filters when Total Parenteral Nutrition Solutions are Supplemented with Vitamins and Trace Elements. *Jpn. J Pharm Health Care Sci.* 2005;31:620-624.
19. Thibault M. Possible Incompatibility between Amino Acids and Copper in Solutions for Pediatric Parenteral Nutrition. *Can J Hosp Pharm*. 2014;67:160-164.

20. Foinard A, Perez M, Barthélémy C, et al. In Vitro Assessment of Interaction Between Amino Acids and Copper in Neonatal Parenteral Nutrition. *JPEN J Parenter Enteral Nutr.* 2015: DOI: 10.1177/0148607115571967.
21. Barnett MI, Cosslett AG, Duffield DA, et al. Parenteral Nutrition. Pharmaceutical Problems of Compatibility and Stability. *Drug Saf.* 1990;5:101-106.
22. Minton AR, Barnett MI and Cosslett, AG Detection of Particulate Material in Parenteral Nutrition Admixtures. *Nutrition.* 1998;14, 251-252.
23. Allwood MC, Martin H, Greenwood M, et al. Precipitation of trace elements in parenteral nutrition mixtures. *Clin Nutr.* 1998;17:223-326.
24. Pluhator-Murton MM, Fedorak RN, Audette RJ, et al. Trace Element Contamination of Total Parenteral Nutrition. 1. Contribution of Component Solutions. *Journal of Parenteral and Enteral Nutrition.* 1999;23:222-227.
25. SmPC Primene®. Available: https://www.gov.uk/government/organisations/medicines-and-healthcare-products-regulatory-agency Accessed: 02.2018.
26. Newton DW and Driscoll DF. Calcium and phosphate compatibility: Revisited again. *Am J Health Syst Pharm.* 2008;65:73-80.
27. Watson D. Piggyback Compatibility of Antibiotics with Pediatric Parenteral Nutrition Solutions. *JPEN J Parenter Enteral Nutr.* 1985;9:220-224.
28. Veltri M and Lee CKK. Compatibility of neonatal parenteral nutrient solutions with selected intravenous drugs. *Am J Health Syst Pharm.* 1996;53:2611-2613.
29. Trissel LA, Gilbert DL, Martinez JF, et al. Compatibility of parenteral nutrient solutions with selected drugs during simulated Y-site administration. *Am J Hosp Pharm.* 1997;54:1295-1300.
30. Trissel LA, Gilbert DL, Martinez JF, et al. Compatibility of Medications With 3-in-1 Parenteral Nutrition Admixtures. *JPEN J Parenter Enteral Nutr.* 1999;23:67-74.

31. Valentino SJ. A case for prodrugs: Fosphenytoin. *Adv Drug Deliv Rev.* 1996;19:311-330.
32. Fox LM, Wilder AG and Foushee JA. Physical compatibility of various drugs with neonatal total parenteral nutrient solution during simulated Y-site administration. *Am J Health Syst Pharm.* 2013;70:520-524.
33. Bouchoud L, Fonzo-Christe C, Klingmüller M, et al. Compatibility of Intravenous Medications With Parenteral Nutrition: In Vitro Evaluation. *JPEN J Parenter Enteral Nutr.* 2013;37:416-424.

FIGURES:

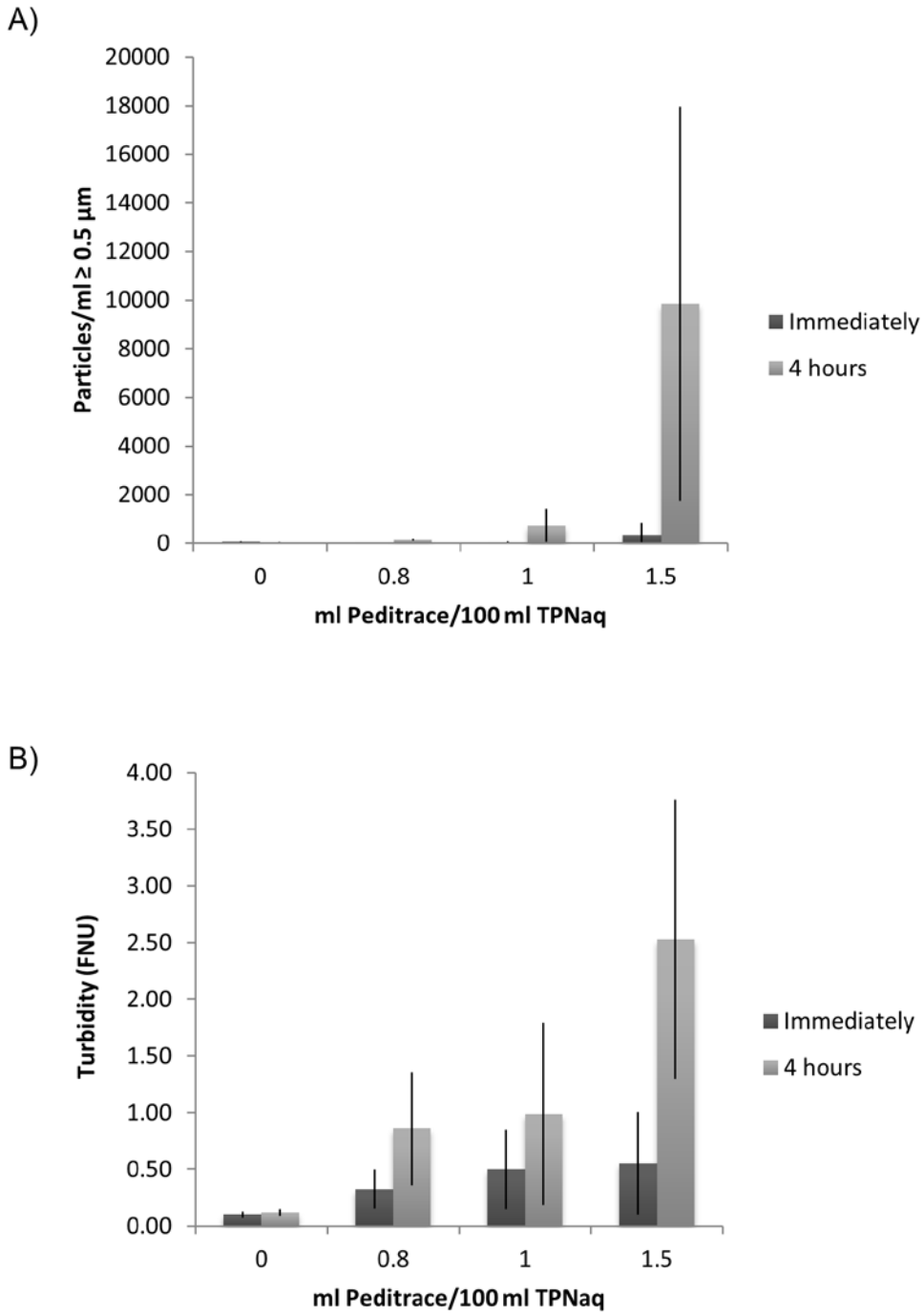

Figure 1: Increasing sub-visual particle counts (A) and turbidity (B) of TPN_{aq} as a consequence of stepwise addition of trace elements (Peditrace®) ($n \geq 3$). Sample preparation and measurements were performed over several days within the shelf life of the TPN_{aq} admixture.

Figure 2: Appearance of filters after filtration of the TPN_{aq} admixture; without addition of trace elements (left) and with ≈ 1.5 ml/100 ml of trace elements (right). A brown color could be seen on filters that had been in contact with the admixture containing trace elements. The color disappeared over time.