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Emulsion Stability of Different Intravenous Propofol Formulations in Simulated Co-Administration with Remifentanyl Hydrochloride

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Abstract

Background: Propofol and remifentanyl often need to be co-administered via the same intravenous catheter line, which predisposes to potential compatibility issues. Our aim was to determine and compare the emulsion stability of three propofol formulations, two with medium chain triglycerides and one with long chain triglycerides, when administered together with remifentanyl hydrochloride.

Methods: Remifentanyl hydrochloride (*Ultiva*®) 50 µg/mL was mixed with two concentrations (10 and 20 mg/mL) of each propofol formulation in mixing ratios 10 + 1, 20 + 1, 1 + 1 and 1 + 20. Emulsion stability was assessed immediately after mixing and 4 hours later by measurements of pH, mean droplet diameter, polydispersity index, and calculating percentage of fat residing in globules > 5 µm (PFAT5).

Results: High PFAT5 values were observed in certain mixing ratios. The correlation between elevated PFAT5 and high propofol concentration (20 mg/mL), when remifentanyl was in abundance and for long contact time indicated that these factors influenced the stability of the propofol emulsions.

Conclusions: Stability differences between the propofol formulations were identified under extreme test conditions. Co-administration of remifentanyl and propofol in

the same i.v. line is safe when propofol is in abundance. Caution is advised when remifentanyl is present in equal parts or in abundance when co-administered with propofol 20 mg/mL.

Keywords: compatibility, safe intravenous administration, anesthesia, droplet growth, destabilisation, PFAT5

Introduction

Propofol is a general anesthetic with a rapid onset of action and a short recovery period. It is often used together with remifentanyl, an opioid analgesic, to induce and maintain anesthesia or sedation. Propofol is formulated as an oil-in-water emulsion because of its low aqueous solubility (Table 1).

It is a common clinical practice that remifentanyl and propofol are co-administered via the same intravenous (i.v.) catheter line. Co-administration of drugs predisposes to potential compatibility issues. Adverse consequences range from i.v. line obstruction to life-threatening embolism. In the case of parenteral emulsions, such as propofol, an incompatibility reaction could manifest as destabilisation of the emulsion. This involves an increase in the diameter size of the propofol oil droplets that ultimately leads to coalescence and phase separation. An early sign of emulsion destabilisation is an increase in droplet size in the large diameter tail of the size distribution [1]. Droplets that reach a diameter of 5 µm or more have an increased tendency to become entrapped in small capillaries, for instance those found in the lungs or the brain, and they may cause embolism [2–5]. Entrapment in the liver, the reticuloendothelial system and several other organs has also been reported [6, 7]. In addition, incompatibilities have been suspected of having a deleterious immunomodulating effect [8, 9].

The available propofol formulations differ in lipid and excipient composition (Table 2). Our hypothesis is that different propofol formulations may behave differently upon contact with remifentanyl.

In clinical practice, infusion flow rates are constantly adjusted to achieve the desired clinical response.

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Table 1: Molecular structure and relevant physico-chemical properties of propofol and remifentanyl.

Property	Propofol	Remifentanyl hydrochloride
Structure
Molecular weight	178.3 g/mol	412.9 g/mol
Aqueous solubility	124 mg/l	591 mg/l
LogP (octanol/water)	3.79	1.75
pKa	11.1	7.1

Table 2: Overview of the compositions of the investigated propofol formulations (Source: SmPC).

Acronym	P-MCT1	P-MCT2	P-LCT
Product name Manufacturer	<i>Propolipid</i> ® Fresenius-Kabi	<i>Propofol-Lipuro</i> ® B.Braun	<i>Diprivan</i> ® Aspen
Soya-bean oil (LCT)	50%	50%	100%
Medium-chain triglycerides (MCT)	50%	50%	
Glycerol	+	+	+
Purified egg phosphatide	+		+
Egg lecithin		+	
Disodium edetate			+
Sodium oleate		+	
Oleic acid	+		
Sodium hydroxide	+		+
Water for injections	+	+	+
pH	7.5–8.5	6.0–8.5	7.0–8.5

This creates an ever-changing relationship between the drugs that are co-administered via the same i.v. line. It is therefore important to examine these drugs in mixing ratios that mimic the relationships that might occur in the i.v. line during infusion. To the best of our knowledge, there is a lack of thorough compatibility studies comparing propofol formulations after mixing with remifentanyl [10–12]. In the case of propofol, an incompatibility can be revealed by a change in the emulsion droplet size. An increase in the content of large sized oil droplets (>5µm) may become critical (exceed the acceptable safety limit) long before it is possible to visually detect emulsion destabilisation.

The aim of this study was to determine and compare the emulsion stability of the three propofol formulations currently used in Norwegian hospitals (*Propolipid*® (P-MCT1), *Propofol-Lipuro*® (P-MCT2) and *Diprivan* (P-LCT)) when administered together with remifentanyl in the same i.v. line.

Material and methods

Materials

The three commercially available propofol formulations used in this study were *Propolipid* (Fresenius-Kabi, Bad Homburg, Germany), *Propofol-Lipuro* (B.Braun Melsungen AG, Melsungen, Germany), *Diprivan* (Aspen Pharma, Dublin, Ireland). They were used at concentrations of 10 mg/mL and 20 mg/mL. Remifentanyl hydrochloride, *Ultiva* (GlaxoSmithKline, Brentford, United Kingdom), 2 mg and 5 mg powder for solution for injection/infusion was used.

Water, unless otherwise specified, was purified by the Milli-Q® integrated water purification system for ultrapure water (Elix®, Merck Millipore, Darmstadt, Germany) with 0.22µm filtration and from now on referred to as MilliQ-water in this manuscript.

Sample preparation

All sample preparation was done aseptically and in a vertical laminar flow bench. Remifentanyl powder was

reconstituted in 9 mg/mL sodium chloride to a final concentration of 50 µg/mL. The calculated volume of remifentanyl was transferred to 50 mL sterile polypropylene centrifuge tubes with CentriStar™ cap (VWR, Radnor, Pennsylvania, USA/Coring Incorporated, New York, New York, USA) via a 0.2 µm sterile syringe filter (Pall, VWR, Radnor, Pennsylvania, USA). The propofol glass container was carefully shaken prior to extraction of the calculated volume, which was transferred to the centrifuge tube containing remifentanyl using a Soft-ject® syringe (Henke-Sass Wolf GmbH, Tuttlingen, Germany).

Remifentanyl was mixed with propofol formulations in the following volume per volume (v/v) mixing ratios respectively: 10+1, 20+1, 1+1 and 1+20. The mixing ratios were chosen to represent the typical drug flow rates in the i.v. line. Each mixing ratio was investigated in three parallels to ensure reproducibility of the results. Control samples consisted of propofol only.

It should be noted that the background level of particle contamination (noise) must be kept at a minimum. This was achieved by selecting tubes and containers with low particle numbers, by rinsing with 0.2 µm filtered MilliQ-water and by always measuring the background noise of the specific batch of MilliQ-water employed for the analyses. The acceptance limit for background noise was set to 50 particles/mL as measured by light obscuration.

Stability analysis of the emulsion

All samples were analysed immediately after mixing and after 4 hours. Emulsion stability was assessed by:

- measuring the pH
- measuring mean droplet diameter (MDD) and polydispersity index (PI) of the droplet size distribution by dynamic light scattering (DLS)
- measuring the number and size of oil droplets larger than 1 µm by light obscuration
- calculation of the percentage of fat droplets larger than 5 µm (PFAT5)

The potential change in the stability of each propofol formulation as a result of mixing with remifentanyl was assessed by comparing all the characteristics of the remifentanyl-propofol mixtures, with those of propofol alone (the control).

pH measurements

The pH was measured for each sample using a pH meter (Mettler Toledo, Columbus, Ohio, USA) at 0 hours and

after 4 hours. The pH-meter was calibrated daily with pH 2.00, 4.00 and 9.00 buffers to cover the full range of pH-values encountered.

Dynamic light scattering

The intensity weighted (I.W.) MDD and PI was determined by DLS with a Zetasizer nano series (Malvern instruments, Malvern, UK). The United States Pharmacopeia (USP) states that a stable i.v. emulsion should not contain droplets with an I.W. MDD of more than 500 nm [1]. PI is a measure of how uniform the size of the emulsion droplets is and ranges from 0 to 1. Values below 0.10 indicate a monodisperse size distribution (uniform droplet size) consistent with a stable emulsion, whereas values above 0.50 are indicative of a very broad size distribution, which is less desirable with respect to emulsion stability. The latter could suggest an increasing droplet size caused by droplet growth [13]. DLS is suitable for measurement of particles in the size range of nm up to maximally 1 µm.

The droplet concentration of propofol emulsions and mixed samples were too high to produce reliable measurements, and the samples were diluted 58–801 times (sample:water) in prefiltered 0.2 µm (Pall, VWR, Radnor, Pennsylvania, USA) tap water (to ensure conductivity) immediately before testing and transferred to polystyrene centrifuge tubes.

Light obscuration

Light obscuration (LO) was used to measure the number and size of oil droplets in the emulsion. In LO (Accusizer 780 Optical Particle Sizer, Nicomp PSS, Santa Barbara, California, USA), particles pass a detection zone whilst being illuminated by a laser beam. The method is also known as single particle optical sizing (SPOS). LO determines droplet sizes above 1 µm, and is therefore mostly interesting for capturing changes in the large diameter tail of the droplet size distribution.

In order to ensure that each droplet was detected separately, samples of remifentanyl + propofol were diluted in MilliQ-water to a final volume of 40 mL in such a way that the number of droplets did not exceed the 9000 «particles»/mL limit required by the instrument. The dilution factor varied from 1:200–1:28 000 (sample:water). Before analysis, the diluted sample contents were evenly distributed by gently shaking the centrifuge tube and resting for 60 seconds to

allow air bubbles to escape. The same procedure was carried out for the controls of propofol.

Based on the measurements of number of the oil droplets of each size, PFAT5 was calculated as described by Gonyon *et al.* [14].

Zeta potential

Zeta potential of the propofol emulsion and mixed samples was measured using a Zetasizer nano series (Malvern instruments, Malvern, UK). The instrument was checked daily with a standard of known zeta potential (-42 ± 4.2 mV). Each sample was diluted in tap water to ensure sufficient conductivity in the solution.

Multivariate data analysis

All results are calculated and reported as mean $\pm$ standard deviation (SD) using Microsoft Excel.

The correlation between variables was evaluated by principal component analysis (PCA) and partial least squares (PLS) regression using Unscrambler® 9.8 (Camo ASA, Trondheim, Norway). PLS models were made based on (a) the full data matrix (144 samples), (b) based on all data immediately after mixing, i. e. excluding the measurements after 4 hours (72 samples), (c) based on all data for propofol 10 mg/mL (72 samples) and finally, (d) based on all data for propofol 10 mg/mL immediately after mixing, i. e. excluding the measurements after 4 hours (36 samples). Prior to analysis, the variation of each variable was scaled to unit variance ($1/\text{SD}$), and the models were cross-validated. The Unscrambler uses Jack-knifing to estimate the uncertainty of the regression coefficients of PLS [15], which for most practical reasons resembles α 0.05. For more information on projection methods, see e. g. Esbensen *et al.* [16].

Results

The background noise in all tested centrifuge tubes and containers both filled with Milli-Q water were very low: 1.0 ± 0.9 particles/mL measured immediately after filling and 2.5 ± 2.4 particles/mL measured after 4 hours. These values were well below the limit of background noise of 50 particles/mL, and demonstrate that the droplets analysed in this study had minimal risk of being influenced

by particle contaminants originating from the water or the containers.

pH

The pH of the reconstituted remifentanil solution was measured to be 3.5, which was in accordance with the information from the manufacturer [17]. The pH values of the three propofol formulations were around 7.5, as expected (Table 2). As shown in Table 3, the pH of the mixtures reflected their composition, i. e. when the mixing ratio contained high amounts of remifentanil the pH shifted towards acidic in contrast to samples with high amounts of propofol where the pH was neutral. Comparing pH-values immediately after mixing and after 4 hours, a slight drop in pH-values was seen for most samples and even some of the propofol controls.

MDD, PI and zeta potential

The MDD varied slightly between the different propofol formulations (Table 3), but was in the range between 165–200 nm, well below the recommended cut-off of 500 nm for injectable emulsions. Small variations were observed between the various mixing ratios and the unmixed controls, e. g. statistically significant difference between 10 mg/mL mixed with remifentanil versus the control for P-MCT1. However, we also found statistically significant differences between different batches of the same propofol formulation, i. e. control samples (data not shown).

All samples showed PI well below 0.1 indicating monodisperse droplet sizes. The zeta potential values of all samples ranged between -20 and -40 mV (data not shown). Of note is the fact that the zeta potential could be different within the range (-30 and -40 mV) from one batch of the formulation to the next one.

PFAT5

All propofol control samples (unmixed propofol), showed PFAT5 values below the 0.05 limit both immediately and after 4 hours (Table 3). This indicated that all three propofol formulations were stable throughout the test period.

In all samples of propofol 10 mg/mL where remifentanil was added in abundance, PFAT5 values were above the recommended 0.05 limit 4 hours after mixing

Table 3: Results of emulsion stability testing for propofol formulations (controls) and after mixing with remifentanyl hydrochloride.

Propofol formulation and concentration	Mixing ratio, remifentanyl HCl + propofol	Time after mixing h	Light obscuration PFAT5	pH	Dynamic light scattering	
					I.W. MDD nm	PI
<i>P-MCT1</i> 10 mg/mL	Control	0	0.004 ± 0.002	7.58	198.7 ± 1.7	0.062 ± 0.026
		4	0.004 ± 0.002	7.46		
	1 + 1	0	0.014 ± 0.008	7.15	190.0 ± 0.5	0.050 ± 0.017
		4	0.015 ± 0.009	6.59		
	1 + 20	0	0.004 ± 0.002	7.14	189.0 ± 1.1	0.058 ± 0.025
		4	0.004 ± 0.002	6.91		
	10 + 1	0	0.032 ± 0.003	3.68	188.1 ± 1.5	0.046 ± 0.020
		4	0.780 ± 0.507*	3.62		
	20 + 1	0	0.026 ± 0.003	3.82	188.4 ± 1.3	0.056 ± 0.015
		4	0.216 ± 0.096*	3.80		
<i>P-MCT1</i> 20 mg/mL	Control	0	0.008 ± 0.002	7.63	202.0 ± 1.3	0.045 ± 0.027
		4	0.007 ± 0.003	7.70		
	1 + 1	0	0.046 ± 0.016	6.09	193.8 ± 1.7	0.068 ± 0.022
		4	0.072 ± 0.022	6.02		
	1 + 20	0	0.007 ± 0.004	7.32	191.2 ± 1.5	0.061 ± 0.022
		4	0.006 ± 0.005	7.07		
	10 + 1	0	0.129 ± 0.034	3.75	192.4 ± 1.6	0.082 ± 0.010
		4	0.590 ± 0.200*	3.76		
	20 + 1	0	0.062 ± 0.017	4.11	196.2 ± 9.5	0.059 ± 0.027
		4	0.651 ± 0.278*	4.04		
<i>P-MCT2</i> 10 mg/mL	Control	0	0.013 ± 0.002	7.32	188.7 ± 1.7	0.079 ± 0.022
		4	0.015 ± 0.005	6.98		
	1 + 1	0	0.072 ± 0.017	5.46	180.3 ± 1.7	0.083 ± 0.019
		4	0.255 ± 0.076	5.38		
	1 + 20	0	0.017 ± 0.005	7.19	180.9 ± 1.1	0.083 ± 0.013
		4	0.014 ± 0.001	7.35		
	10 + 1	0	0.048 ± 0.001	3.59	181.3 ± 1.2	0.085 ± 0.010
		4	0.248 ± 0.113*	3.55		
	20 + 1	0	0.026 ± 0.007	3.73	180.9 ± 1.3	0.090 ± 0.020
		4	0.560 ± 0.178*	3.72		
<i>P-MCT2</i> 20 mg/mL	Control	0	0.012 ± 0.008	7.49	184.0 ± 1.5	0.080 ± 0.006
		4	0.011 ± 0.007	7.53		
	1 + 1	0	0.122 ± 0.048	5.77	175.6 ± 1.0	0.095 ± 0.011
		4	0.555 ± 0.129*	5.99		
	1 + 20	0	0.014 ± 0.004	7.73	176.9 ± 0.9	0.089 ± 0.021
		4	0.013 ± 0.004	7.51		
	10 + 1	0	0.167 ± 0.078	3.70	174.3 ± 1.5	0.078 ± 0.013
		4	0.945 ± 0.089*	3.62		
	20 + 1	0	0.024 ± 0.003	3.74	181.5 ± 1.4	0.085 ± 0.012
		4	0.269 ± 0.096*	3.73		
<i>P-LCT</i> 10 mg/mL	Control	0	0.013 ± 0.003	7.39	175.4 ± 0.9	0.078 ± 0.024
		4	0.013 ± 0.003	7.33		
	1 + 1	0	0.031 ± 0.013	5.41	175.9 ± 1.2	0.075 ± 0.013
		4	0.026 ± 0.000	5.35		
	1 + 20	0	0.015 ± 0.002	7.31	175.6 ± 1.5	0.059 ± 0.018
		4	0.015 ± 0.002	7.17		
	10 + 1	0	0.033 ± 0.014	3.62	174.8 ± 1.9	0.069 ± 0.021
		4	0.095 ± 0.035	3.59		
	20 + 1	0	0.017 ± 0.001	3.58	174.2 ± 1.7	0.080 ± 0.020
		4	0.037 ± 0.013	3.50		

(continued)

Table 3: (continued)

Propofol formulation and concentration	Mixing ratio, remifentanyl HCl + propofol	Time after mixing h	Light obscuration PFAT5	pH	Dynamic light scattering	
					I.W. MDD nm	PI
P-LCT 20 mg/mL	Control	0	0.014 ± 0.004	7.09	168.2 ± 1.9	0.067 ± 0.016
		4	0.014 ± 0.002	7.22		
	1 + 1	0	0.063 ± 0.006	5.17	169.6 ± 0.9	0.074 ± 0.017
		4	0.117 ± 0.012	5.15		
	1 + 20	0	0.016 ± 0.002	7.08	168.5 ± 1.3	0.066 ± 0.019
		4	0.016 ± 0.002	7.04		
	10 + 1	0	0.065 ± 0.014	3.73	166.5 ± 1.0	0.073 ± 0.021
		4	0.291 ± 0.010*	3.69		
	20 + 1	0	0.041 ± 0.004	3.57	167.5 ± 1.0	0.071 ± 0.017
		4	0.240 ± 0.010*	3.62		

*Sample had to be diluted to meet the instrument's coincidence limit of 9000 particles/mL.

(*P-MCT1* = Propolipid®, *P-MCT2* = Propofol-Lipuro®, *P-LCT* = Diprivan®, PFAT5 = Percentage of fat residing in globules larger than 5 µm, I.W. = Intensity weighted, MDD = Mean droplet diameter, PI = Polydispersity index. Mean ± standard deviation).

(long contact time); this happened immediately after mixing in samples of propofol 20 mg/mL irrespective of the mixing ratios of remifentanyl and propofol (Table 3).

Principal component analysis (PCA) showed that PFAT5 displayed a positive correlation with remifentanyl content in the mixing ratio (high value of ratio and high PFAT5), time after mixing (high value of time and high PFAT5) and negative correlation with pH-values of the sample (high value of pH located on the opposite side of origo from PFAT5) (Figure 1(A)). Propofol concentration was somewhat correlated with PFAT5, but to a lesser extent than the other factors as shown by the loading located much closer to origo on the first two principal components. The distribution of samples in the score plot showed three distinct groups along the first principal component (PC1). The group with the highest positive values on PC1 (right end of the x-axis) displayed a positive correlation with PFAT5, whereas the group with the highest negative values (left end of the x-axis) displayed a negative correlation with PFAT5. This group displayed a positive correlation with the pH values of the samples. It should be noted that all propofol formulations are present in all three groups; however, an overrepresentation of *P-MCT2* (green color in Figure 1(B)) might be seen in the group with high PFAT5-values.

The influence of each variable on PFAT5 was analysed by PLS regression (Figure 2). The regression coefficients from PLS models of all factors containing all samples (full matrix) showed that propofol concentration, mixing ratio, mixing time and pH had a significant influence on PFAT5 (Figure 2(A)). High propofol concentration (20 mg/mL), long contact time (4 h after mixing) and low

pH-values (high amounts of remifentanyl) were positively correlated with high PFAT5 values.

Another interesting finding was that *P-LCT* showed a negative correlation with PFAT5 values whereas *P-MCT2* showed a positive one. No significant correlation between *P-MCT1* and PFAT5 values was identified.

Since long contact time between remifentanyl and propofol seemed to have a detrimental effect on the PFAT5, it was interesting to investigate the data from the immediate time point separately. Therefore, data obtained 4 h after mixing were left out and a new PLS model was made for the PFAT5 measured immediately after mixing. The regression coefficients showed the same correlations with PFAT5 as before (Figure 2(B)), even though most of the PFAT5 values for immediate samples were within the 0.05 limit (Table 3). Due to the identified correlations of high propofol concentration and long contact time with high PFAT5, PLS analyses were performed on the data set of propofol 10 mg/mL separately (Figure 2(C)), and propofol 10 mg/mL immediately after mixing separately (Figure 2(D)). Similar trends of the regression coefficients were again seen to correlate to high PFAT5.

Discussion

Propofol is very slightly soluble in water, and is, therefore formulated as an oil-in-water emulsion. Injectable emulsions are composed of oil droplets in water stabilized by one or more emulsifiers. The droplets have a charged surface securing the necessary repulsion between the drop-

Figure 1: Results from a principal component analysis (PCA) of PFAT5 (percentage of fat residing in globules larger than $5\mu\text{m}$) for all mixtures of remifentanyl with the various propofol formulations. (A) correlation loading plot showing the correlation between the factors. (B) score plot showing the projections of the samples.

P-MCT1 = Propolipid, **P-MCT2** = Propofol-Lipuro, **P-LCT** = Diprivan, P conc = propofol concentration, Ratio = mixing ratio of remifentanyl and propofol, Time = time after mixing propofol with remifentanyl, determined PFAT5 and pH.

Figure 2: Balanced and weighted (BW) regression coefficients from partial least squares (PLS1) regression analyses of investigated variables on the measured PFAT5 (percentage of fat residing in globules larger than $5\ \mu\text{m}$) in mixtures of propofol and remifentanyl. Significant factors are those with error bars not crossing zero. Propofol formulation ($P\text{-MCT1}$ = Propolipid, $P\text{-MCT2}$ = Propofol-Lipuro, $P\text{-LCT}$ = Diprivan), P conc = propofol concentration, Ratio = mixing ratio of remifentanyl and propofol, Time = time after mixing propofol with remifentanyl, determined pH. (A) Full data matrix. (B) All formulations but only data immediate after mixing, i. e. excluding measurements of PFAT5 4 hours after mixing. (C) All formulations of 10 mg/mL propofol. (D) All formulations of 10 mg/mL propofol immediate after mixing i. e. excluding measurements of PFAT5 4 hours after mixing.

lets, thereby, avoiding coalescence and phase separation [18]. Emulsions are sensitive to physicochemical changes, such as changes in pH, temperature or variation in electrolyte contents (e. g. oppositely charged counter ions). In a destabilised emulsion, an increase in droplet size will occur [14, 19, 20]. Since intravenous infusion of enlarged droplets encompass potentially serious risks, droplet stability is essential for injectable emulsions [2–9].

Knowledge on propofol emulsion stability when co-administered with remifentanyl is sparse. None of the few studies that have been conducted is able to show conclusive evidence about the compatibility between propofol and remifentanyl or lack of such [10–12]. No studies have analysed both 10 mg/mL and 20 mg/mL propofol even though both concentrations are used regularly in the clinical setting. Neither have a direct comparison of different propofol formulations been carried out.

To capture possible changes in emulsion stability we used different analysis methods, including dynamic light scattering, measurements of pH and zeta potential, and analyses of size and number of large oil droplets using light obscuration followed by calculation of PFAT5. Even though significant differences in MDD could sometimes be identified between the control and mixed samples, despite

all being well below $500\ \text{nm}$, this should not be directly interpreted as incompatibility. The mean droplet size is not the best parameter to assess changes in stability due to droplet growth [21]. As mentioned above, the first signs of droplet growth appears in the large diameter tail of the size distribution, and a considerable amount of large droplets will have to be formed before it can be recognized as an increased MDD. In the current study, MDD and PI were measured to keep an eye on the bulk properties, which was useful since small differences could be observed between different batches of the same formulation, but PFAT5 is the parameter that provides the information on the large diameter tail region. Defined as the percentage of fat droplets larger than $5\ \mu\text{m}$, increased PFAT5 values suggest destabilisation of emulsion. According to USP <729> PFAT5 values should lie below 0.05 (i. e. 5% of droplets below $5\ \mu\text{m}$ in size) to ensure emulsion stability, from an infusion safety perspective [1].

A slight decrease in pH-values was observed for most samples after 4 hours contact time. It is known from literature that lipid emulsions, when exposed to air, may undergo oxidative processes releasing free fatty acids and lowering pH [22]. Steger and Mühlebach showed that emulsions based on LCT and LCT/MCT

degrade at different rates under light exposure at room temperature [22]. Therefore, lipid phase stability should not be forgotten when evaluating emulsion stability.

The experimental area was spanned out with the aim of making the results as clinically relevant as possible. The maintenance dose of propofol is 5–150 µg/kg/min and that of remifentanyl is 0.008–0.25 µg/kg/min. This is in concordance with local guidelines at Oslo University Hospital, Norway [23], the American College of Critical Care Medicine's revised guideline "Clinical practice guidelines for the management of Pain, Agitation and Delirium (PAD)" [24], and information provided in the section General anesthesia: maintenance, in UpToDate [25]. This gives a wide range of possibilities of mixing ratios when adjusting the dose according to the response of the patient. To also accommodate for the possible scenario of bolus doses, the mixing ratios spanned from 20 + 1 to 1 + 20 for remifentanyl: propofol. This was discussed with local clinicians, taking anesthesiology both during surgery and at intensive care into account.

The infusion rates of the drugs are determined by the desired level of sedation and the contact time of the drugs in the i.v. line is therefore difficult to predict. We therefore, analysed all samples immediately after mixing and 4 hours after mixing remifentanyl and propofol. We did not identify any values of PFAT5 above the recommended limit of 0.05 immediately after mixing with remifentanyl for all three formulations at a propofol concentration of 10 mg/mL, but elevated PFAT5 values were seen in most samples after 4 hours (Table 3). This is indicative of emulsion destabilisation at long contact times, which can lead to adverse implications for the patient during surgery that last longer or in cases where the infusion rates are very slow, e. g. for children. In fact, the infusion rates in paediatric patients might be much longer with contact times of the drugs exceeding the experimental conditions covered by this study.

For all three formulations, the high propofol concentration (20 mg/mL) was more prone to the formation of larger droplets after mixing with remifentanyl (Table 3). This phenomenon was even more marked after 4 hours, but also significant immediately after mixing (Figure 2). The direct correlation of PFAT5 with propofol concentration, and samples where remifentanyl was in abundance and pH was low (i. e. high ratio of remifentanyl) indicated that these factors influence the stability of propofol emulsions. Gersonde *et al.* also reported high PFAT5 values for two MCT-based propofol formulations (*Propofol-Lipuro* and *Propofol MCT*) at 20 mg/mL upon mixing with remifentanyl [11]. They measured the samples 24 hour after mixing. Nemec *et al.* found an aggregate formation immediately after mixing equal parts

remifentanyl either with *Propofol LCT* 2% (20 mg/mL) or *Propofol MCT* 1% (10 mg/mL) and recommended not to mix remifentanyl directly with propofol [10].

Our regression models suggested that the different propofol formulations responded differently to mixing with remifentanyl. It seemed like the P-MCT2 formulation (*Propofol-Lipuro*) was positively correlated with PFAT5 and associated with high values of PFAT5 immediately after mixing with remifentanyl and also after 4 hours whereas this was not the case for the other MCT-formulation (*Propolipid*) or the LCT-formulation (*Diprivan*) (Figure 2(A–D)). This deviates from the reports from the manufacturer B. Braun, as they did not see any sign of emulsion destabilisation in their analysis of their MCT-formulation (*Propofol-Lipuro* 1%) mixed with remifentanyl performed after one hour storage. Their mixing ratios were 10 + 1, 1 + 1 and 1 + 10 [26]. For *P-LCT* we identified high PFAT5 values for both concentrations but only 4 hours after mixing with remifentanyl (Table 3). Humbert-Delaloye *et al.* reported no sign of emulsion destabilisation when visually inspecting *Disoprivan* (LCT) 20 mg/mL mixed with remifentanyl in glucose (50 mg/mL) in the concentration 0.12 mg/mL [12], but visual examination is not an accurate method to identify early signs of emulsion destabilisation. Even though our findings suggest that different propofol formulations can react differently upon mixing with remifentanyl, the difference in the lipid phase alone (MCT vs LCT) could not explain these differences; the complete formulation and/or the manufacturing process are the most likely determining factors.

We did not see any high values of PFAT5 in the mixing ratio of 1 + 20, i. e. propofol in abundance, neither immediately nor 4 hours after mixing. In intensive care units, propofol 20 mg/mL is frequently used, and at this concentration, we found high PFAT5 values for all three propofol formulations, both immediately and after 4 hours, in mixing ratios 1 + 1 and 10 + 1, that is when remifentanyl was present in equal parts or in abundance. This demonstrates that high propofol concentrations are more sensitive to reactions with remifentanyl and caution is advised when remifentanyl is present in equal parts or in abundance.

Stewart *et al.* concluded that from a chemical stability perspective of the drugs, it was safe to mix remifentanyl and propofol in a polypropylene syringe and store for up to 36 hours, but mixed in the same polyvinylchloride bag the mixture should be used within 3 hours [27]. Our results showed that from a physical compatibility perspective it is not safe to store remifentanyl mixed with propofol in the same container, such as premixing in a syringe, since both long contact time and mixing ratios of remifentanyl > propofol increased the risk of emulsion

instability. In fact, the importance of always including an emulsion stability test in compatibility studies involving propofol was emphasized by Cherin and Smiler in a letter to the editor as a response to the study of Stewart *et al.* [28]. If premixing remifentanyl and propofol is desired, propofol 10 mg/mL should be used, the ratio of remifentanyl to propofol should be dominated by propofol, and the mixture should be utilised within 4 hours, as we lack stability data beyond 4 hours contact time.

Our findings need to be interpreted with the following limitations and strengths in mind: The samples had to be strongly diluted in order to be analysed. This may have affected the physicochemical properties of the emulsions since flocculates/aggregates that otherwise would gradually coalesce and destabilise the emulsion may have been broken up by the dilution process. However, the control samples of propofol alone were diluted to the same extent. Another limitation is that by performing sample preparation and analysis in the lab environment and not in a classified clean room, the risk of particle contamination from the surroundings as well as from the equipment could not be entirely eliminated, although particle content in the MilliQ-water and main containers were checked. Particles, if in the right size range, could be interpreted as emulsion droplets. The clinical relevance of a rigid cut off limit of 5 µm could be discussed [29]. Oil droplets are more flexible and malleable than solid rigid particles, such as precipitates of crystals, and might be better tolerated by the human body. Furthermore, the 5 % threshold of the acceptable amount of enlarged droplets can also be discussed, and it might be worth mentioning that the PFAT5 monography of the USP does not have an equivalent in the European Pharmacopeia. Emulsion stability was only analysed at two time points, immediately after mixing and 4 hours after mixing, and there will be clinical scenarios that were not accounted for in this study. Infusion rates, especially for the paediatric population, could exceed 4 hours, and as shown, extended contact times could lead to emulsion destabilisation. Last, but not least, our study was set up to simulate a Y-site administration of remifentanyl and propofol and could not be directly converted to the dynamic situation when drugs are co-administered via the same i.v. line.

Conclusions

Differences in emulsion stability depending on the propofol formulation were identified under the most extreme

test conditions in our study (long contact times and excess of remifentanyl in the mixing ratio). The differences could not be explained by type of propofol lipid phase alone. Nevertheless, all tested propofol formulations were found to be stable after mixing with remifentanyl in ratios where propofol was in abundance. Our results indicate that co-administration of remifentanyl and propofol 20 mg/mL in the same i.v. line when remifentanyl is present in equal parts or in abundance should preferably be avoided. Based on the results after 4 hours contact time, i. e. development of larger droplets and a drop in pH, premixing of propofol and remifentanyl in syringes or as admixtures should, if possible, be avoided. Since destabilisation of the emulsion may increase the risk of large fat droplet infusion, we recommend taking differences in emulsion stability between propofol formulations into account when switching between different propofol products.

Abbreviations

PFAT5	Percentage of fat droplets larger than 5 µm
I.W.	Intensity weighted
MDD	Mean droplet diameter
PI	Polydispersity index
USP	United States Pharmacopeia
DLS	Dynamic light scattering
LO	Light obscuration
PCA	Principal component analyses
PLS	Partial least squares
MCT	Medium chain triglycerides
LCT	Long chain triglycerides
HCl	Hydrochloride
i.v.	intravenous
SmPC	Summary of product characteristics
P-MCT1	Propolipid®
P-MCT2	Propofol-Lipuro®
P-LCT	Diprivan®

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