Intravenous drug co-administration in neonatal and paediatric intensive care patients – examining clinically relevant multi-drug compatibility

Thesis for the degree Philosophiae Doctor

by

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"You can't be afraid to fail. It's the only way you succeed - you're not gonna succeed all the time, and I know that." Lebron James.

List of Abbreviations

BPD	Bronchopulmonary dysplasia
BSA	Body surface area
CMC	Critic micelle concentration
CVC	Central venous catheter
DLS	Dynamic light scattering
EMA	European medicines agency
ESPHGAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
EU	European Union
FNU	Formazin nephelometry units
i.v.	Intravenous
I.W.	Intensity weighted
LAF	Laminar air flow
MDD	Mean droplet diameter
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
OUH	Oslo University Hospital
PCA	Principal component analysis
PDA	Patent ductus arteriosus
PDCO	Paediatric committee
PDI	Polydispersity index
PEG	Polyethylene glycol
PFAT5	Percentage of fat residing in globules > 5 μm
Ph.Eur.	European Pharmacopoeia
PICU	Paediatric intensive care unit
PN	Parenteral nutrition
PVC	Peripheral venous catheter
ROP	Retinopathy of prematurity
SD	Standard Deviation
SIMCA	Soft independent modelling by class analogy
USP	United States Pharmacopeia

List of Papers

The present work is based on the following papers, which are referred to by their Roman numerals:

I Nezvalova-Henriksen, K., Nilsson, N., Østerberg, C.T., Staven Berge, V. and Tho, I. Y-Site Physical Compatibility of Numeta G13E with Drugs Frequently Used at Neonatal Intensive Care. *Pharmaceutics.* 2020; 12(7): 677.

II Nilsson, N., Storesund, I., Tho, I. and Nezvalova-Henriksen, K. Co-administration of drugs with parenteral nutrition in the neonatal intensive care unit-physical compatibility between three components. *European journal of paediatrics.* 2022; 181(7): 2685–2693.

III Nilsson, N., Nguyen, V., Nezvalova-Henriksen, K. and Tho, I. Exploring a case of incompatibility in a complex regimen containing Plasma-Lyte 148 in the pediatric intensive care. *Pediatric Anesthesia*. 2022; 00: 1- 8 (early online).

IV Nilsson, N., Nezvalova-Henriksen, K., Bøtker, J.P., Højmark Andersen, N., Strøm Larsen, B., Rantanen, J., Tho, I. and Brustugun, J. Co-administration of intravenous drugs: Rapid troubleshooting solid form composition of precipitate in a multi-drug mixture using on site Raman spectroscopy. Submitted manuscript

Publications not included in the Thesis

i Nilsson, N., Nezvalova-Henriksen, K. and Tho, I., Emulsion stability of different intravenous propofol formulations in simulated co-administration with remi-fentanil hydrochloride, *Pharmaceutical Technology in Hospital Pharmacy.* 2019; 4: 77-87.

ii Staven Berge, V., Nezvalova-Henriksen, K., Nilsson, N., Andersson, Y., Brustugun, J. and Tho, I. Er legemidlene kompatible? *Norsk Farmasøytisk Tidsskrift.* 2021; 2: 32-36.

iii Nezvalova-Henriksen, K., Holm Huseby, T., Nilsson, N, Kjønniksen, I. and Tho, I. Frequently acquired drugs in neonatal intensive care and their physical compatibility. *Acta Paediatra*. 2022; 111: 2307–2314.

Abstract

Neonatal and paediatric intensive care patients are in need of numerous intravenous (i.v.) drugs. The majority of drugs, including parenteral nutrition (PN) and buffered electrolyte solutions, used in the intensive care setting are administered via the central venous catheter. Due to limited venous access ports two or more drugs have to be co-administered in the same i.v. line and a major challenge associated with co-administration of multiple i.v. drugs is the risk of physical incompatibility. Incompatibilities may be presented as precipitation, effervescence, changes in colour, or for emulsions, destabilisation, of one or more of the components that are co-administered. Consequences range from transient catheter obstruction to lethal embolism. This Thesis investigated the compatibility of drugs including PN and buffered electrolyte solutions with focus on the neonatal and paediatric population. In addition, this Thesis explored new methodology, such as Raman spectroscopy, for rapid detection and identification of precipitates in multi-drug mixtures.

Numeta G13E, the PN used for preterm neonates, was tested in two-component mixtures with vancomycin, fentanyl, paracetamol, dopamine, cefotaxime and morphine, respectively, which all were shown to be compatible. In addition, Numeta G13E was tested in three-component mixtures with morphine+cefotaxime and morphine+dopamine. No signs of precipitation nor signs of emulsions destabilization were seen.

The investigation of a five-component case of incompatibility from the paediatric intensive care unit where fentanyl, ketamine, midazolam and potassium chloride had been co-administered with the buffered electrolyte solution Plasmalyte, revealed that both midazolam and ketamine were precipitating, which could be explained by reduced solubility due to pH change governed by the buffered electrolyte. Midazolam also precipitated when replacing Plasmalyte with Plasmalyte Glucos, but to a much lower degree corresponding to the more suitable pH in this version of the product. Fentanyl and potassium chloride were compatible with both Plasmalyte and Plasmalyte Glucos.

Raman spectroscopy was shown to be a powerful tool to identify the nature of precipitated compounds in a multi-drug mixture of ampicillin, calcium chloride, cefotaksime, ceftriaxone, metoclopramide and paracetamol. Using the same model system, single particle Raman microscopy could be used to identify a sub-visual precipitated particle as ceftriaxone calcium

in a three-component mixture of calcium chloride, cefotaxime and ceftriaxone. These results are promising and show that Raman spectroscopy could be a useful tool for compatibility assessment of complex i.v. regimes.

Sammendrag

Neonatale og pediatriske intensivpasienter har behov for en rekke intravenøse (i.v.) legemidler. De fleste legemidler, inkludert parenteral ernæring (PN) og bufrede elektrolyttløsninger som brukes i intensivavdelingen, administreres via sentrale venekateter. På grunn av et begrenset i.v. innganger må ofte to eller flere legemidler administreres i samme i.v. kateterslange og en stor utfordring knyttet til slik administrering av flere i.v. legemidler, er risikoen for fysisk uforlikelighet. Uforlikeligheter kan være utfelling eller destabilisering av emulsjonen av en eller flere av komponentene som administreres i samme inngang. Konsekvensene kan variere fra forbigående kateter obstruksjon til fatale embolier. I denne avhandlingen undersøkes forlikeligheten til legemidler inkludert PN, og bufrede elektrolyttløsninger med fokus på den neonatale og pediatriske befolkningen. For rask påvisning og identifisering av utfellinger i blandinger med flere legemidler utforskes det i denne avhandlingen, Raman spektroskopi som en metode innen forlikelighetsanalyse.

Numeta G13E, PN brukt til premature nyfødte, ble testet i to-komponent blandinger med henholdsvis vankomycin, fentanyl, paracetamol, dopamin, cefotaksim og morfin, som alle viste seg å være kompatible. I tillegg ble Numeta G13E testet i trekomponentblandinger med morfin+cefotaksim og morfin+dopamin. Det var ingen tegn til utfelling eller destabilisering av emulsjonen.

I et tilfelle av uforlikelighet ved en barneintensiv avdeling, der en fem-komponent blanding med fentanyl, ketamin, midazolam og kaliumklorid var blitt administrert i den samme kateterslangen som den bufrede elektrolyttløsningen Plasmalyte. Det viste seg at både midazolam og ketamin felte ut, hvilket kan forklares av redusert løselighet på grunn av pH-endring styrt av den bufrede elektrolytten. Midazolam felte også ut når Plasmalyte ble erstattet med Plasmalyte Glucos men i lavere grad og kan forklares i at Plasmalyte Glucos har en lavere pH hvilket er mer gunstig for løseligheten av midazolam. Fentanyl og kaliumklorid var forlikelige med både Plasmalyte og Plasmalyte Glucos.

Raman spektroskopi viste seg å være et godt verktøy for å identifisere utfellingen ceftriakson-kalsium fra en blanding av flere legemidler bestående av ampicillin, kalsiumklorid, cefotaxim, ceftriakson, metoklopramid og paracetamol. Raman spektroskopi viste seg å også kunne identifisere en sub-visuell utfelt partikkel som ceftriakson-kalsium i en tre-komponent

blanding av kalsiumklorid, cefotaksim og ceftriakson. Disse resultatene er lovende og viser at Raman spektroskopi kan være et nyttig verktøy for vurdering av forlikelighet ved komplekse i.v. regimer.

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1. Background

In 2019 there were a total of over 17,000 patient stays in an intensive care units in Norway, which included paediatric intensive care unit (PICU) (1). Since paediatric patients could in some hospitals be admitted together with adults at general intensive care units, the exact number of paediatric intensive care patients in Norway could not be retrieved. However, it is known that 95,200 children were admitted to a hospital in 2019 in Norway (2), and data from an Australian study found that 1.5 % of all paediatric patients admitted to hospitals included time in the PICU. Transferring the Australian percentage to Norwegian numbers would mean that approximately 1400 paediatric patients spend time in the intensive care unit. Out of the 52,000 children born in Norway in 2020, there were 6900 (13.3 %) patient stays at neonatal intensive care units (NICU) (3). The patient stays also include change of hospital or readmissions, and therefore, double registries could occur, and the number of actual patients may be slightly lower.

Intravenous (i.v.) drug (medications, fluid and parenteral nutrition) regimes are important parts of the treatment of the neonatal and paediatric intensive care patient. The development in medicine has been huge and more severe cases can be treated. Most drugs are given intravenously because the critically ill patient is sedated and not able to swallow tablets, has unpredictable absorption from the intestinal tract or because the formulation or characteristics of the drug itself dictates the necessity of i.v. administration. Neonatal and paediatric intensive care patients are often prescribed numerous i.v. drugs, many of them receiving more than 16 drugs daily (4). Since many of the drugs are administrated as continuous infusions, two or more drugs are often co-administrated in the same i.v. line. Co-administration of drugs in the same i.v. line could increase the risk of incompatibility reactions due to differences in physico-chemical properties of the drugs. Physical incompatibility denotes a physical change due to mixing and can be change of solution colour or turbidity, precipitation (formation of solid particles), or growth of lipid droplets in an emulsion destabilising the emulsion. Chemical incompatibility is when drugs undergo chemical reactions that most often leads to degradation, such as hydrolysis, reduction or oxidation.

Small capillaries in the human body have a diameter of 5-7 micrometers (5), and therefore, particulate drug delivery system intended for i.v. injection (*e.g.* drug carriers or injectable lipid

emulsions), should be in the nanometer range (6, 7). Large particles and enlarged lipid droplets (sizes above the capillary diameter) could lead to catheter occlusion or when entering the body these could be trapped in organs and lead to thromboembolic incident, in the worst case with fatal consequences (8, 9). Critically ill neonatal or paediatric patients are fragile and vulnerable and it could be critical to inject large particles or fat droplets into the bloodstream (10). The naked eye can only detect particles or droplets from approximately 50 micrometre in size (11). It is therefore important to be aware that it is not always possible to detect precipitation visually. This is even more difficult in the case of emulsions.

Decision-supporting tools and databases, for example Micromedex IV compatibility (12) and Stabilis (13), have been developed in order to help the health care professionals to find out if a given combination of drugs will be compatible or not. This enhances the chances of safe co-administration of two drugs. However, a known problem is that studies can show conflicting information, information is incomplete for a given combination or information is lacking (14). In addition, information on more than two drugs co-administered is generally lacking. The test conditions are typically based on adult dose and infusion regimes, which can be very different for paediatric and neonatal patients. Last but not least, most studies are old and outdated, or conclusions are based only on analyses by visual inspection. Considering the limits of what the eye could detect, visual inspection may not be sufficient to detect particles of a size that can be captured in and block the smallest capillaries.

The lack of information on compatibility of frequently used combinations in NICU and PICU complicates safe and practical administration of drugs. Therefore, this Thesis have investigated physical compatibility of drugs in concentrations and infusion regimes that is used for these patients. To further enable safe co-administration of drugs, this Thesis also explored new methodology for rapid detection and identification of precipitates in multi-drug mixtures.

2. Introduction

For the purpose of this dissertation the term i.v. drugs encompasses i.v. medications, parenteral nutrition (PN) and i.v. fluids. All three categories are defined as drugs according to Norwegian drug regulation (15).

2.1 Paediatric patients

Paediatrics is the part of medicine that deals with medical care of children and is a relatively young speciality with a history of two centuries (16). Before this it was mostly the family, friends or midwifes who took care of sick children. Neonatology was introduced in 1960 as a subspecialty of paediatrics, and deals with medical care of newborn infants (17). Treating respiratory distress syndromes and the ability to provide nutrition intravenously were two key factors in the development of neonatal care.

European medicines agency (EMA) divides the paediatric population into following age groups (18):

- Preterm newborn infants (see below)
- Term newborn infants (0-27 days)
- Infants and toddlers (1 month to 23 months)
- Children (2 – 11 years)
- Adolescents (12 – 16 or 18 years)

A newborn is also called a neonate and the neonatal period is the first 4 weeks of life. If the newborn is born before 37 weeks of pregnancy, it is defined as a preterm (19, 20). Preterm neonates are sub-categorised, based on gestational age (first day of the pregnant women's last menstrual period to day of birth):

- Moderate to late preterm (32-37 weeks)
- Very preterm (28-32 weeks)
- Extremely preterm (less than 28 weeks)

Prematurity is also categorised by birth weight:

- Low birth weight < 2500 gram
- Very low birth weight < 1500 gram
- Extremely low birth weight < 1000 gram

Childhood is an extraordinary period with growth and development where the physiological processes are maturing rapidly with advancing age. Especially infants and toddlers bodily changes are rapid and profound. For healthy infants, the birth weight doubles at 6 months and triples at the age of 1 year. Physical development pertains to bodily growth, fine and gross motor skills and the abilities of various organs of the body. Important aspects that determine the progress of physical development in physical and brain changes are development of reflexes, motor skills, sensations, perceptions, learning skills and health issues (21). The development of the brain and nervous system begins a few weeks after conception and is thought to be complete by early adulthood. The better developed the brain and nervous systems are, the more complex behavioural and cognitive abilities children are capable of and new physical capabilities will improve the life of the child (22). Minor illnesses like flu and coughs may also help children learn empathy, or how to understand someone else's discomfort and distress. On the other hand, children suffering from long-term illness is associated with negative impact on quality of life, development delays, pain, and emotional distress (23).

2.1.1 Neonatal and paediatric intensive care patients

Neonatal and paediatric intensive care units provide the highest level of care to children. The patients are, among other things, in need of highly advanced, customised and complex medical care (24). Patients receive intensive care treatment from a wide range of health care specialists, and the patients are followed continuously with close monitoring of heart rate, blood pressure and other vital parameters. If needed, patients can receive organ support for example ventilation assistance, renal replacement treatment or therapeutic hypothermic treatment. Typically, the neonatal or paediatric intensive care patient has one designated nurse that always observes and supervises the patient. Preterm and neonates could if needed be placed in an incubator that will protect the baby and give optimal temperature and humidity conditions for their development. An incubator helps the baby to regulate body temperature, protect from light and sound and adds an extra barrier between the baby and the environment to reduce the chance of infections.

Complications of preterm birth is the cause of approximately 1 million deaths each year worldwide and preterm birth rates are increasing (25). The inequality between high-income

and low-income countries is huge, where in the western world most preterm-born babies survives while in low-income countries preterm babies still die due to lack of adequate neonatal care (26, 27). The two biggest risk factors of dying in the neonatal period is low birth weight and low gestational age. During the first month of life over two-thirds of neonatal deaths occur. Out of preterm who was born with a birth-weight below 1500 gram, 50 percent dies within the first 3 days of life. In a Norwegian study from 2017, Stensvold *et al.* showed that the survival of preterms that was born in week 23 were 35 % and if born in week 24 the survival was increased to 58 % (28). For neonates and even more profound in preterm infants the organs and their functions are immature. Complications and their frequencies vary depending on gestational age and body weight and are and common complications include (28-31):

- Hypothermia. Rapid heat loss occurs in preterm infants because relatively large body surface area leads to excess heat loss and low storage of body fat
- Respiratory abnormalities and includes respiratory distress syndrome, bronchopulmonary dysplasia (BPD), neonatal apnea, pulmonary haemorrhage
- Retinopathy of prematurity (ROP)
- Intraventricular haemorrhage (bleeding into the fluid-filled areas, or ventricles, surrounded by the brain)
- Cardiovascular abnormalities where patent ductus arteriosus (PDA) and low blood pressure is the most common problems. PDA is a heart defect where the foetal extra blood vessel that bypass the lungs does not close after birth and an extra load of oxygenated blood goes back to the lungs, which makes the lungs work harder to handle the extra blood volume
- Necrotising enterocolitis (NEC)
- Infections. Late onset sepsis and other complications associated with an increased risk of infection included prolonged intubation, BPD, prolonged intravascular access, PDA, and NEC

There has been a drastic decrease in mortality rates in the PICU, and patients who earlier died now survives for multifactorial reasons where medical advancements are playing a key role (32). Burns *et al.* concluded in their study from 2014 that the mortality rate has been halved over the last two decades at paediatric intensive care units in the United States (33).

Namachivayam *et al.* also found that more patient survive over the last 30 years at the PICU at Royal Children´s hospital in Melbourne, but concomitant to survival there was an increase in the proportion of survivors with moderate (dependent on care) or severe (totally dependent on care) disability (34). In summation, this means more patient are treated at PICU.

To treat neonatal and paediatric patient admitted to intensive care units is a huge task. Treatment is complex and to meet the needs of the patient the treatment has to be individualised and is therefore difficult to both predict and to plan in detail. The typical patient is vulnerable with constant changes in condition for either the better or worse. Many decisions are to be taken by several health care professionals and many procedures should be handled during the whole stay and many of the procedures and tasks have to be done simultaneously. Drugs are one important part in the treatment of neonatal or paediatric intensive care patients. The clinical care team needs to get information about the status of the patients, and therefore, a variety of tests of for example body fluid samples or different imaging tests are needed. Breathing and heart rate are monitored with stickers on the chest that are connected to wires. Blood oxygen levels has to be checked regularly with either a pulse oximetry attached to one finger or through an intra-arterial catheter. If the patient is in need of extra help to breathe, they are connected to a breathing machine, also called ventilator. An endotracheal tube (a plastic tube through the mouth or nose) or a tracheostomy (a plastic tube inserted directly through the skin into the larynx) is connected to the ventilator on the other end. The care team must also care, handle, inform and educate the parents or guardians of the patient.

2.1.2 Nutrition and fluid need

The general need of nutrition and fluid changes as the newborn grow, with decreasing needs per kg bodyweight with increasing age (35, 36). In the intensive care setting, critical illness induces both metabolic and endocrine changes and needs, such as catabolism and insulin resistance. In addition, enteral feeding difficulties are common and could add to worsen the situation even more (37).

The preterm infant has low substrate storage of both macronutrients (carbohydrates, proteins, and fat) and micronutrients (vitamins, trace elements and electrolytes) due to insufficient time of growth. Electrolytes are also lost through the immature kidneys that are unable to

sufficiently reabsorb electrolytes. To avoid catabolism and nutritional deficit, it is fundamental to support the preterm with an appropriate electrolyte and fluid management (38, 39). Due to gut immaturity, low capacity to control body temperature and high need of nutrition that promote growth have led to early start of PN and are to be lifesaving (40). A summary of fluid and nutritional need for preterm, neonates and children is found in Table A1 in the Appendix.

2.1.3 Drug treatment in neonatal and paediatric patients

Most drugs in children are dosed according to body weight (mg/kg), by age or by scaling the dose from adults by calculating body surface area (BSA) (mg/m^2). Within the Paediatrics, a well-known mantra is that children are not small adults. Children has its own range of independence and horizon. Paediatric patients are not one homogenous group since their physiological changes are rapid and subject to both inter- and intra-individual variabilities resulting in altered pharmacokinetics and pharmacodynamics (41). These variabilities can influence the individual dosage of drugs during the development of the newborn, toddler or child.

Main pharmacokinetic parameters (absorption, distribution, metabolism and excretion) fluctuate in response to growth and with the development of the child, yielding for many drugs a pharmacokinetic profile that is vastly different in children compared to adults (42). Preterm and neonates have relatively increased intragastric pH and this could increase the absorption of enteral administered acid-labile medications, such as ampicillin due less hydrolytic ring-opening, whereas weak acids (for example phenobarbital) could have decreased absorption (43, 44). The newborn consists of 80 % water at birth, which decreases to around 60 % after 6-9 months, which requires higher dosing of water-soluble medications in neonates (for example gentamicin and vancomycin). Other physiological factors that are changing with age are reduced gastric emptying, reduced intestinal transit time and immature transporter activity (45). Other routes of administration of medications are also altered for example the stratum corneum is thinner for preterm and neonates, which could increase the absorption of dermal medications. Differences in developmental aspects affect the capacity of the body to metabolise medications, in particular due to the cytochrome p450 enzymes (CYP450) in neonates corresponding to a lower percentage of its adult activity, affecting the optimal medication dosage. If the medication is mainly metabolised by a CYP450, the effect of

the medicine would be lower for the first week of life but higher later on since the CYP450 activity will increase for each week of life. Maturation of the kidneys is a continuous process and is completed in early childhood. However, for preterm and neonates the renal immaturity is profound and will change drastically during the first months in life. Elimination of for example gentamicin demands either dose reduction or longer dose intervals which changes from week to week in preterm infants.

In the development of many drugs the neonatal and paediatric patients and their needs have not been taken into account. The paediatric committee (PDCO) of EMA is a scientific committee founded in 2007 in response to the new European Union (EU) paediatric regulation (46, 47), and is responsible for activities on medicines for the paediatric patient, to enforce and support the development of age-appropriate medicines for the paediatric population. Still many medications are not available in optimal doses, suitable dosage forms, administration volumes, dosage form size, taste, and type and choice of excipients. When a drug is prescribed for a different purpose than what EMA approved, *e.g.* used in children younger than the approved age or the dosage form has been manipulated (tablets crushed, injections given orally etc.), the use is called *off-label*. Teigen *et al.* found that 83 % of the hospitalised paediatric patients received *off-label* medications (48). For the paediatric patients, the health care practitioner is left with professional experience to assessing optimal medication usage, taking the characteristics of each patient into consideration.

I.v. drugs are common in neonatal and paediatric critical care and are used for life-sustaining medicines and are given to patients for different reasons. It could be because the medication itself is not suitable for oral administration where for example the medication will be broken down in the gastrointestinal tract, have very short half-life or have variable absorption when stable blood concentration of the medication is important. It could also be that the patient is not able to swallow the medication, is sedated or on ventilator or have reduced bowel function.

2.1.3.1 Medications

Medication therapy is a powerful tool to improve the outcome in patients, which also holds true in the neonatal and paediatric patient, and the balance is to have the maximum effect with the minimum amount of side effects (49). For many patients medication therapy is life-saving

but the neonatal and paediatric patient is three times more likely to experience a potential adverse drug event than the adult population (50). Typical medications that are used to the neonatal and paediatric intensive care patient are vasoactive (affect the blood pressure and/or the heart), sedatives (to put the patient in decreased consciousness or in a controlled period of unconsciousness (general anaesthesia), pain-reliefs and antibiotics (4, 51, 52). The neonatal and paediatric intensive care patient are in need of many medications and most of them are as mentioned, given intravenously. Many medications are for stability reasons provided as concentrates or powder for injection or infusion. How to reconstitute and dilute the medication and which concentration of the medication is suitable for the patient have to be considered along with infusion rate and infusion time. Many hospitals or specific wards will have support tools to ensure appropriate medication administration for their patients, such as the national guide "blandekort" in Norway (53). Safe administration is essential since neonatal veins are small and fragile, and i.v. therapy could affect the veins negatively or lead to extravasation, which occurs when fluid from an i.v. line leaks into the surrounding tissues. Commonly used neonatal infusions prone to extravasation are PN, calcium, potassium, bicarbonate, and glucose in high concentrations. Some i.v. medications are also well known for their potential to cause extravasation for example vancomycin and vasoactive medications (54).

2.1.3.2 Fluids

I.v. fluid therapy is fundamental in acute care and is frequently administered for replacement of intravascular volume and restoration of hemodynamic stability in the critically ill patient (55). There are many fluids are available with some differences in applications and properties. The optimal composition of a fluid is dependent on the needs of the patient and could differ in osmolarity and pH (56). A crystalloid fluid is an aqueous solution of electrolytes, glucose or other small, water-soluble molecules and are often isotonic to human plasma (57). Isotonic sodium chloride (NaCl 0.9 %) is the most well-known crystalloid. Possible negative effects of NaCl on acid–base status and plasma tonicity encouraged the development of buffered electrolyte solutions, where the most widely used being Ringers lactate (or acetate) and Plasmalyte in which some of the negative ions (chloride) is replaced with a buffer to affect the acid-base equilibrium in less degree (58-60). However, none of these solutions is completely physiological and crystalloids often vary in electrolyte concentrations including calcium,

magnesium, and potassium. None of these solutions exactly matches that of human plasma and all may have unwanted effects (55).

Glucose is an i.v. fluid that is given to patients who have low levels of blood sugar or are dehydrated and where isotonic glucose (5 %) is the most common. Many neonates are prescribed glucose infusion since hypoglycaemia especially in the early neonatal period where normal adaptive mechanisms like glucogenolysis and gluconeogenesis are immature in neonates and infants, and hypoglycaemia predisposes to long-term neurological damage (61). Since fluid restriction is common for neonates, glucose 10 % is an i.v. fluid of choice to provide maintenance fluid requirements. It is important to notice that higher glucose concentrations than 5 % are hypertonic when determining the appropriate site for infusion. One should also be aware that isotonic glucose has lower pH than isotonic NaCl. Both glucose 5 % and NaCl 0.9 % may also be used for the reconstitution and dilution of drugs.

2.1.3.3 Parenteral nutrition

PN is i.v. nutrition and may include protein, carbohydrate, fat, electrolytes, vitamins and trace elements, which in total could be up to 50 different ingredients (62). The first clinical application of PN in human was in the late 1960s when Dudrick *et al.* successfully nourished 6 adults (63). Later on in the 70`s the development of a complete bags with both macronutrients and micronutrients were achieved (64). The use and the spectre of PN products have since then been in constant development, and today include individualised and standardised pre-manufactured or on-site manufactured for different paediatric population, even preterm neonates (65).

Most preterm are in need of PN due to low capacity to regulate body temperature produce, body heat, heat loss and low storage of body fat. To prevent developmental failure, it is recommended for the extremely preterm start with PN as soon as possible after birth (39). Neonates, infants and children could be in need of PN for numerous reasons like gastro-intestinal issues and persistent nausea. To reach the nutrition goals and at the same time not overhydrate the patient, the PN products must be highly concentrated. There are pre-manufactured standardized PN available in either 2-in-1 (glucose and protein) or 3-in-1 (glucose, protein and lipid) chamber bags (Figure 1). Depending on the patient requirements,

the sealing between the chambers are broken to allow the content of two or all three chambers to mix as part of preparation. It is important to know that these 2-in-1 or 3-in-1 chamber bags are not containing vitamins (both water- and fat-soluble) or trace elements. These micronutrients are added to the bag according to the manufacturer's specification before use. If the patient is in need of extra electrolytes these could either be added to the bag within the maximum addition limits with respect to stability information or be given as a separate infusion. A ready mixed PN with all macro- and micronutrients is often known as TPN as in *total parenteral nutrition*. However, since what really constitute total nutrition could be a matter of discussion, all PN also fully supplemented containing lipid will be addressed as PN in this Thesis. PN is with all its ingredients a complex system and have buffer properties with a pH of around 5-7. It is mainly the amount and concentration of amino acids that determines the pH of the PN especially (66).

Figure 1. *Example of a parenteral nutrition in a 3-in-1 chamber bag used in this Thesis. The bag contains lipid emulsion, glucose and amino acids in separated chambers, and is supplemented with vitamins (water- and lipid-soluble), trace elements and sometimes electrolytes before use.*

2.1.4 Administration of i.v. drugs

Vascular catheters are indispensable in intensive care for administration of i.v. drugs but also for purposes, such as blood pressure monitoring and blood sampling. Insertion of an

intravascular catheter is a common invasive procedure in neonatal and paediatric intensive care units. Technology in catheters is improving and vascular access in smaller and more sick infants has been made possible (67). Drugs could be administered as an injection over seconds or minutes. In other situations the drugs have to be administered over longer time *i.e.* intermittent infusion or as a continuous infusion.

Physiochemical properties of the drug could be reasons that impedes rapid administration of drugs with high osmolality (> 500-600 mOsm/L) or with pH < 4 and pH > 10, which could be painful or could cause thrombophlebitis (68, 69). Vasoactive, highly concentrated or high volume medications should be administered via a central venous catheter (CVC, Figure 2a), and injections or small volume drugs are often administered via a peripheral venous catheter (PVC). A CVC is inserted in a large vein near the heart and in the newborn an umbilical venous catheter could be inserted (Figure 2b).

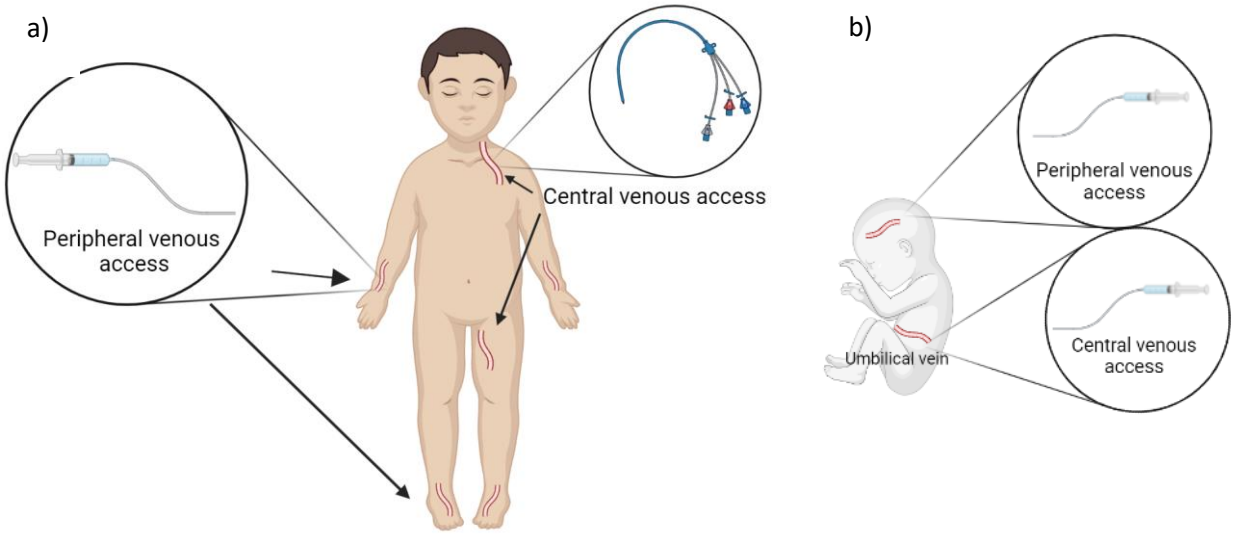


***Figure 2**. Examples of central and peripheral venous access sites in a) children and b) newborn patient (created with BioRender.com)*

A CVC could have one or more lumen and each lumen runs beside each other inside the catheter and has staggered exit ports that prevent the drugs of being in contact between each lumen (Figure 3). A multi-lumen CVC has several lines, one per lumen, but only one venous access point. A PVC is inserted in a small peripheral vein and provides one line via one venous

access point. Obtaining venous access in neonates and children are difficult by anatomical and physiological factors in particular due to veins are small and fragile, which makes the i.v.-catheters are thinner in size (67, 70).

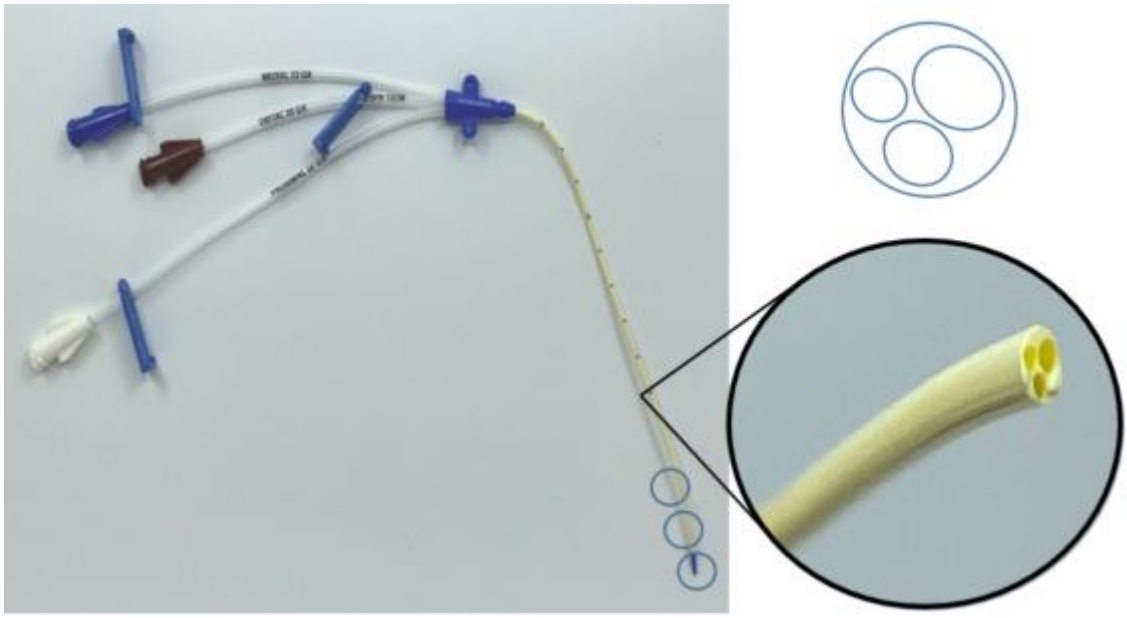


***Figure 3**. A three-lumen central venous catheter with staggered exit ports (highlighted with small blue circles). Cross-section of the catheter illustrates the three lumens inside (Photo: Asbjørn Lunnan).*

Central venous catheters in neonatal and paediatric patients often have not more than one or two lumens due to risk of infections and thrombosis or due to small blood vessels, especially, in the neonates (70, 71). When the patient is in need of more i.v. drugs than available i.v. access ports, the drugs are forced to be co-administered in the same i.v. line. Drugs could be co-administered by adding three ways stopcock connectors to the i.v. line (Figure 4 and Figure 5).

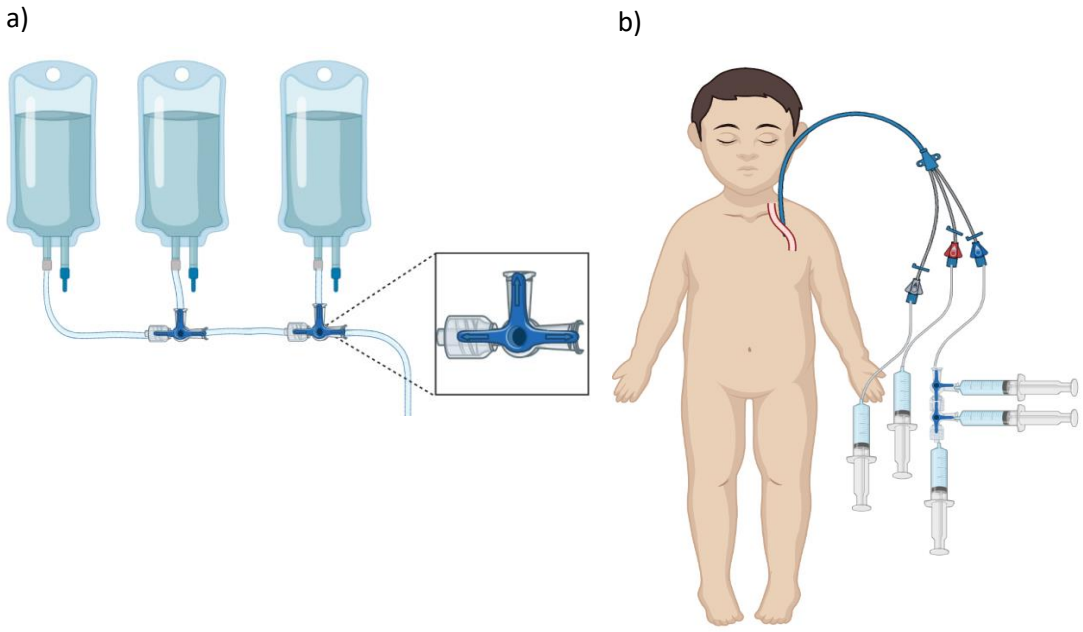


Figure 4. *a) Three drugs are connected to the same intravenous (i.v.) line with three-way stopcock connector (magnified) that facilitates for co-administration of drugs in the same i.v. line. b) Three-lumen central venous catheter with two drugs in each lumen (grey and red) and in the third lumen (blue) three drugs are co-administered in the same i.v. line. (Created with BioRender.com).*

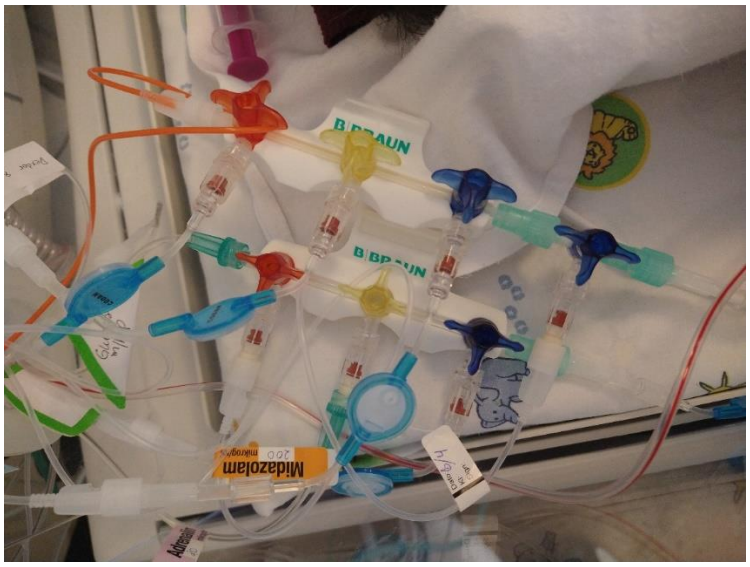


Figure 5. *Overview of a two-lumen central venous catheter with several three-way stopcock connectors (red, yellow and blue) that facilitates for co-administration of drugs in the same intravenous line. (Personal photography).*

2.2 Solubility

Solubility is the ability for a compound (solute) to be molecularly dissolved in a specified medium (solvent) at a specific temperature (72). The solubility is the thermodynamic equilibriums concentration of a saturated solution of the compound in the solvent with excess compound present, and reflects the maximum amounts of compound that the solvent can hold in solution. When it comes to drug solubility, this often relates to the solubility of the drug in aqueous medium at room or body temperature. Solubility is given as a concentration, *e.g.* mg/mL, molarity or %. The solubility of a drug compound depends on its structure (functional groups, charge) and the solvent (polarity, temperature, composition). The structure of the compound determines the hydrophilicity or lipophilicity, hydrogen bonding ability, pKa-value and ability to ionize. Other conditions that influence the solubility are related to the solvent, such as pH, co-solvents, additives, ionic strength and the environment, such as temperature, pressure, mixing rate and kinetics.

The extent of solubility ranges widely, from infinitely soluble to poorly soluble and practically insoluble, and the European Pharmacopoeia (Ph.Eur.) defines solubility in parts of solvent required per parts of solute (Table 1) (73) . In most other sources, such as PubChem, DrugBank and scientific literature, solubility is given as the concentration of the solute in the solvent or parts of solute in parts of solvent. The term *insoluble* is often applied to *poorly* or *very poorly* soluble compounds, even though they do have a low solubility.

***Table 1**. European Pharmacopoeia (Ph.Eur.) solubility definitions and corresponding solubility ranges (Ph.Eur. 11.0).*

Descriptive term	Part of solvent required per part of solute	Solubility range (mg/mL)
Very soluble	Less than 1	≥1000
Freely soluble	From 1 to 10	100-1000
Soluble	From 10 to 30	33-100
Sparingly soluble	From 30 to 100	10-33
Slightly soluble	From 100 to 1000	1-10
Very slightly soluble	From 1000 to 10.000	0.1-1
Practically insoluble	10.000 and over	<0.1

For drugs to be able to cross biological membranes and barriers in the body, *e.g.* for oral drugs be absorbed, they need to be dissolved. Most drugs are either weak acids or weak bases, and therefore, do not completely ionise in a solution. These drugs are most soluble in its ionised form, which is related to the pKa of the drug and the pH of the surrounding (more discussed in 2.2.1 and 2.2.2). There will be an equilibrium between the ionised and unionised form, and it is the unionised form that crosses the membrane. Increased levels of drug in the ionised form drive the concentration gradient and more of the unionised form permeates the membrane. For drugs with poor aqueous solubility, the solubility could be enhanced by chemical modifications like salt formation, by change of pH, or use of a buffer to keep pH at the desired pH-level. Other methods to enhance solubility could be to use surfactants, co-solvents or other solubilisers. *In vitro* and in the drug formulation all these approaches are feasible, but it should be kept in mind that the *in vivo* solubility will be influenced to a major part by the environment, which for i.v. drugs is the blood flow and the physiological pH. When it comes to co-administration of drugs, it is important to be aware of possible interaction between drug formulations that meet in the same i.v. line, and how these products can influence the solubility of each other. Large changes of pH or a buffer with an undesired pH-level can reduce drug solubility and cause precipitation, which ends up being infused into the patient.

2.2.1 pH

pH is the negative logarithm of the concentration of hydrogen ions (H^+) in a solution (72). The pH-scale is logarithmic and ranges from 0 to 14. A change of one unit changes the concentration of hydrogens ions tenfold and a reduction in pH of 0.3 doubles the H^+-concentration. To change the pH of the solution is the most common way to promote the drug being a weak acid or base to be ionised, and therefore, be in the water-soluble form. Basic drug compounds are proton (H^+) acceptors, and are more soluble at acidic pH where the ionised form is in abundance, whereas acidic drug compounds are proton donators and the ionised form are predominant at high pH (Figure 6) (74). At the pKa value, the drug will be 50 % ionised.

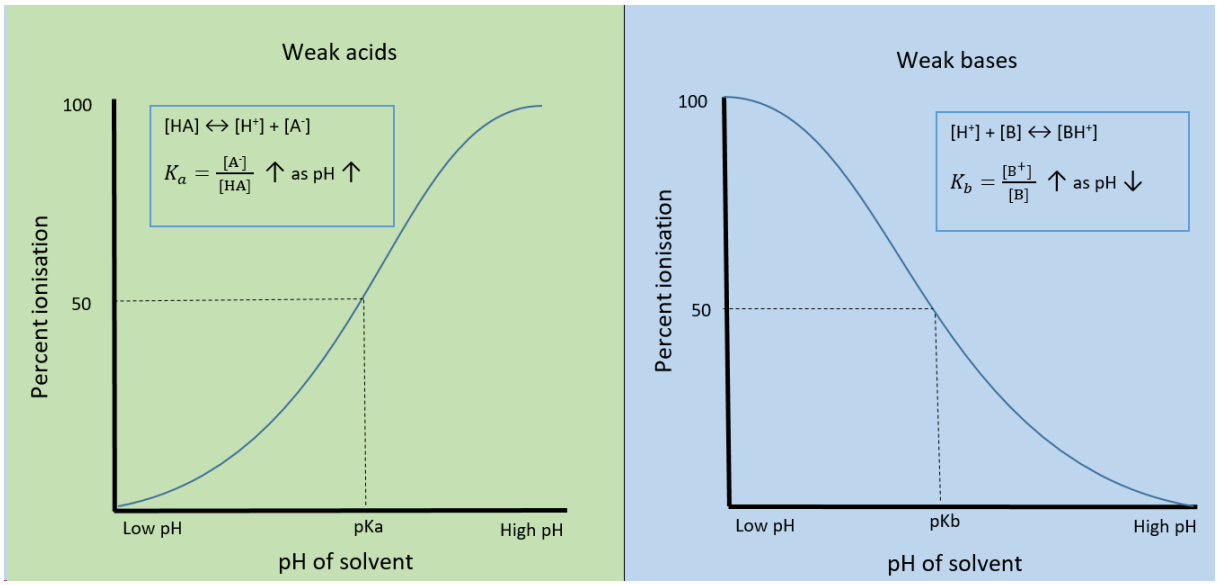


***Figure 6**. Ionisation of weak acids and weak bases with changing pH. Ka is the dissociation constant for an acid (A) and Kb is the dissociation constant for a base (B). H is a proton. (Adopted from quizlet.com).*

2.2.2 Ka, pKa, Kb and pKb

The dissociation constants of the week acids and bases (Ka, and Kb) and their negative logarithm (pKa, and pKb) are useful parameters when predicting whether a species will donate or accept protons at a specific pH value (72). They describe the degree of ionization of an acid or base, and are true indicators of acid or base strength because adding (neutral) water to a solution will not change the equilibrium constant. Ka is the equilibrium dissociation constant of an acid (equation 1) (74).

$$Ka = [A^-][H^+]/[HA] \quad (1)$$

where HA is a weak acid and A^- is the conjugate base of the acid.

To scale down high or low values of Ka, it is common to take the negative logarithm Ka that gives pKa. pKa is a number that shows how weak or strong the acid is and the smaller pKa, the stronger the acid. pKa is used to see the direction of the acid-base reaction *i.e.* helps to predict the ionisation of a drug at a specific pH. Similarly, pKb is the negative log of the base dissociation constant (Kb).

pKa and pKb are related by the simple relation (equation 2):

$$pKa + pKb = 14 \quad (2)$$

The relationship between pKa and pH is described by the Henderson-Hasselbalch equation (equation 3):

$$pH = pKa + \text{logarithm}_{10}([\text{conjugate base}]/[\text{weak acid}]) \quad (3)$$

It is frequently seen in Pharmaceutical sciences that the term pKa is used to denote the negative logarithm of the acid-based dissociation constant regardless of the drug being a weak acid or a weak bases (75).

2.2.3 Excipients

Co-solvents are often organic solvents used to make lipophilic drugs more water-soluble (76). Typical examples are ethanol, propylene glycol, and polyethylene glycol (PEG). Dilution of solutions that contain co-solvents risk diluting the dissolving effect so much that the poorly soluble drug precipitate, because it is still not highly enough diluted to keep the drug in aqueous solution. One prominent example is trimethoprim/sulfamethoxazole concentrate (Co-trimaxole® (77)), a solution for infusion that contains ethanol and propylene glycol as co-solvents. If trimethoprim/sulfamethoxazole is insufficiently diluted, trimethoprim, which is very slightly soluble in water (78) cannot remain in solution. Following the dilution guidelines is essential for concentrates containing co-solvents.

Surfactants are amphiphilic molecules that will improve the solubility of poorly soluble substances by reducing the surface tension between the lipophilic molecule and the aqueous solvent. At concentrations above the critic micelle concentration (CMC), the surfactant will form micelles that solubilise the lipophilic drugs inside the surfactant vesicles. Diluting a drug product below CMC of the surfactant could lead to precipitation of the drug in the same manner as for the co-solvents.

2.2.4 Parenteral nutrition as intravenous oil-in-water emulsion

PN is one example of complex i.v. lipids, which are formulated as oil-in-water emulsions that are stabilised by an emulsifier, most often phospholipids, such as lecithin from egg yolk. The emulsion is stabilised through negatively charged droplets that are kept apart due to

electrostatic repulsion (79). A pure lipid injectable emulsion has a pH of 6-9 and changes during shelf-life, and will be most stable at its initial pH of 9 (80). During storage, the pH approaches 6 and the repulsive forces are weakened or neutralised due to reduced ionisation of the phospholipids (81). Most 3-in-1 PN admixtures have lower pH values after mixing of the chambers, normally pH will be in the range from 5.5-6.5. This low pH, in addition to the presence of electrolytes, reduces the surface charges of the oil droplets. A reduction in surface charge, will lead to less repulsion between the droplets, and they are more likely to interact through Van der Waals attractive forces. Electrolytes can promote flocculation of the droplets and with less repulsions droplets are more likely to merging or coalescence, and a growth in the droplets size can be observed in the large diameter tail of the droplet size distribution (>5 µm) (Figure 7). The process is irreversible and the destabilised emulsion will eventually lead to oiling out and complete phase separation (82). Typically, the pH is lower in paediatric 3-in-1 admixtures than in the corresponding adult products, mainly because of different composition of amino acids where especially arginine, histidine, and lysine are important determinants of buffer capacity (66). However, this also makes the neonatal and paediatric products more prone to stability issues related to the lipid emulsion.

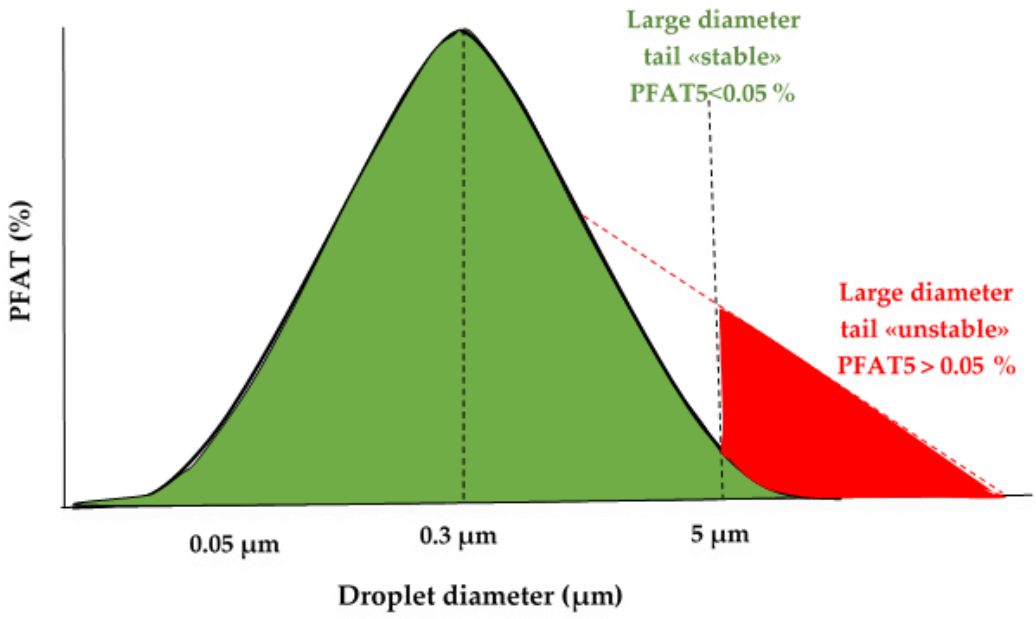


***Figure 7**. Normal droplet size distribution of a parenteral lipid emulsion with large diameter tail. The green area indicates the lipid droplet population with a lower proportion (PFAT5 < 0.05 %) of large droplets (>5 µm). The red area indicates the droplet population with a larger amount of large droplet (>5 µm) (PFAT5 > 0.05 %) typical for an unstable emulsion. The figure has been inspired by Driscoll (83).*

Stability of PN could be altered by light, temperature, amounts of each component, and changes in pH. PN are less stable than each component (macronutrient) on its own, which is why admixtures often are provided in separate chambers inside a bag instead of all-in-one bags. Stability issues are related to the risk of precipitation, degradation or emulsion destabilisation of the different compounds in the PN-bag. Monovalent ions, sodium and potassium, can be present in higher amount than di-and trivalent ions, such as calcium, magnesium and phosphate. Formation of a calcium and phosphate precipitate is the most feared instability in PN compounds. There are cases where crystallisation of poorly soluble calcium and phosphate have led to deaths caused by embolism (9, 84, 85). Three phosphate species could exist in a solution and each of them can form calcium salts with different solubility (Figure 8). Phosphoric acid has three pKa-values, namely 2.2, 7.2 and 12.4, and when pH is around 5.5, the more soluble monobasic form ($H_2PO_4^-$) predominates and forming calcium dihydrogen phosphate in reaction with calcium (equation 4). If the pH reaching a physiological pH of 7.4, the dibasic phosphate species (HPO_4^{2-}) dominates and could form the less soluble calcium monohydrogen phosphate (equation 5) (86).

$$Ca^{2+} + 2\ H_2PO_4^- \leftrightarrow Ca[H_2PO_4]_2 \quad \text{which have a solubility of 18 mg/mL} \qquad (4)$$

$$Ca^{2+} + HPO_4^{2-} \leftrightarrow CaHPO_4 \quad \text{which have a solubility of 0.3 mg/mL} \qquad (5)$$

Most commercially available PN today has a pH of approximately 5.5 and at this pH, the more soluble monobasic form of phosphate predominates. The use of organic or inorganic salts of both calcium and phosphate does matter where the inorganic forms are much more prone to precipitate. The inorganic calcium chloride is more likely to dissociate than the organic calcium gluconate and more Ca^{2+} will be available to interact with dibasic phosphate (87). The organic phosphate is covalently bound to a glycerol. which obstructs binding to calcium ions and less precipitation will be formed then for the organic form of phosphate (88). The PN manufacturer always provide maximum levels of safe additions of calcium and phosphate to avoid formation of precipitates. There are several other factors that also could influence the formation of calcium and phosphate precipitate; for example the buffer capacity of amino acids and formation of insoluble complexes of both magnesium and amino acids with calcium or phosphate (86). Higher temperature has also been described to promote the formation of the

precipitate (86). When mixing PN with medications or fluids, it is important to consider these stability issues.

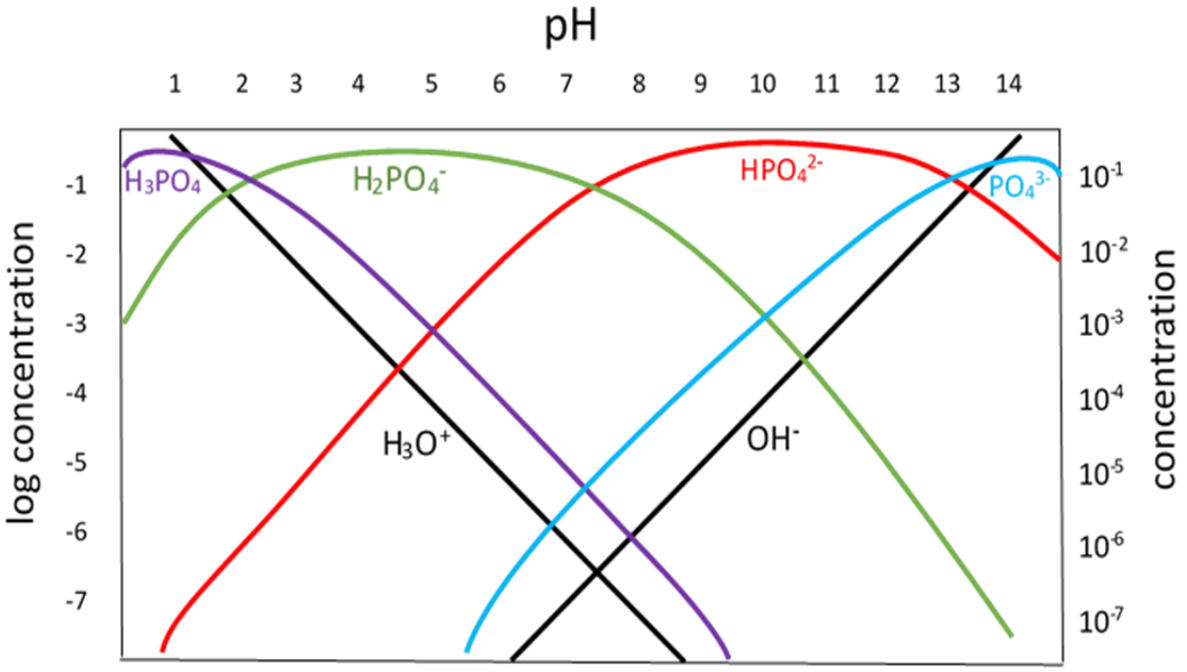


Figure 8. Formation of phosphate species depending on pH value. The figure is inspired by Newton and Driscoll (87).

2.3 Intravenous drug compatibility

I.v. drug compatibility is when drugs meet in the same i.v. line or are mixed in a container without affecting each other. I.v. drug incompatibility is when drugs affect each other in a way that results in a change in physical or chemical property, which ultimately could have a negative impact for the patient (89, 90). There exists a risk of incompatibility whenever i.v. drugs are mixed. Incompatibilities are categorised as physical or chemical (91). Physical incompatibility is when drugs are mixed together and cause formation of solid particles (particulate formation, precipitation, or haze), colour change or gas evolution, or when there is an increase of larger droplets indicating emulsion destabilisation. Chemical incompatibility occurs when mixing of drugs results in a chemical reaction, such as hydrolysis or oxidation, which affects the potency of the active ingredient or uncontrolled (undesired) degradation products are formed. In this Thesis, **physical compatibility** is investigated and Table 2 summaries typical causes of physical incompatibility (92, 93). Other factors affecting compatibility are order of mixing, drug concentration, contact time, temperature, light, oxygen etc. (94).

***Table 2**. Summary of causes of physical incompatibility. This table is inspired by Staven et al. and Newton (92, 93).*

Cause of incompatibility	Description	Examples
Acid-base reactions	More than 90 % of drugs are weak acids or bases (92). Degree of ionisation is based on pH of the solvent and pKa-values(s) of the drug molecule. Best solubility dependent on the pH relative to pKa. Ampholytes, which have both acidic and basic groups, are least soluble at their isoelectric point (pI) Buffered electrolyte solutions can affect solubility of drugs if the pH of the buffer is not optimal with respect to the pKa of the drug.	Furosemide (pH=8.9-9.3, pKa 3.5 (95)) precipitates when mixed with norepinephrine (pH=3.0-4.5) (96). Morphine (pH 3-5) precipitates after mixed with ampicillin (pH=8.0-10.0) (52). Acyclovir (pH 11) precipitates in parenteral nutrition (pH 5-7) (97). Ampicillin (pKa 2.5 and 7.2, pI=4.9). Plasmalyte (pH=7.0) with midazolam (pH=2.9-3.7) (Paper III). Ringer-lactate (pH=6) with thiopental (pH=10.2-11.2 and pKa=7.55) (98).
Polyvalent ions	Formation of salts of di- and polyvalent ions, as calcium, magnesium, phosphate and sulphate are generally less soluble than monovalent ions (for example sodium and potassium). Di- and polyvalent ions could also form precipitates with drug molecules.	The most clinically known precipitation is monohydrogen phosphate ($CaHPO_4$) that can occur when excessive amounts of calcium and phosphate is added to parenteral nutrition (87). The reaction of calcium and ceftriaxone (8).
Salting out	A drug could be salted out when highly hydrated inorganic ions (e.g. Cl^-, K^+ or Na^+) deprive organic ions and molecules of adequate water molecules to remain dissolved.	At a certain ionic strength, the water molecules are no longer able to support the charges of both the ions and the proteins. The result is the precipitation of the least soluble solute, such as proteins and large organic molecules (99).
Dilution of co-solvents	Dilution of solutions of poorly soluble drugs to an extent where the co-solvent loses its effect.	Ethanol loses its effect as co-solvent when trimethoprim/sulfamethoxazole is diluted with aqueous solution (53).
Destabilisation of emulsions	Positively charged electrolytes or acid neutralises the negatively charged surface of the lipid droplet which leads to reduced electrostatic repulsion between the droplet with the result of an increase in droplet size (100).	Parenteral nutrition is formulated as oil-in-water emulsions and can be destabilised when mixed with thiopental and ondansetron (7, 97).

From a physico-chemical drug compatibility standpoint, each drug should have to be given in a separate i.v. line: since patients often need many drugs simultaneously, the need for more access ports is inevitable. However, due to the fact that neonatal and paediatric patients have fragile and small veins, it is undesirable to insert more venous access ports. Injections and short time infusions could be given successively in the same i.v. line one after the other, for example some antibiotics and pain-relieving medications and flush before and after with a compatible fluid. But since especially neonates have fluid restrictions, this amount of fluid for flushing the line could be too much making this not a viable option. The only option left is to co-administer drugs together in the same i.v. line. When many drugs are given as continuous infusions it means that in reality more than two drugs have to be co-administered in the same i.v. line.

2.3.1 Detecting and predicting incompatibilities in the clinical setting

If drugs are co-administered in the same i.v. line, visual inspection of the i.v. line may reveal whether the drugs are compatible or not. But many changes (particles or droplets growth) are sub-visual and cannot be seen by the naked eye (101). Melchore *et al.* showed that it was almost impossible to detect particles under 50 μm in size by visual examination (102). Since capillaries in the body have diameter sizes from 5 μm, sub-visual particles or droplets in or around this size are therefore of interest to be able to detect. The blood vessels in especially neonates are small, the i.v. line has to be even thinner and could be as small as 26 gauge (G), which corresponds to an outer diameter of 0.6 mm (inner diameter not specified). This makes visual inspection even more challenging. Preterm neonates are vulnerable and are not ready to face their environment and must be protected from the outside world. They have to be in an incubator and parts of the i.v. lines are inside the incubator where the preterm should not be disturbed more than necessary, further add to how difficult it is to inspect the line for incompatibilities. In addition, in 2019 new guidelines demanded that for PN containing lipids, bags and i.v. lines should be protected from light when administered to patients younger than 2 years. This has made visual inspection almost impossible. PN containing lipids are opaque, with a milky white appearance. It is difficult to visually detect signs of precipitation or emulsion destabilisation upon visual inspection. Figure 9 illustrates how challenging it can be, here exemplified by precipitation of heparin that could be observed after co-administration

with PN (Numeta G13E). All this emphasises the importance to know before administration which drugs are compatible, and especially for combinations with PN.

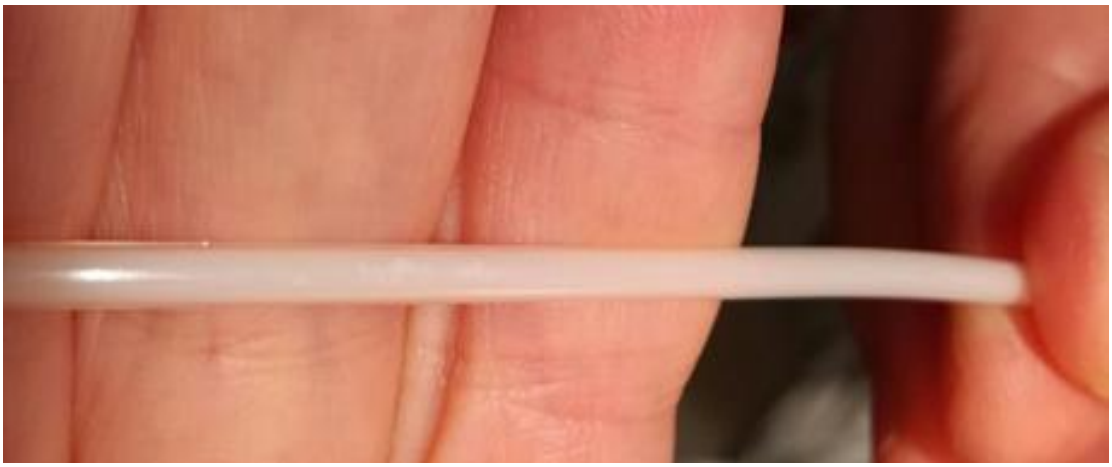


***Figure 9**. Example of incompatibility in a paediatric intravenous (i .v.) line where parenteral nutrition (Numeta G13E) have been co-administered together with heparin. A white precipitation can be seen in the i.v. line as "white flakes". (Personal photography).*

2.3.2 Sources of information on the compatibility of i.v. drugs

Information of i.v. drug compatibility is scarce. Most studies are on adult doses and infusion rates and this data are not safe to extrapolate to paediatric patients. As mentioned earlier, children have fewer access ports and are in need of as many i.v. drugs as adults. This forces health care professionals to administer many drugs in the same i.v. line. Studies on multi-drug infusions are almost absent even if many drugs are administered simultaneously (103). A study where they performed focus group interviews with nurses revealed that few i.v. access ports and lack of information were the two biggest challenges associated with co-administration of drugs (104). Kalikstad *et al.* found in 2010 that in almost three out of four cases the drug combinations used in neonates in Norway were either incompatible or lacked information of drug compatibility (105). Recently, Fernandez-Pena *et al.* reported that there was a gap of knowledge for drug combinations used at neonatal intensive care units in Spain (2021) where no compatibility information were found in 46 % of the studied drug combinations (106).

Many hospitals or health care institutes have created compatibility chart that includes the most frequent locally used i.v. drugs (Figure 10). These charts are typically created from information from literature on compatibility studies and/or databases (12, 13, 107). These charts are useful to easily find compatibility information of specific combinations of two drugs.

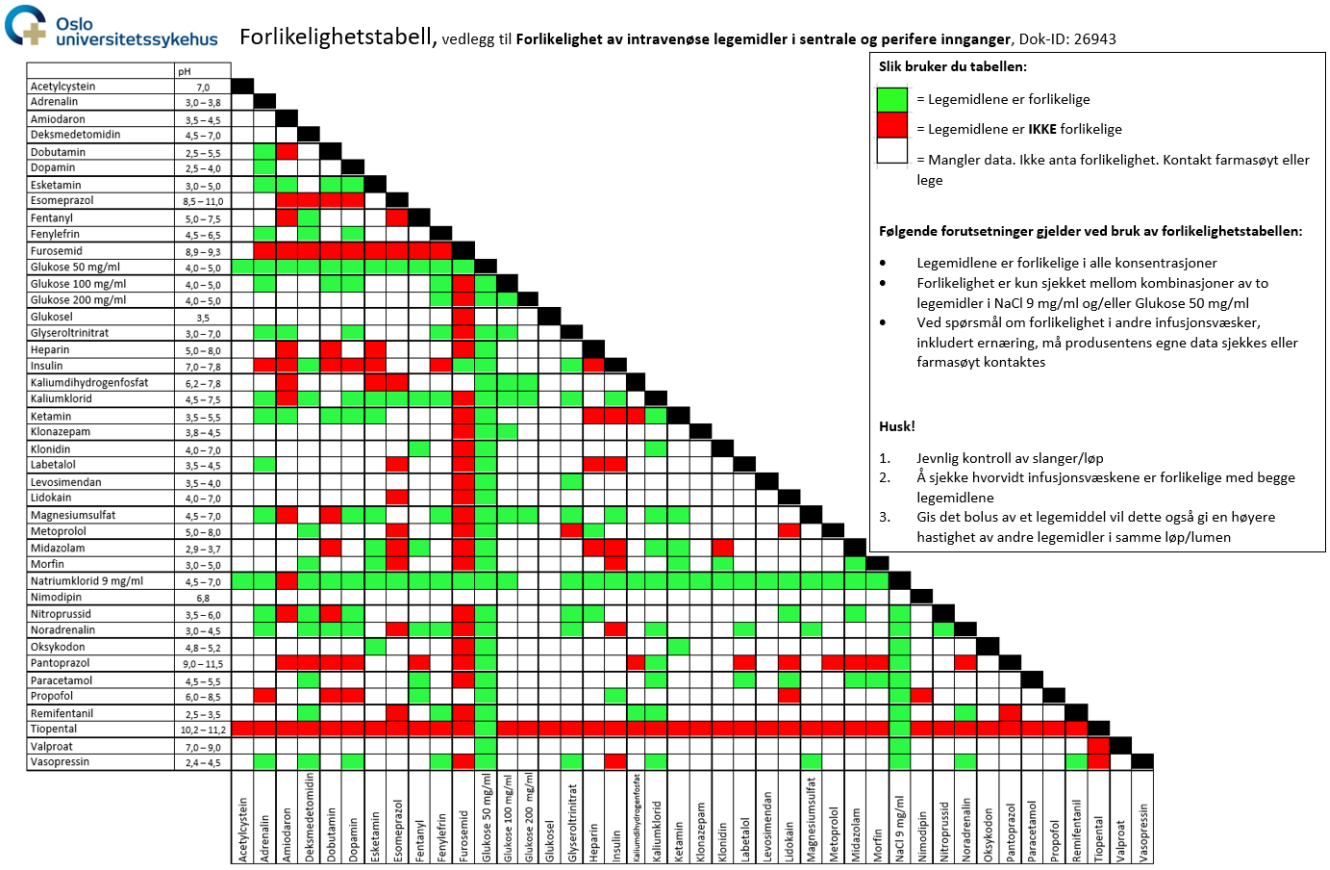


Figure 10. *Compatibility chart from Oslo University Hospital (108).*

Decision-supporting databases, for example Micromedex (12), Blandbarhetsdatabasen Västra Götalandsregionen (107) and Stabilis (13) have been developed in order to help the health care professionals to find out if a combination of drugs are compatible or not. This enhances the chances to co-administer two drugs in a safer way. If a combination of drugs have been studied the databases gives a recommendation of which drug combinations are compatible or incompatible. These recommendations are either that the combination is compatible, not compatible, conflicting data or information is missing. Studies are performed on specific drug combinations, in specific concentrations and it presupposes that the user have chemistry knowledge in order to translate this information to the clinical setting. This interpretation includes finding studies on the same drug concentration and infusion rates as the case that is of interest. One also needs to consider what alterations in pH can do in the reaction of the drugs. Databases and other sources regarding compatibility issues could conclude differently or most often, there is non-existent information or the information is incomplete (109). In other

words, available sources are difficult to interpret and does not always present an answer to the user.

Another weakness of existing information is that many studies are outdated and have only been performed by visual examination of drug mixtures. As discussed above, this limits the detection of sub-visual particles due to the eyes disability to be able to detect smaller particles than around 50 μm. Many studies only examined a mixture of two drugs in a 1:1 volume ratio *i.e.* 1 mL of drug A mixed with 1 mL of drug B. Different volume ratios could influence the interaction between two drugs differently when they meet in the line, for example altering the pH of the final of the mixture. The 1:1 volume ratio is not relevant to the clinical setting where two or more drugs that are co-administered in the same i.v. line have different infusion rates (read: not in a 1:1 volume ratio). In addition, the infusion rates are frequently altered due to the clinical response or because infusion rate related side effects appears.

There is no consensus in literature nor in the clinical setting on what should a recommendation of compatibility be evaluated. It is up to the reader to evaluate if the database result are relevant for the actual setting. Questions of which analysis have been used in the study and which volumes and concentrations of each drug or solution have been used could be of relevance that the user think through when using information from databases. This means that it is still up to each user to take responsibility for the results from the search and reflect if it fits into the real scenario in clinical practice.

2.3.3 In-line filters

Many guidelines recommends the use of an in-line filter, which is connected to the i.v. infusion set. Filters could be effective to capture particles and air bubbles before it enters the body. The filters have different sizes from 0.22 μm up to 1.2 μm where the larger filter is used for products containing lipids. If the infusion is connected to an infusion pump, an alarm will signal when the filter is clotted and in this way the filter could help to detect a precipitation.

Studies have showed that filters could reduce thrombophlebitis (110) but also reduce the incidence of sepsis, thrombosis and necrotizing enterocolitis in neonatal patients (111). Perez *et al.* showed that in-line filters was effective to prevent administration of precipitates to

critically ill paediatric patients (112). Jack *et al.* reported that the use of in-line filter both reduced severe complications and the length of stay at paediatric intensive care units (113). Ball strongly recommends the use of in-line filter since the filter protects from precipitates, microbiological contamination and air bubbles (114). On the other hand a Cochrane review from 2015 did not conclude with any significant effect of in-line filters for the prevention of morbidity or mortality in neonates (115), but the review had excluded the study from Jack *et al.* since they had included children up to 18 years. Because of inconclusive information the use of filter differs between locations also within Norway.

2.4 Compatibility testing

I.v. drug compatibility testing has been of interest for decades and Lawrence A. Trissel published the first Handbook on injectable drugs in 1977 (116). In 1983, Trissel criticised studies for having poor study design where materials, methods and test conditions were not satisfactory described (117). Still there is no consensus in how compatibility testing should be designed. In a review article, Kanji *et al.* suggested that future studies should apply the same methodology in order to be able to compare outcomes (109). Staven *et al.* developed in 2016 a compatibility test program for physical compatibility of drugs (7). But still compatibility studies uses different methodology and different acceptance criteria's and this could be the reason to why conflicting data exists.

The Ph.Eur. and United States Pharmacopoeia (USP) define standards and specify limits for marketed products (73, 118), but neither defines any criteria to ensure compatibility when two or more marketed products meets in the same infusion line, since this is most often an *off-label* use of the products. Nevertheless, the Pharmacopoeia specifications can be useful tools also in a compatibility context. For instance, the Ph.Eur. contain monographies with specified limits of particulate contents in marketed injections and infusions as well as methods to determine particulate contamination of visible particles (119) and sub-visual particles (120). Monograph 2.9.19 *Particulate contaminations –sub-visual particles* specifies limits for number of particles/mL of a size >10 μm and 25 μm. It might be reasonable to expect that *off label* use of drug products should not result in a significantly higher particle burden than the licenced products itself, especially when it comes to particles that potentially can block capillaries; thus, it is frequently

seen in literature that the particle content in mixed samples are compared to the Pharmacopoeia specification for sub-visual particles (121, 122).

In addition, USP also has a monography on injectable lipid emulsions <Chapter 729 *Globule size distribution of pure lipid injectable emulsions*> introducing two parameters to characterize such emulsions, namely the mean droplet diameter (MDD) and the large dimeter tail droplets (>5 µm) with their specified limits; MDD should be below 500 nm and the volume-weighted, percentage of large diameter fat globule (>5 µm) limits of the dispersed phase (called PFAT5) must not exceed 0.05 % (123). Driscoll *et al.* reported an increased probability for emulsion destabilisation for PFAT5 above 0.4 % (124). Since PN also contain electrolytes that could affect the surface charge and the flocculation of droplets which is different to pure lipid injectable emulsions (0.05 %), Staven *et al.* suggested to set the acceptance level of PFAT5 for PN at 0.4 % (125).

The methods summarized below are frequently used in compatibility testing in literature.

2.4.1 Tyndall effect

The Tyndall effect is the light scattering of small particles in suspension upon illumination with a focused light beam. The Tyndall effect can be utilised to enhance detection of sub-visual particles suspended in a fluid. When projecting a focused light beam or a laser beam through the fluid the scattered light from particles becomes visible due to the Tyndall effect (11, 126, 127) (Figure 11). Staven *et al.* showed that it is a subjective method with varying reliability, and recommended that it should not be used as the only method to evaluate compatibility (11).

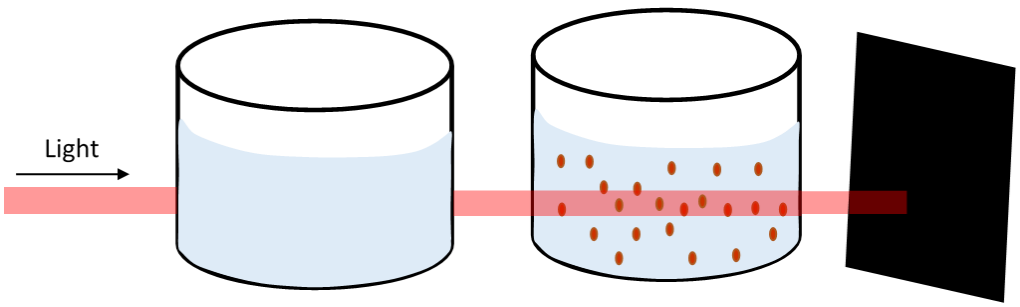


Figure 11. *When a focused light beam hits particles in a suspension, the particles scatter and reflect light which makes the beam visible.*

2.4.2 Light obscuration

Light obscuration or single particle optical sensing, is a method to optically count and size-determine particles and oil droplets. When a diluted liquid suspension is passed between a light source and a detector in a stream (Figure 12), the individual particles block or obscure the light beam and the reduction of light intensity corresponds to the count of the particles or oil droplets (7). By comparing the signal with a calibration curve, the size of the particles or oil droplet can be determined. The sample has to be diluted to a concentration that ensures that the detector counts one single particle at the time. Larger particles or oil droplet (approx.1.5 μm) are detected by the amount of light they obscure (block) to the extinction detector, while smaller particles are detected by the intensity of light scattered toward the scattering detector.

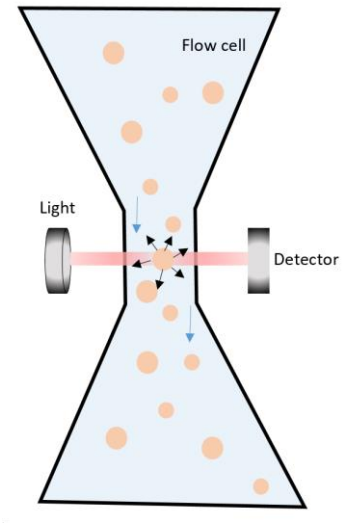


Figure 12. *Light obscuration where particles passes through a laser diode and each particle can be counted and sized individually. The detector could be either scattering or extinction. The illustration is modified from* Particle technology labs.

2.4.3 Turbidity

Turbidimetry is an optical measurement that is used to detect the presence of suspended particles (7, 127, 128). The turbidity is measured as the loss of intensity of transmitted light due to the scattering effect of the suspended solid particles. The detection angle relative to the incident light beam and the number of detectors has an impact on detection of particles and the ability to compensation for interference, such as colour. The 90 degree detection angle is often referred to as the nephelometric detection angle (Figure 13), and is the most commonly used because of

its sensitivity to a broad range of particle sizes. Formazin Nephelometric Unit (FNU) measure the transmitted light scatter signal at 90 degrees from the incident light beam. The higher the FNU value the greater the turbidity. Ratio turbidimetric determination with more than one detector is frequently occurring; the primary detector typically at 90 degrees angle and other detectors at various angles including an attenuated; backscatter, and forward scatter angles.

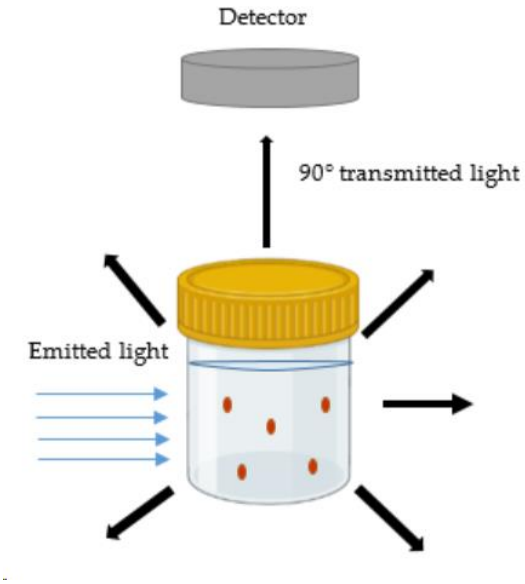


Figure 13. *Turbidity determined by the loss of intensity of transmitted light due to scattering effects of suspended particles detected at an angle of 90 ° from the incident beam. The illustration was inspired of PhysicsOpenLab.*

2.4.4 Dynamic light scattering

Dynamic light scattering (DLS) measures the scattered light caused by the Brownian motions of small particles (sub-micron) in dispersion (129). The velocity of the Brownian motions is related to the size of the particles - the larger the particles the slower the Brownian motions. As the particles move the intensity of the scattered light is measured, and is shown as fluctuations in intensity over time. Different parameters can be derived. Most used are Z-average mean (cumulants mean; intensity based) and polydispersity index (PDI).

DLS is most suitable for determination of particle sizes in the sub-micron range, which based on their small sizes are not at any immediate risk for occluding capillaries, unless they aggregate. However, DLS has been used for measurement of droplet size of emulsions, which

are supposed to be less than 500 nm in parenteral emulsions. For this purpose, the intensity weighted (I.W.) MDD and PDI are assessed (7, 130-132).

2.4.5 Zeta potential

Zeta potential is a physical property exhibited by any particle in suspension (133). Theoretically, it is the electrical potential in the double layer at the slipping plane, which is the interface separating the bulk fluid from the surface of the particle. From a practical point of view it is the difference in potential between the dispersion medium and the stationary layer of fluid attached to the particle. The zeta potential is an expression of the magnitude of the electrostatic interaction (repulsive or attractive forces) that are between dispersed particles or droplets in a given fluid, and can be used to optimize stability of a disperse system. Zeta potential can be measured by micro-electrophoresis through charge migration in an electric field (133). Zeta potential measurements has been used to estimate stability of PN emulsions in a compatibility setting with i.v. drugs (7, 130). A pure lipid injectable emulsion typically has a zeta potential of -30 mV to – 50 mV (100). A change in zeta potential towards 0 could indicate less repulsions and therefore increased risk of droplet growth *i.e.* unstable emulsion.

2.4.6 Raman spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique based on the principle of the inelastic light scattering off a sample irradiated with an intense monochromatic light source, usually a laser. Most of the radiation scattered from the sample has the same wavelength as that of the incident light (Rayleigh scattering, elastic), but a small fraction (about 10^{-6}- 10^{-8}) of the incident photons is scattered from the sample with a shifted wavelength; this scattered light is known as Raman scatter or inelastic light scatter (134). The change in wavelength of the incident light and the scattered light is called the Raman shift and is related to the molecular vibrations within the sample, which are related to the nature of the bindings of the compound.

Raman spectroscopy can be used for qualitative and quantitative applications. Raman spectroscopy provides its own unique molecular fingerprint in the region between 300 and 1900 cm^{-1}, and can be used for identification or authentication testing of raw materials,

excipients and active pharmaceutical ingredients (APIs) in drug products, *e.g.* for quality control (135). It is a rapid and non-invasive analytical method that also can be used in process analytical technology (135).

Raman spectroscopy has the potential to be a powerful tool in i.v. compatibility testing, but has not been investigated for this purpose before. It has been used for rapid *in situ* quality control of chemotherapeutic drugs in infusion bags (136, 137).

3. Aim of the project

The overall goal of the project was to increase safety for critically ill children that receive complex i.v. drug regimes. This should be achieved through investigations into the physical compatibility of i.v. drugs (medications, PN and fluids) in a clinically relevant simulated y-site administration test set up with focus on neonatal and paediatric patients. In addition, to explore new methods for improved detection and identification of precipitates in multi-drug mixtures.

The specific aims of this Thesis were:

- To investigate the compatibility of PN with medications that are frequently co-administered in the same i.v. line at neonatal intensive care units, in pairs of two components (paper I and II) and three-components (paper II)

- To identify the source of incompatibility in a clinical case of precipitation observed between an buffered electrolyte solution given in a multi-drug mixture containing five components, and to further explore the compatibility of this complex regime (paper III)

- To explore the possibility to detect and identify a precipitate in a multi-drug mixture using Raman spectroscopy (paper IV)

4. Experimental conditions

4.1 Materials used in the Thesis

Table 3 (page 46) provides an overview of the drugs investigated with their physico-chemical properties. Their chemical structures are found in Figure 14 (page 47). Plasmalyte, Plasmalyte Glucos and Numeta G13E, all from Baxter, were included in studies. Their compositions can be found in Appendix Table A2 and Table A3, respectively. Table 4 (page 48) summarizes all tested combinations with their respective papers.

4.2 Methods for selection and preparation of test substances

4.2.1 Selection of drugs

This Thesis focus on the investigation of compatibility of i.v. drugs frequently used in the neonatal and paediatric intensive care setting. Drugs were chosen based on their actual use and in concentrations and infusion rates used in the neonatal intensive care unit/paediatric intensive care unit (NICU/PICU). This information as well as which combinations that were co-administrated were recorded at neonatal and paediatric intensive care units at Oslo University Hospital (OUH). This was done by going through the electronic medication management records for several patients (anonymised) but also by bedside registration, through discussions with nurses, physicians and clinical pharmacists at each unit. The data registration was approved by the patient and user ombud. Examples of registration can be found in Appendix Table A4 and A5. No personal information that could be traced back to a single patient was recorded. Also, frequently acquired drugs at NICUs in key hospitals in the South-Eastern district of Norway during 2019 and 2020, was used in the selection process (52). It should be mentioned that the candidate is a paediatric clinical pharmacist with long experience in the intensive care setting.

Additional and supplemental information, such as dilution medium, dose, concentrations and infusion rates were found in national dilution guidelines (53), local dilution guidelines, local syringe pump protocols and NeoFax (138). As mentioned, a summary of medications investigated for compatibility in paper I-IV are found in Table 4.

***Table 3**. Summary of drugs, their concentrations, excipients and physico-chemical properties.*

Drug product, concentration	Active ingredient	Excipients*	pH*	Active ingredient	
				pKa**	Solubility parent compound****
Ampicillin 34 mg/mL	Ampicillin sodium	-	8.0-10.0	2.55, 7.25	Sparingly soluble
Calcium chloride 13.25 mg/mL	Calcium chloride dihydrate	-	5.5-7.5		Freely soluble
Cefotaxime 40 mg/ mL	Cefotaxime sodium	-	5.0-7.5	2.2, 3.2, 10.9	n.a
Ceftriaxone 50 mg/ mL	Ceftriaxone sodium	-	6.7***	2.7, 3.2, 4.1, 10.7	n.a.
Dopamine 2 mg/ mL	Dopamine hydrochloride	Sodium pyrosulphate, sodium chloride	2.5-4.5	9.3	Freely soluble
Fentanyl 10 μg/mL and 50 μg/mL	Fentanyl citrate	Sodium chloride, hydrochloric acid / sodium hydroxide	5.0-7.5	8.99	Practically insoluble
Ketalar 10 mg/mL	Ketamine hydrochloride	Benzethonium chloride, sodium chloride	3.5-5.5	7.5	n.a.
Metoclopramide 5 mg/ mL	Metoclopramide hydrochloride		4.5-6.5	9.4	Very slightly soluble
Midazolam 5 mg/mL	Midazolam hydrochloride	Sodium chloride, hydrochloric acid 10 %	2.9-3.7	6.6	Practically insoluble
Morphine 0.2 mg/ mL	Morphine hydrochloride	Sodium chloride, hydrochloric acid	3.0-5.0	8.21	Very slightly soluble
Paracetamol 10 mg/ mL	Paracetamol	Mannitol, sodium citrate trihydrate, glacial acetic acid	4.5-5.5	9.4	Sparingly soluble
Potassium chloride 1 mmol/mL	Potassium chloride	Water for injection	4.5-7.5	-	Freely soluble
Vancomycin 5 mg/ mL	Vancomycin hydrochloride	-	2.5-4.5	2.6, 7.2, 8.6, 9.6, 10.5, 11.7	Sparingly soluble

* SmPC, ** PubChem,*** Micromedex ,**** weak acid or base, unionized form in water. n.a=not available

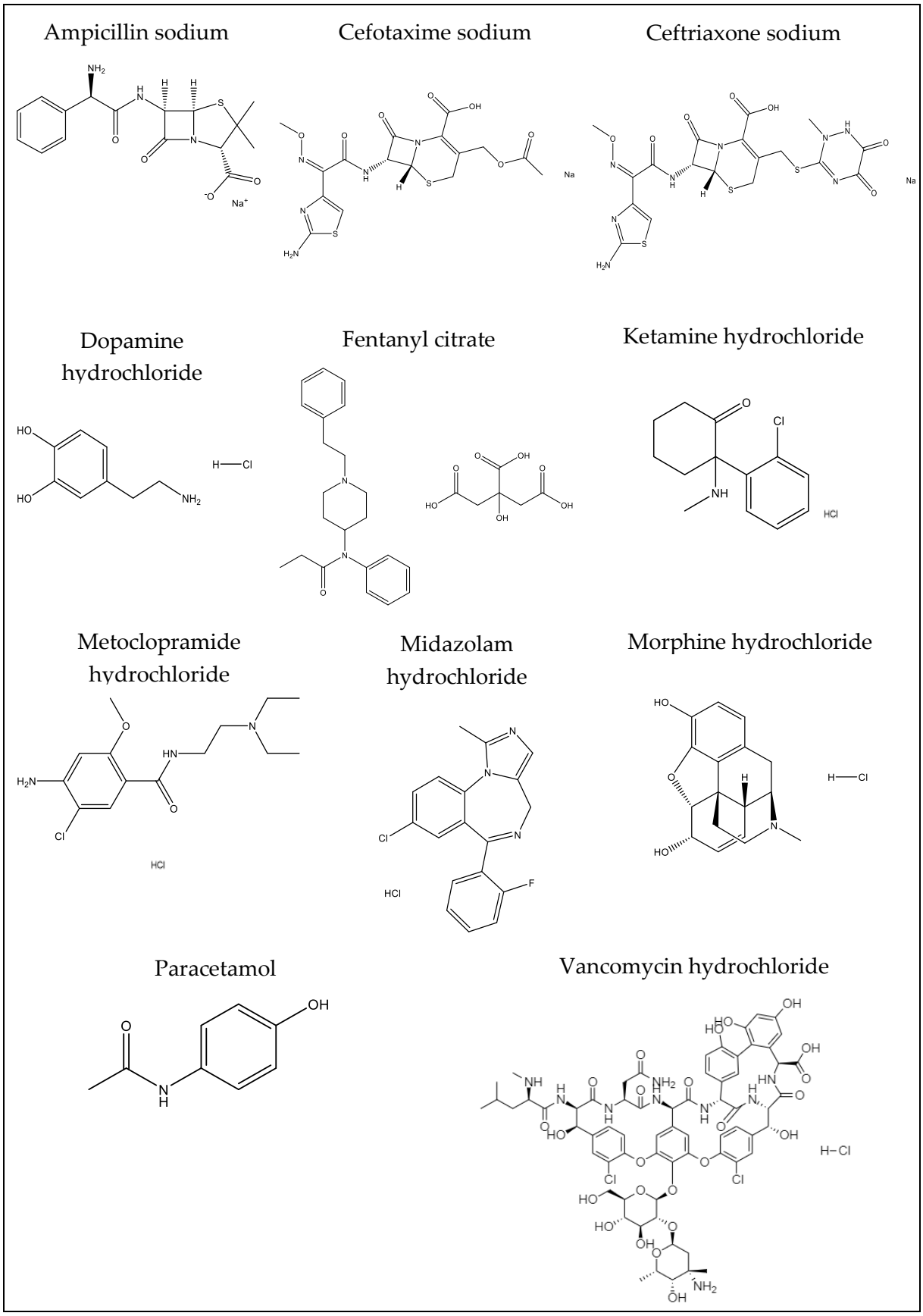


Figure 14. *Molecular structure of drugs in paper I-IV.*

***Table 4**. Overview of tested combinations and in which papers (I-IV) they were studied.*

Medication	Ceftriaxone	Morphine	Numeta G13E	Plasmalyte	Plasmalyte Glucos
Ampicillin	IV				
Calcium chloride	IV				
Cefotaxime	IV	II	II		
Cefotaxime + Calcium chloride + Paracetamol + Metoclopramide + Ampicillin	IV				
Cefotaxime + Morphine			II		
Dopamine		II	II		
Dopamine + Morphine			II		
Fentanyl			I	III	III
Fentanyl + Midazolam + Potassium chloride + Ketamine				III	III
Ketamine				III	III
Metoclopramide	IV				
Midazolam				III	III
Morphine			II		
Paracetamol	IV		I		
Potassium chloride				III	III
Vancomycin			I		

4.2.2 Reconstitution, dilution and sample preparation

All drugs that were provided as powders or concentrates were reconstituted and/or diluted to the desired concentration in an appropriate solvent, and the PN mixed and supplements added according to guidelines as described in 4.2.1. All samples and controls used in paper I-III were prepared in a particle controlled environment in a laminar air flow bench (LAF-bench). Samples were prepared by mixing estimated volumes (further described in 4.2.3) in low particle content centrifugation tubes, except for samples intended for visual examinations, which were mixed directly in cleaned and sterilised flat bottom glass tubes (Tyndall tubes).

To enable potential precipitate detection, Milli-Q water was used to substitute the lipid constituent of Numeta G13E. This admixture was referred to as *aq*Numeta G13E+. Only trace elements were added to *aq*Numeta G13E+ and no vitamins because the water-soluble vitamins discolour the solution and may lead to analytical problems and lipid soluble vitamins are insoluble in water (For composition see Table A3 in the Appendix). To test emulsion stability, Numeta G13E was mixed with both trace elements and vitamins. Maximum amounts were added according to manufacturers' recommendations to mirror the extreme case scenario in clinical practice. This version was referred to as Numeta G13E+ (For composition see Table A3 in the Appendix).

4.2.3 Calculation of mixing ratios

The most common mixing ratio in compatibility studies is when one part of the medication A is mixed with one part of medication B, which gives a mixing ratio of 1+1. Allen *et al.* suggested that equal volumes (1+1) was the actual mixing ratio when two fluids where administrated in the same i.v. infusion set at the same rate in a drip chamber (139). More correctly using pumps, mixing ratios will alter depending on the infusion rates of the fluids that are administrated in the same i.v. line. If one mL of fluid A is mixed with 20 mL of fluid B, the concentration of fluid A will be lower due to dilution effect and the mixing ratio will be 1+20.

To mimic the potential mixing ratios that could arise when two medications are meeting in the i.v. infusion set, the most extreme mixing ratios were calculated. Concentrations of the drugs used in the hospital setting for doses for patients in weight classes between 0.5 kg and 10 kg (to extend the experimental space as much as possible) at neonatal intensive care units and

between 10 and 50 kg for paediatric intensive patients, were used. In addition to mixing ratio of 1+1 (and 1 more part for each additional medication depending on how many components were tested) additional mixing ratios were calculated. Information on drug concentration and doses were retrieved from international and local guidelines (140-143). From the dose-range of the drug, the highest and lowest infusion rates were estimated. Nutritional need of amino acids, glucose, fat, calories, electrolytes and fluid for neonates and paediatric patients were retrieved from ESPHGAN (35, 36, 144-147) and infusion rates for both 8 hour and 24 hour were calculated. Mixing ratios were retrieved by dividing the lowest infusion rate of component A and the highest infusion rate of component B and vice versa. This was repeated for each weight class. Among all mixing ratios obtained, the two most extremes (A>B and A<B) were chosen testing together with 1+1. Calculation of mixing ratios are described in more details in paper I.

This approach formed the base for paper I-II and the exploring part of paper III**.** The test substances for paper III originated from a reported incidence of precipitation from a multi-drug administration together with a buffered electrolyte solution. The administration case was replicated with exactly the same mixing ratios and further explored to other ratios and complementary buffered electrolyte with glucose.

An entirely different approach was used in paper IV. A model system that is known to produce precipitation was chosen and mixed in equal molar concentrations assuming that the stoichiometry of formed poorly soluble salt was 1:1. To challenge the analytical methods to detect the precipitation, structurally similar as well as therapeutically relevant drugs for co-administration was added to the mixture in relevant concentrations.

4.3 Short overview of applied test methods

A brief description of methods used in paper I-IV is given below. A battery of analytical methods was used in paper I-III for compatibility testing. These are methods frequently occurring in literature and the full set of methods were suggested by Staven *et al.* to make a robust foundation for assessment of physical compatibility (7). Table 5 (next page) provides an overview of the analytical methods used to capture incompatibility.

***Table 5**. Overview of analytical methods used for detection of potential precipitation and emulsion destabilisation.*

Detect	Analytical method	What to look for	Advantages and disadvantages
		Precipitation testing	
Visual particles	Focused light beam Laser beam	Tyndall effect: coherent laser line, sign of haze, particle growth, colour change, gas evolution	+ Easy to perform - Low validity and low reliability
Sub-visual particles	Single particle measurement by light obscuration	Particle number and size: growth in the size-range of 0.5-50 µm	+ Provides particle number/ mL of each particle size - Measures everything as particles even air bubbles and contamination
Particles, colour or gas	Turbidity	Potentially dispersed particles or colour as compared to controls	+ A more standardised method than visual inspection
pH	pH measurements	Change in pH as compared to controls and taking pKa values into consideration	+ Allows theoretical evaluation of solubility of the drug and the risk of precipitation in the new environment
		Emulsion destabilisation testing	
Sub-visual droplet sizes	Single droplet size measurements by light obscuration	Oil droplet growth and the calculation of PFAT5	+ Assess changes in the large diameter tail of droplet size distribution - The samples need to be diluted and destabilised emulsions could be reversed
	Dynamic light scattering	Mean droplet diameter (MDD) Polydispersity index (PDI, the broadness of the droplet size distribution)	+ Assess the performance of the assemble of droplets - Most suitable for detection of droplets in the nanometre range
Droplet charges	Zeta potential	Change as compared to controls	+ Change towards zero alert destabilisation
	pH measurements	Change in pH as compared to controls	+ Neutralization of droplet charges alert destabilization

4.3.1 Visual examination

Visual examination was performed as a supporting method. Two sources of focused light were used to aid the detection of potential particles based on the Tyndall effect. The focused fiberoptic light source was most suitable to detect larger particles (e.g. crystal growth), colour changes and gas bubbles, whereas the laser beam was most suitable for detection of microprecipitates. It was essential to examine the samples in random order (*i.e.* blinded) and to compare samples with controls.

4.3.2 Single particle optical counting

This technique was used to count particles as particles formed by precipitation in an aqueous phase and count droplets to assess stability of the o/w emulsion in PN. For these measurements, the single particle optical counter Accusizer (Accusizer Syringe Injection Sampler, Optical Particle Sizer, PSSNICOMP, Billerica, MA, USA) was used.

Sub-visual particles were assessed using in summation mode. The dispersion of particles needs to be appropriately diluted to ensure that particles pass the sensor as single particles and not clusters of particles giving rise to increased particle size. In compatibility testing, the dispersion cannot be diluted since this may dissolve precipitated particles. All samples were therefore measured undiluted. In cases with massive precipitation, this was recognised by detector overload (>9000 particles/mL over 0.5 μm). The results were analysed in the following fractions: total number of particle/mL > 0.5 μm, > 5 μm, > 10 μm and > 25 μm. The two largest fractions were selected inspired by the specification in Ph.Eur. 2.9.19 when it comes to large volume parenteral (max 25 particles/mL > 10 μm and max 3 particles/mL > 25 μm), >5 μm was included since this is the size range of the smallest capillaries and > 0.5 μm based on Staven *et al*. (7). A high total number (above 1500-2000 particles/mL > 0.5 μm) was regarded as alarming.

Droplet counting of diluted samples containing o/w PN emulsion was performed in extinction mode in order to assess the potential increase of droplets size and number in the large diameter tail of the size distribution. This would be an indication of emulsion destabilisation. The size fraction of droplets >5 μm was extracted from the results and used for calculation of PFAT5 (4.3.3).

4.3.3 Percentage of fat residing in globules larger than 5 μm (PFAT5)

According to Driscoll *et al.* destabilization of PN emulsion is likely to occur when more than 0.4 % of the oil droplets in the emulsion have a diameter > 5 μm (148). There are several steps to calculate the percentage of large droplets with large diameter *i.e.* PFAT5 (equation 6-8). This is briefly summarized below and explained in more details in paper I.

Droplet number and droplet size measurements were extracted from the light obscuration results.

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

where D: diameter in centimetres and ESV: is expressed in cubic centimetres.

$$\text{Total spherical volume (TSV)} = \text{number of droplets x ESV} \quad (7)$$

$$\text{PFAT}_5 = \frac{[\text{TSV (cm}^3) \times \text{Density} \left(\frac{\text{g}}{\text{mL}}\right) \times \text{Dilution factor}]}{[\text{Sample volume (cm}^3) \times \text{Final oil composition} \left(\frac{\text{g}}{\text{mL}}\right)]} \quad (8)$$

Density: density of the oil for the PN was approximated to be 0.92 mg/mL

Dilution factor: dilution of the samples was done in two steps. First, the emulsion was diluted when mixed with drug, which reflects the mixing ratio. Secondly, the samples were diluted before analysis in order for the instrument to count each droplet separately.

Final oil composition: amount of lipids in PN (including fat from lipid soluble vitamin component) and this was estimated to be 0.030 g/mL for Numeta G13E with the supplemented amounts of vitamins.

4.3.4 Turbidity

Change in turbidity (2100Qis Turbidimeter, Hach Lange GmbH, Duesseldorf, Germany) between mixed samples and unmixed controls was used as a nonspecific way of detecting changes that could be traced to the mixing of the components, namely physical incompatibility. Such changes could be formation of particles, gas or colour changes. Differences between mixed samples and controls of 0.2-0.3 FNU were taken as a sign of incompatibility.

4.3.5 pH-measurements

pH was measured in all samples and compared to controls. In samples with only aqueous phase, a pH difference of more than 1 pH-unit in the mixed sample compared to the unmixed control, was regarded as alarming. pH changes and pKa values of the drugs were instrumental in theoretical evaluation of solubility and risk of precipitation.

pH measurements in mixed samples containing PN emulsion was compared to the unmixed PN control, and more acidic pH values in the mixed samples could be an alert for destabilization, since this could lead to protonation of the emulsifier. Change in the surface charges of the oil droplets would mean less electrostatic repulsions and emulsion destabilisation due to increased chance of oil droplet coalescence.

4.3.6 Dynamic light scattering

Measurement of MDD was performed on diluted (1:500-1:1000) samples using a Zetasizer Nano series (Malvern Instruments, Malvern, UK). Acceptance criteria for a stable emulsion were set to not contain droplets with a MDD of more than 500 nm and a PDI below 0.20. A PDI above 0.50 indicate a very broad size distribution, and could be a sign of an unstable emulsion due to growth in droplet size (149).

4.3.7 Zeta potential

Samples were diluted (1:500-1:1000) prior to measurement with the Zetasizer Nano-series using a dipcell electrode. Zeta potential was measured in all samples and compared to controls. A change in zeta potential towards zero could be an indication of an unstable emulsion.

4.3.8 Raman spectroscopy

In this Thesis, two different Raman instruments were employed; one for averaging over larger powder samples, whereas the other was specialised for studies of single particles. The first one, a Kaiser RXN1 Microprobe (Kaiser Optical Systems, Ann Arbor, MI, USA) with a PhaT-probe (Kaiser Optical Systems), controlled by HoloGRAMS software, was used for the identification of the precipitate formed in a multi-drug mixture, *i.e.* after mixing of ampicillin sodium,

ceftriaxone sodium, cefotaxime sodium, calcium chloride dihydrate, metoclopramide, and paracetamol. The other, a Horiba Jobin-Yvon T64000 Raman Instrument equipped with a confocal microscope was used for the identification of a single sub-visual precipitated particle after mixing calcium chloride dihydrate, cefotaxime sodium and ceftriaxone sodium. In the first case the spectra were analysed using multivariate statistical analysis (more in 4.4.2) to identify a match between the known library spectra and the analysed precipitate. The second case was used as a refinement, emphasizing that identity can be determined based on single particle analysis.

4.4 Statistics

4.4.1 Acceptance criteria

In order to evaluate potential incompatibility in paper I-III, several well established methods were used with acceptance criteria for each analytical method along with taking the physico-chemical properties of drugs into consideration.

In paper I-III, all samples were presented as mean (n=3) with their standard deviation (SD). Testing for statistical significance was considered and also performed in several cases, but in the end the practical meaning of statistical significant differences also had to be considered. Significant differences may be less important, whereas non-significant differences indicating a trend can be important. Therefore, the results from the compatibility testing were always compared to an acceptance limit instead of isolated statistical methods. For example, unless the particle counts exceeded the acceptance level including those for the larger particle fractions, an increased number of small particles were not regarded as a safety risk, even if a statistically significant difference to the control could be proven.

4.4.2 Multivariate analysis

In paper IV, multivariate analysis was employed to evaluate spectral data from Raman spectroscopy and proof the identity of the «unknown» material as a match with the reference spectra.

The correlation between variables was evaluated by principal component analysis (PCA). PCA reduce the amount of spectral data from Raman analysis but still keeping the variability in the

spectral data. Before PCA, all spectral data were transformed by standard normal variant (SNV) in order to remove intensity differences unrelated to the sample composition and the spectra were subsequently mean centred.

Soft independent modelling by class analogy (SIMCA) was used to classify the spectrum from the "unknown" precipitate with spectra from the raw materials (drugs).

In order to identify which of the drugs could be the parent drug of the precipitate, Pearson correlation coefficient was used to compare Raman spectra. Pearson correlation coefficients, r^2, were directly calculated between the spectra of the individual raw materials (reference) and the "unknown" sample. These correlation coefficients were calculated on the average spectrum made on the "unknown" sample against the average spectra of the individual raw materials, including ceftriaxone-calcium, using the measured intensity for the same wavenumber in all the Raman spectra. The Pearson correlation coefficients were calculated as the proportion of explained variance, r^2, and was shown as a percentage.

5. Summary of papers

Paper I investigated the compatibility of the neonatal 3-in-1 PN (Numeta G13E), together with three frequently used medications; paracetamol, vancomycin and fentanyl, respectively. There were no signs of precipitation nor emulsion destabilisation for neither paracetamol, vancomycin nor fentanyl when mixed with Numeta G13E each in three mixing ratios reflecting potential mixing ratios in the i.v. lines of preterm neonates.

Paper II investigated the compatibility of both two-components and three-components mixtures for Numeta G13E (PN for preterm neonates) with either one medication (morphine, dopamine or cefotaxime) or with two medications (morphine+dopamine or morphine+cefotaxime). In addition, the medication-medication compatibility of morphine with cefotaxime and dopamine, respectively was investigated. No sign of neither precipitation nor emulsion destabilisation were seen in doses and infusion rates relevant for neonates. The results were reassuring since other studies have shown both morphine and dopamine to be incompatible with some PN. However, these studies were not adapted to neonates and used higher concentrations of the drugs and the compositions of the PN differed from Numeta G13E.

Paper III investigated a case from the PICU where precipitation was seen when five drugs where co-administered in the same i.v. line to a patient. The drugs involved were fentanyl, ketamine, midazolam, potassium chloride and the buffered electrolyte solution, Plasmalyte (pH 6.5-8.0). Since the solubility of midazolam is dependent on a low pH, the corresponding buffered electrolyte solution containing glucose (Plasmalyte Glucos) with a pH of 4.0-6.0 also was investigated. When replicating the full case, an instant precipitation could be visually observed but also captured in high particle counts and high turbidity. As theoretically predicted, midazolam precipitated when mixed with Plasmalyte due to the reduced solubility of midazolam at neutral pH. The analysis also revealed that ketamine precipitated when mixed with Plasmalyte, but to a lesser extent than midazolam. Altogether, the results from this five component mixture concluded that midazolam and ketamine should not be co-administered with Plasmalyte. In addition, midazolam was evaluated to be incompatible with Plasmalyte Glucos. Fentanyl and potassium chloride were found to be safe to co-administer

with both Plasmalyte and Plasmalyte Glucos. The study emphasize the importance of pH in buffered solutions used for hydration.

Paper IV explored the use of Raman spectroscopy to identify the solid form identity of precipitation of ceftriaxone-calcium from a multi-drug mixture. The mixture consisted of ceftriaxone sodium, calcium chloride dihydrate, cefotaxime sodium, ampicillin sodium, metoclopramide and paracetamol. The precipitate from the multi-drug mixture was called "unknown". The Raman spectrum of the "unknown" was compared to reference spectra of each of the medications and ceftriaxone-calcium, respectively. Multivariate analysis using principle component analysis (PCA) and SIMCA classification clearly identified the positive correlation of the "unknown" with ceftriaxone-calcium but it was also possible to recognize resemblance with ceftriaxone sodium. The proportion of explained variance using Pearson correlation coefficients for ceftriaxone-calcium, ceftriaxone sodium and cefotaxime sodium was 97 %, 48 % and 8 %, respectively, whereas for paracetamol, metoclopramide and ampicillin sodium the correlations were below 2 %. This showed with high significance that the "unknown" sample was ceftriaxone-calcium. In addition, if a reference spectrum of ceftriaxone-calcium had not been available, the relatively high correlation of the "unknown" to ceftriaxone sodium (48 %), it would have been possible to predict that ceftriaxone was part of the precipitate. Furthermore, Raman spectroscopy was also showed to be able to detect and identify the solid form of sub-visual particles, in sizes of 25 μm, which potentially could block capillaries. These findings showed that Raman spectroscopy could identify the origin of a precipitate from a multi-drug mixture and are promising for use in compatibility testing. In the hospital setting, Raman spectroscopy could be a powerful tool to identify which drug(s) that would precipitate in a multi-drug mixture so that they could be eliminated from co-administration, thereby, increasing patient safety.

An overview of compatibility results from this Thesis can be found in Table 6.

***Table 6**. Summary of investigated combinations and compatibility results. Paper I= yellow, paper II= green, paper III=pink, paper IV=orange, and open white square= not tested. *diluted in glucose 50 mg/mL, **undiluted, ***diluted in sterile water. C= compatible, I=incompatible.*

Medication	Concentration	Numeta G13E	Plasmalyte	Plasmalyte Glucos	Ceftriaxone 50 mg/ml	Morphine 0.2 mg/ml
Ampicillin	34 mg/mL***				C	
Calcium chloride	13.25 mg/mL *** (=90 mM)				I	
Cefotaxime	40 mg/mL*	C			C	C
Cefotaxime + Morphine	40 mg/mL* + 0.2 mg/mL*	C				
Cefotaxime + Calcium chloride + Paracetamol + Metoclopramide + Ampicillin	40 mg/mL 13.25 mg/ml*** 10 mg/mL** 5 mg/mL** 34 mg/ml***				I	
Dopamine	2 mg/mL*	C				C
Dopamine + Morphine	2 mg/mL* + 0.2 mg/mL*	C				
Fentanyl	10 µg/mL*	C				
	50 µg/mL**		C			
Fentanyl + Midazolam + Potassium chloride + Ketamine	50 µg/mL** + 5 mg/mL** + 1 mmol/mL** + 10 mg/mL*		I	I		
Ketamine	10 mg/mL*		I			
Metoclopramide	5 mg/mL**				C	
Midazolam	5 mg/mL**		I	I		
Morphine	0.2 mg/mL*	C				
Paracetamol	10 mg/mL**	C			C	
Potassium chloride	1 mmol/mL**		C			
Vancomycin	5 mg/mL*	C				

6. Discussion of main results

The results will be discussed with focus on the increasing degree of complexity that is reflected from paper I to paper IV.

6.1. Behaviour of fluids in a dynamic infusion system

I.v. infusions are dynamic flow of fluid driven by syringe pumps or by volumetric pumps. When one drug is meeting another drug in a i.v. line, for instance using stopcock connectors, the drug solutions meet in a specific ratio depending on the infusion rates (Figure 15a). What happens when two drug solutions meet in an infusion line may be compared to two rivers of different sizes that may flow at different speeds and meet to form a larger river. This can be seen in the photo in Figure 15b. The two colours of the rivers clearly show that they flow side by side after the meeting point in some sort of laminar flow. The degree of mixing after the meeting point depend on interruptions creating turbulence and force the fluids to mix. This is a matter of physical laws, which is beyond the scope of this Thesis.

a) b)

Figure 15. *a) Two syringes connected with a stopcock connector, which both should illustrate two solution meeting in the same channel or i.v. line b) picture of two rivers meeting. (a) Created using BioRender.com b) freely available for download from www.pexels.com.*

Early in this work, a dynamic setup was explored (unpublished data). Syringe pumps, infusion lines and stopcocks used in the paediatric clinic was set up in the laboratory (Figure 16). Two solutions were pumped at various infusion rates, one of them coloured with Brilliant blue to more easily follow the behaviour when the solutions met in the line.

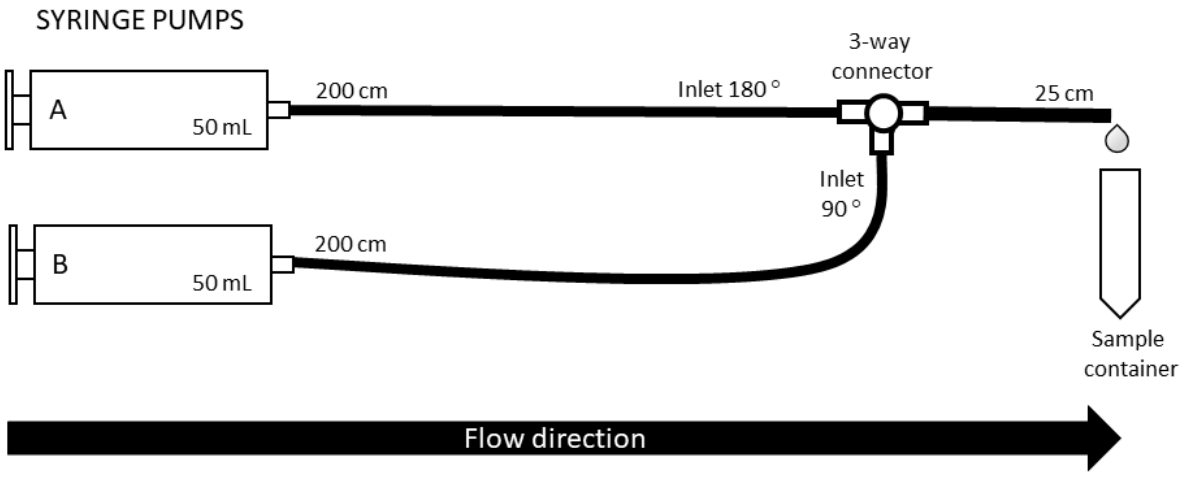


Figure 16. *Illustration of infusion setup replicating a possible clinical scenario for paediatric patients.*

The photos in Figure 17 show three different scenarios, the two infusions meeting at the same rate 1:1 (a), coloured solution at slower infusion rate 1:3 (b) and finally, the coloured solution at higher infusion rates 3:1 (c). Characteristic for all is the laminar appearance of the solutions inside the lines; it does not appear to be a great deal of mixing of the colours. This suggests that the contact between the two solutions is limited to the contact area in the middle where the two solutions go side by side. In a static test setup using test tubes, the two volumes will be completely mixed with the implications that the dilution effect will be larger but also the contact area. These factors could be determining for whether a drug remain in solution or precipitates, hence, the compatibility result obtained in such a test. Based on the encouraging results from the colour experiment, this setup was used to test incompatible combinations to verify the test conditions. However, the analytical methods used to assess precipitation all require certain volume of liquid, and collecting liquid in a tube for 20 minutes did not provide any advantage over direct mixing of volumes in a tube. During sample collection, the liquid mixed completely and the same limiting factors were encountered in the tubes (unpublished data). In literature, there is examples of dynamic compatibility testing using dynamic image analysis employing the Qicpic particle analysis instrument with a Lixell module both from the company Sympatec GmbH (Germany) (150). The instrument was equipped with a high-speed camera, which captured images up to 500 frames per second, allowing real time measurements of particle content.

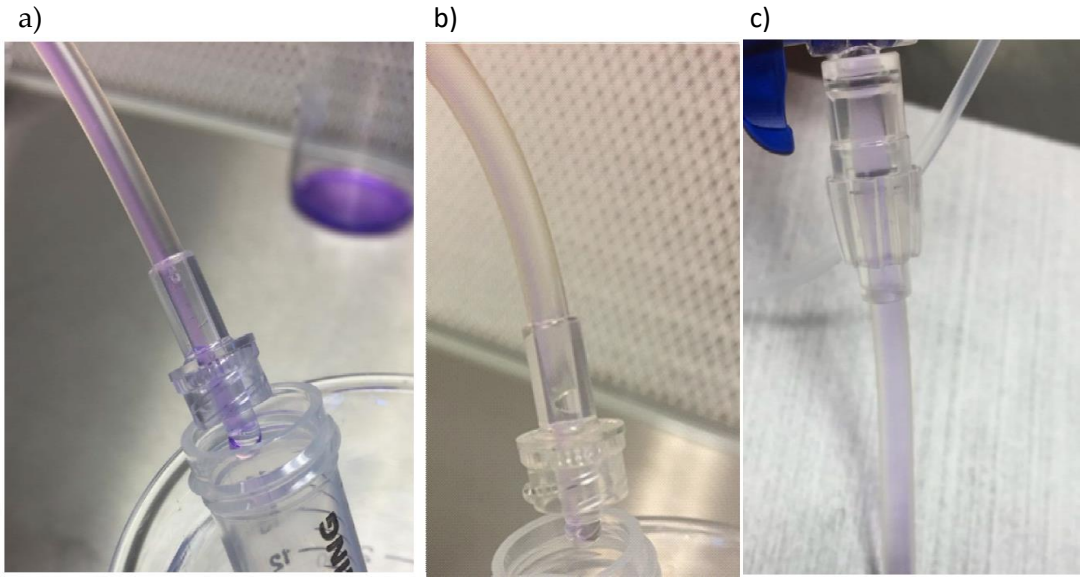


Figure 17. *Pictures showing the laminar flow of a solution with Brilliant blue together with a transparent solution in the infusion line (coloured solution given first in ratio). a) 1:1, b) 1:3 and c) 3:1 (unpublished data). Photo: Anette Lima Hansen.*

In this Thesis a static testing of volume ratios that were estimated to meet in the line, based on typical drug concentrations, doses and infusion rates in neonates and paediatric patients in various weight classes were used. The mixing ratios were selected in an approach trying to expand the experimental area that the results could represent. 1+1 was always included, since this is a mixing ratio that occur in most literature, dating back to Allen *et al.* (139). This was a practical approach, but a static test-setup can never fully represent what is happening in a dynamic infusion.

6.2 Physiochemical considerations

Compatibility of two medications may perhaps be comprehensible (6.2.1). The complexity of co-administration of several drugs in the same i.v. line is huge. How the reoccurring changes in infusion rates of each drug in an ICU setting will affect the pH in the contact area, and hence, the solubility of each drug is not easy to predict nor to investigate. However, since several drugs are co-administered in the clinical setting, it is of great importance to investigate these scenarios. Therefore, three, five and six components mixtures where either buffered electrolytes (section 6.2.2) or PN (section 6.2.3) were one of the components, was studied. These are cases that represent complex therapy regimes frequently used in NICU/PICUs.

6.2.1 Potential precipitation between medication and medication

Morphine hydrochloride was found to be compatible with both cefotaxime and dopamine (Paper II). In 1985, Nieves-Cordero *et al.* tested the compatibility between different narcotics and several antibiotics and found morphine sulphate to be compatible with both ampicillin and cefotaxime (151). They mixed the samples with equal parts of each medication and used visual examination to analyse the samples for precipitation. In Norway morphine hydrochloride is used while most other countries uses morphine sulphate (151, 152). The main difference between the salts of morphine was that morphine hydrochloride has a slightly higher aqueous solubility than morphine sulphate (1:17.5 and 1:15.5, respectively) (153), which amplifies the results of cefotaxime being compatible with morphine hydrochloride (paper II). Cefotaxime has a pH of 5.0-7.5 (154) and morphine has a pH of 3.0-5.0 (155) with a pKa of 8.21. Due to the broad ranges of potential pH-values for both medications, the mixture of the medications could theoretically give a pH of 7.5. At this pH, morphine would be approaching the pKa of morphine and morphine molecules would be more deprotonated. However, the measured pH of cefotaxime was 5.40 and the pH of morphine was 4.55, and the pH of the mix of both medications was 4.88-5.29, depending on mixing ratio. In this range, morphine would be in its protonated state and not be at risk of precipitating with cefotaxime. This shows, in addition to knowing the pKa-values, the importance of knowing the actual measured pH of the medications and the pH of the mixture of the medications to predict solubility. The pH range given in the SmPC by the manufacture is not enough. In another study (not included in this Thesis), both ampicillin and benzylpenicillin were found to be incompatible with morphine hydrochloride (52). Most probably had both ampicillin and morphine precipitated due to the pH of the mixture (9.0-9.4) was in a range where precipitation could occur for both medications. Morphine with a pKa of 8.21 would be less ionised at the pH of the mixture. On the other hand, at this pH ampicillin with pKa of 2.55 and 7.25 (156) will have an ionised carboxylic acid group but a less ionised amine group, which would make the medication less soluble than if pH would have been below the basic pKa. Since most medications are weak acids or bases, this reasoning emphasise that pH and solubility are two of the most important factors when it comes to compatibility issues.

Dopamine has, according to the manufacture a pH-range 2.5-4.5 and a pKa of 8.93 (157). From a theoretical perspective, dopamine should not precipitate when mixed with morphine. The

measured pH after mixing dopamine with morphine was 3.8-4.3 and mirrored the mixing ratios in the study. Both dopamine and morphine were within a pH-range that promotes ionisation of both medications and no precipitation was observed.

The five-component mixture of ceftriaxone, ampicillin, cefotaxime, metoclopramide and paracetamol did not show any sign of precipitation by visual inspection using focused light and polarised light microscope (paper IV). Calcium chloride mixed with ampicillin, cefotaxime, metoclopramide and paracetamol, respectively did also not precipitated using the same visual methods as the five-component mixture. How to predict and calculate pH in a multi-drug mixture is not straightforward but would be very relevant when theoretically exploring the consequences a change of pH could have on each drug. The pH and pKa of the medications are presented in Table 3 (page 46). Most probably the pH would be around 7.5 since no precipitation were seen visually. At a pH of 7.5, the weak bases are still ionised and ampicillin would be on the basic side but as seen when mixed with morphine, ampicillin was reaching the basic pKa and should show sign of lower solubility due to deprotonation of the amine group. If a larger volume of ampicillin were mixed with, for example paracetamol, the pH would most probably be towards the pH of ampicillin (9.3) whereas paracetamol would be at its pKa and 50 % would be unionised and less soluble.

The precipitation of ceftriaxone-calcium is not pH-mediated but calcium and ceftriaxone have formed a very poorly soluble salt or complex (158). Infusion of this ceftriaxone-calcium complex has led to lethal consequences, and because of this, for young children it is even advised not to treat the patient with calcium-containing products within the same time frame as ceftriaxone (159). In section 6.3, an innovative approach using Raman spectroscopy in compatibility studies will be discussed.

6.2.2 Potential precipitation with buffered electrolyte solution

In the local PICU a case of incompatibility was reported where fentanyl, ketamine, midazolam, and potassium chloride had been co-administered together with the buffered electrolyte solution Plasmalyte *i.e.* a five component mixture (paper III). The nurses who treated the patient and observed the precipitation tried to solve the problem by elimination of ketamine but noted that precipitation still occurred. The nurses concluded that Plasmalyte was the

problem. Plasmalyte Glucos is often used in the PICU so it was also of interest to investigate. The pH of Plasmalyte (pH 6.5-8.0) and Plasmalyte Glucos (pH 4.0-6.0) both products contain a buffer that work to maintain the pH at the product pH in contact with weak acids or bases. From a theoretical perspective, midazolam could be the contributor of the precipitation in the five-component mixture. The solubility of midazolam is decreased at higher pH (see Table 7) where the ring structure of midazolam is closing and becomes more lipophilic (Figure 18) (160). Therefore, Plasmalyte may contributes to push and maintain, since it contains a buffer, the pH at a level where midazolam solubility is least soluble, whereas the corresponding product with glucose has a more favourable pH for keeping midazolam in solution.

Table 7. *Water solubility of midazolam hydrochloride at different pH at room temperature (161).*

pH	Solubility in water (mg/mL)
6.2	0.24
5.1	1.09
3.8	3.67
3.4	10.3
2.8	> 22

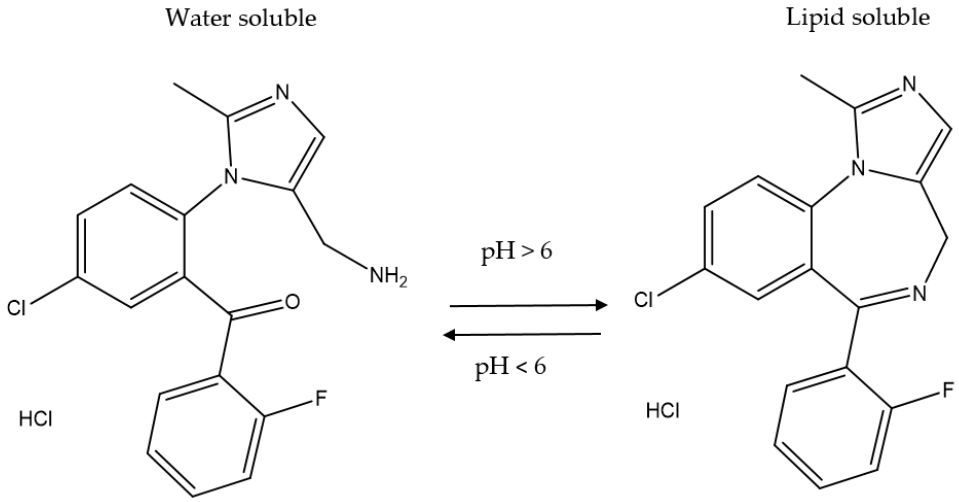
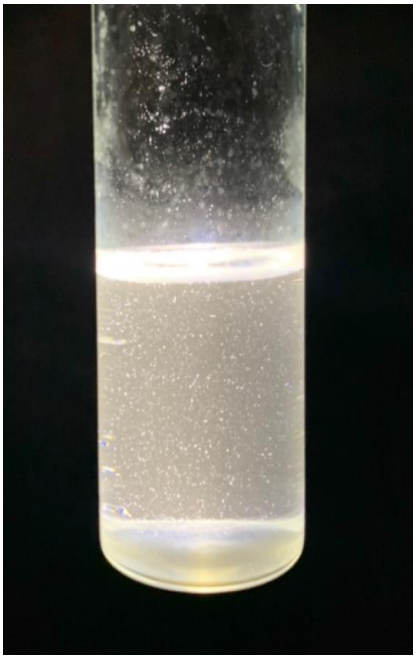


Figure 18. *Change in midazolam structure with increasing pH. Medication products for parenteral administrations are produced as hydrochlorides with a pH around 3.5 to ensure aqueous solubility. At deprotonation, a new ring structure is formed and the aqueous solubility is strongly reduced (ChemDraw Professional 19.1).*

When replicating the case, all analysis methods showed a massive precipitation. The investigation showed that midazolam precipitated when mixed with Plasmalyte with high particle count in all investigated particle sizes and high turbidity results. Interesting findings in addition to midazolam, was that ketamine also contributed to the precipitation when mixed with Plasmalyte. Dawson *et al*. found ketamine compatible with Plasmalyte but they used a lower concentration of ketamine (0.250 mg/mL) in one mixing ratio of 1+30, which even further diluted ketamine to a concentration of around 8 ng/mL (162). In paper III, the concentration of ketamine was 10 mg/ml. In addition, they only checked the samples by visual inspection against a light background, and therefore unable to detect sub-visual particles (<50 μm), which still could represent a safety risk. Ketamine has a pKa of 7.5 (163) and is provided as a product with pH 4.6 (paper III), after mixing ketamine and Plasmalyte the pH was measured to 6.1-6.3, which was increased towards the measured pH of Plasmalyte at 7.0. The closer the pH gets to the pKa of ketamine, the higher percentage of the molecules will be deprotonated (non-ionised) leading to a lower solubility of ketamine.

Based on that Plasmalyte Glucos is often prescribed in PICU and due to the pH difference between Plasmalyte and Plasmalyte Glucos, it was of interest to investigate Plasmalyte Glucos for the same therapy regime. Midazolam was also found to precipitate with Plasmalyte Glucos but in less degree and it was more difficult to see the Tyndall effect using both focused light and a laser pen (see Figure 19 and Figure 20). However, particle count revealed the number of particles in the size range of 10 μm was over the acceptance levels, in addition to high turbidity.

a)



b)

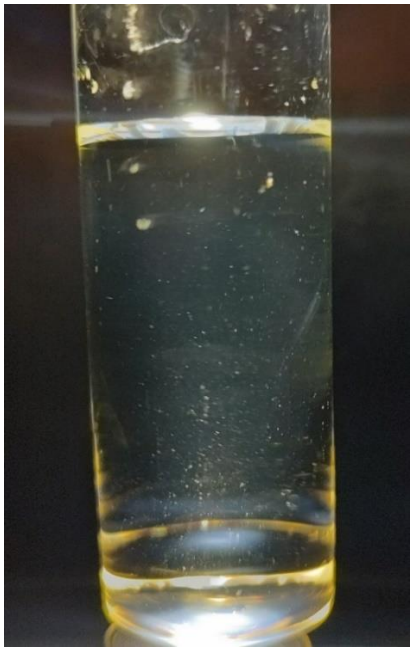


Figure 19. *Precipitation after mixing a) midazolam 5 mg/mL and Plasmalyte and b) midazolam 5 mg/mL and Plasmalyte Glucos. Both in a) and b) the drugs were mixed in equal parts (1+1).*

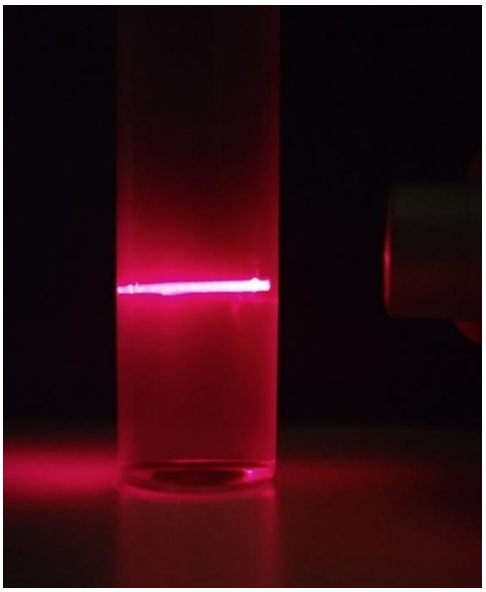


Figure 20. *Plasmalyte and midazolam 5 mg/mL mixed in 1+1 with a clear Tyndall effect by laser beam.*

Could information from paper III be valid for other fluids with buffer properties? Vallée *et al.* studied compatibility when Lactated Ringer´s injection (pH 6.0-7.5) (164) was mixed with other drugs in a 1+1 mixing ratio where they found ketamine 50 mg/mL to be incompatible and midazolam 5 mg/mL to be compatible (165). Vallée *et al.* used the same concentration of

midazolam but five times higher concentration of ketamine as in paper III. Maybe the high concentration of ketamine could be the reason to why they found ketamine to precipitate where more molecules were present that could be deprotonated when the pH of the mixture were reaching the pKa of 7.5 for ketamine. Lactated Ringer's injection also contains calcium (6 mmol/L), which is of interest when it comes to compatibility. How calcium could affect the compatibility of ketamine and midazolam was not mentioned. However the authors mentioned that they found it remarkable that ceftriaxone did not show any signs of incompatibility when ceftriaxone and calcium are known to form a massive and lethal precipitate (8). Several factors may explain the differences between Vallée *et al.* and the results from this Thesis other than different buffered electrolyte solutions, for example contact time, concentrations, diluents, manufacturers, excipients and analytical methods used. This suggests that it is not possible to evaluate pH and solubility alone as reasons for incompatibility, and that the analysis of one buffered electrolyte solution will not directly be applicable for another buffered electrolyte solution with different composition and/or pH.

6.2.3 Potential precipitation with Numeta G13E

Since, PN is a complex mixture with both macro- and micronutrients and ensured stability is challenging, co-administration of drugs in the same i.v. line as PN represents the next level as compared to simple solutions, especially if the PN contains lipids. PN containing lipids is a two-phase system formulated as an o/w emulsion with a milky white appearance where it is difficult to detect precipitated particles because of the oil droplets. For compatibility testing, Staven *et al.* mixed lipid-containing PN with the medication and centrifuged the samples in order to separate the lipids from the aqueous portion (7). The samples remained slightly turbid after removal of the lipid layer and they concluded that it was caused by remaining traces of phospholipids and other remnants. Based on the difficulties removing the lipid part, they concluded that it was better to not use the lipid part in samples investigating potential precipitation, and replace the lipid compartment with purified water (11). Therefore, in paper I and II when testing PN for precipitation after mixing with medication, the lipid-part was replaced with purified water (Milli-Q water) in equal volume. This replacement should not affect the buffer properties of the PN since amino acids are the most important contributor to the pH of the PN. By removing the lipid phase, the solubilising agent for lipophilic drugs could

potentially promote precipitation. It is important to consider this when replacing the lipid part the results are based on a solution that is not exactly the same as when co-administer PN with drugs to the patient.

Numeta G13E has a pH of 5.4-5.7 (166) and the buffer properties is mainly due to the amino acids. Regardless mixing ratio or whether the lipid part was present or not, the measured pH remained in the range 5.4-5.9 when mixed with paracetamol, vancomycin, fentanyl, morphine, cefotaxime and dopamine, respectively or in three-component mixtures (paper I and II). This was also regardless of time after mixing (immediately or 4 hours).

The manufacture of Numeta G13E have performed a study with paracetamol but in the concentration of 5.77 mg/mL and in mixing ratio of 1:5 (paracetamol:NumetaG13E) (167). In order to study how paracetamol and Numeta G13E are given to neonatal patients, paper I investigated the compatibility with paracetamol 10 mg/mL in three mixing ratios. Since paracetamol have a pH of 4.5-6.5 (168) the risk of pH-dependent incompatibility when mixed with Numeta G13E should be small and no sign of precipitation was found in this study. Staven *et al.* had previous concluded that the Numeta G16E, a PN used for newborn infants and toddlers, to be compatible with paracetamol (169). The only deviant they found was that in one of the three tested generic paracetamol formulations, they saw an increased turbidity but since the control sample of pure paracetamol also had elevated turbidity, this observation could be traced to one of the excipients. The specific paracetamol formulation contained hydroxyethyl starch, which form a colloidal dispersion that spreads the light and will give rise to increased turbidity. Since an explanation for the deviating result was identified, it could be concluded that paracetamol mixed with Numeta G16E was compatible. The paracetamol used in paper I was from the same manufacturer, but a new formulation without hydroxyethyl starch and low turbidity was observed for the unmixed control as well as the mixed samples**.** It could be mentioned that two of the excipients in the current paracetamol formulation have shown to be incompatible when mixed with other solutions. Excipients could be of involved in incompatibility, and therefore, switching between generic drug products should be handled with care taking excipients and formulations into account.

Fentanyl was found to be compatible with Numeta G13E (paper I). As a weak base with pH of 5-7.5 and a pKa of 8.99 (170), the measured pH of the fentanyl formulation was 4.81 keeping fentanyl in the ionized and soluble form. The measured pH of the mixture of fentanyl and Numeta G13E was 5.4, which maintain fentanyl solubility. Fentanyl is very slightly soluble with a solubility of 0.74 mg/mL but since fentanyl is very potent, the concentration used in the hospital is up to 50 μg/mL, which is much below the solubility limit, and solubility issues was not a problem for fentanyl.

Vancomycin had a pH after reconstitution of 3.2 (paper I**)**. Vancomycin is an amphoteric glycopeptide with six pKa values, both acidic and basic (see Table 3, page 46**).** Vancomycin is freely soluble with a solubility of greater than 100 mg/mL in water, but the solubility has been shown to decrease at pH above 7 (171). This is caused by the deprotonation of the amine groups, the R-NH3 (pKa=7.2) and R-NH2-R (pKa=8.6); when pH is reaching the pKa-values the solubility decreases (172). Since Numeta G13E has a pH around 5.5 and buffer properties, the PN governs the pH when mixed with vancomycin; pH of the mixtures was measured to be 5.5 in all mixing ratios and vancomycin remain in the protonated and soluble form. Other studies did also not report any signs of precipitation when vancomycin was mixed with different PN admixtures (97, 171).

The investigation of potential precipitation of two-component mixture of Numeta G13E with dopamine and cefotaxime, respectively, and three-component mixtures Numeta G13E with morphine and cefotaxime or morphine and dopamine, (paper II) found Numeta G13E to be compatible with both two- and three- component mixtures. When mixing cefotaxime, morphine and Numeta G13E the pH was measured to be from 5.44 to 5.81, which mirrored the pH of Numeta G13E and was not surprising since all components were on the weak acidic side and PN has buffering properties. The same pattern was seen for the two-component mixtures with either cefotaxime or morphine with Numeta G13E. It was not surprising that no precipitation was seen in any of the two- nor three-component mixtures.

Trissel *et al.* found cefotaxime 20 mg/mL, morphine 1 mg/mL, fentanyl 12.5 μg/mL and 50 μg/mL, respectively to be compatible with nine different PN-admixtures in a 1:1 mixing ratio whereas they found that dopamine 3.2 mg/mL lead to precipitation in two of the PN-

admixtures (97). In paper III, the pH after mixing dopamine 2 mg/mL and Numeta G13E was measured to be around 5.8, irrespective of amount of dopamine in the tested mixing ratio. The same theoretical discussion as above regarding the buffer properties of Numeta G13E would be valid for dopamine. Dopamine remains predominantly in its ionised and soluble form in a weak acidic environment (see Table 3 in the introduction, page 46). It should be noted that the concentration of dopamine was higher in their studies than the 2 mg/mL used in paper III. It should also be added that Trissel *et al.* centrifuged the mixture of drug and PN, and removed the lipid layer with a pipette. Thereafter they inspected the aqueous phase visually for precipitation. They could not perform turbidity, particle count or size measurement due to the aquatic phase was very turbid caused of the presence of surfactants and other emulsion components (97). This is why removing the lipid-phase seems and replace it with water seems to be the best way to investigate for precipitation in 3-in-1 PN admixtures.

6.2.4 Potential emulsion destabilisation

When analysing samples containing lipid emulsions it is of essence to prepare the samples so that the analytical instruments could detect any destabilisation. All samples containing lipid emulsions had to be diluted before light obscuration analysis. However, dilution in itself could affect the emulsion and a potential flocculation of droplets can be redispersed. Flocculation occurs more frequently in emulsions with high electrolyte content, such as PN (100). Nevertheless, flocculation is a reversible process that does not necessarily lead to coalescence and phase separation. It is important to have in mind when evaluating emulsion stability.

Emulsion stability, *i.e.* how the surface charge of the oil droplets are affected when the emulsion is mixed with drugs may be difficult to predict theoretically. Since lower pH can reduce (neutralise) the surface charge of the oil droplets, determining pH could provide an indicator of emulsion stability. A pure lipid injectable emulsion typically has a zeta potential of -30 mV to – 50 mV (100). Vancomycin (in paper I) and dopamine (paper II) had low pH-values that potentially could change the droplet surface charge (see Table 3, page 46**)**. In the emulsion stability tests for paper I and II, zeta potential analysis were performed (unpublished data). The zeta potential values for unmixed samples of Numeta G13E was found to be approximately -30 mV in paper I and in the same range for the tested mixing ratios with vancomycin. In paper II, the zeta potential of the unmixed Numeta G13E was found to be

approximately -26 mV and the mixed samples with dopamine ranged between -26 and -30 mV. It is generally recognised that colloidal systems with a large zeta potential, positive or negative, would be stable and less prone to aggregation. However, when it comes to the zeta potential of PN emulsions this is more challenging (100). First, the emulsion has to be diluted to allow determination of the zeta potential. In the above referenced experiments, the emulsion was diluted in water, meaning that the estimated zeta potential is in fact that of the oil droplets in water, and not (truly) in the emulsion; hence, the values should not be directly interpreted as the stability of the emulsion in a mixed sample with the medication. Secondly, PN emulsions contain a lot of electrolytes and therefore carries current, which contributes to sample heating and electrolysis effects making the values less trust worthy. Even though the experiments of Numeta G13E with vancomycin or dopamine, respectively, indicated little change after mixing, there is a need for additional descriptors to evaluate the emulsion stability. Staven *et al.* concluded that measurements of zeta potential did not add more information than already obtained by simple pH-measurements, which is a fast, more available analysis that provides trust worthy results (7). Finally yet importantly, pH can be measured directly in the mixed sample without any dilution.

PFAT5 is a recognised descriptor for stability of injectable lipid emulsions, which also is applied in compatibility assessment to capture signs of emulsion destabilisation with an acceptance limit of up to 0.4% (7, 148). The PFAT5 of Numeta G13E reference samples was determined to be approximately 0.2% in paper I. Small increases in PFAT5-values was observed for some mixing ratios for paracetamol and vancomycin when mixed with Numeta G13E, respectively (paper I). All samples mixed with fentanyl were within the acceptance limit. For the combinations with slightly increased PFAT5 values, the pH-values were within the acceptance levels. The samples where paracetamol and vancomycin had too high PFAT5-values also had larger standard deviation than for the rest of the samples, indicating that there was large variation between the replications. The reason for this is unknown, but it could be flocculation of droplets that was not redispersed before analysis. As mentioned above, flocculation occurs more easily in emulsions with high electrolyte content and is a reversible process that does not necessarily mean that the emulsion is destabilised. Since the PFAT5 values were not very high and not consistently in samples with high amount of medication in the mixing ratio or only after long mixing times, this seems like a plausible explanation.

Another explanation would be that the samples could have been contaminated. Infusion times of paracetamol and vancomycin are shorter (<< 4 hours) to the neonates and it was concluded that in total the elevated PFAT5 was not to be seen as a case of incompatibility. On the other hand, Stawny *et al.* concluded vancomycin 3.7 mg/mL, which is a lower concentration than 5 mg/mL used in paper I, to be incompatible with two out of five different PN admixtures designed for adult patients (171). Incompatibility was not determined based on PFAT5, but on high polydispersity index (PDI) combined with an increased droplet diameter; 10 % of all lipid emulsion droplets had globule sizes over 4000 nm (= 4 μm). They reported deviations only in the four hour samples in growth of droplet size but pH, mean droplet diameter and visual inspection were within the acceptance levels. Vancomycin had in two occasions slightly elevated PFAT5-values (paper I) but was considered to not be clinically relevant due to infusion rates of vancomycin are shorter than the testing time (4 hours). In addition, this Thesis used a battery of test methods and the conclusion was made based on all methods as a whole. This further accentuates the need of a standard in how to analyse and determine emulsion stability for compatibility analysis of PN and drugs.

Fentanyl did not show any sign to destabilise the emulsion of Numeta G13E (paper I). This was in line with findings from other studies on compatibility on fentanyl with PN (97, 173). The manufacture of Numeta G13E have information on compatibility with fentanyl 3.6 μg/mL in mixing ratio of 10+1 (167) but this concentration of fentanyl is lower than the concentration used at the hospitals. Fentanyl have a pKa-value on the basic side (8.99) and the pH of 5.4 for Numeta G13E promotes solubility of fentanyl. The solubility of fentanyl base is reported to be 0.74 mg/ml. However, fentanyl is a very potent opioid, and since the clinical concentration used is in μg/ml, the solubility of fentanyl is promoted.

When morphine and Numeta G13E were mixed with cefotaxime or dopamine, respectively or as three component mixtures no emulsion destabilisation were seen (paper II). The main reason for this could be that the pH of all combinations were within the range of 5.7, which is the pH of Numeta G13E (166). If pH would have been lowered due to addition of other drugs the surface charge of the lipid droplets could have been decreased. The consequence of decreased surface charge of droplets could be that van der Waals attractive forces could make droplets fuse into larger droplets. None of the medications lowered the pH when mixed with

Numeta G13E. Trissel *et al.* saw an immediate emulsion destabilisation in all nine PN admixtures (all aimed at adult use) after mixed with morphine sulphate 15 mg/mL (97). On the other hand, other studies had found this combination compatible (173). In paper II, the hydrochloric salt of morphine was tested in the concentration of 0.2 mg/mL both as a two-component mixture with Numeta G13E but also as a three component mixture where cefotaxime and dopamine, respectively were added. Reassuringly, no sign of emulsion destabilisation was seen. Trissel *et al.* suggested in their study that the emulsion destabilisation effect of morphine was to be concentration dependent which the results in paper II contributes further to (97).

PN contains charged electrolytes, which could influence the surface charge of the lipid droplets and reduce the repulsions between the droplets that may coalesce and/or form flocculation or loose aggregation of lipid droplets. This potential aggregation could be seen as enlarged droplets. It is also believed that oil droplets are flexible and soft, and therefore it is believed that enlarged oil droplets would be of less danger to be entrapped in small capillaries than precipitated solid particles (174).

6.2.5 Use of filters

In order to have an extra safety precaution, in-line filter could help to prevent the infusion of particles. The guideline from Medicines for children network Norway recommend the use of filter to paediatric patients for all i.v. injections and infusions (175). The guideline at the NICU at OUH states that the use of filter is advised in all injections and infusions for children (176). In Figure 21 a set up for filter use is shown. When drugs are to be co-administer different filters are to be used where a white filter (pore size of 0.2 μm) should be used for medications and other transparent fluids and a blue filter (pore size of 1.2 μm) should use for emulsions. In addition to prevent particles to enter the body, a blocked filter arise an increased pressure in the infusion set which trigger the syringe pumps to alarm and could be an indication of precipitation. As discussed earlier, this was the reason to how the precipitation of the five-component co-administration investigated in paper III was revealed.

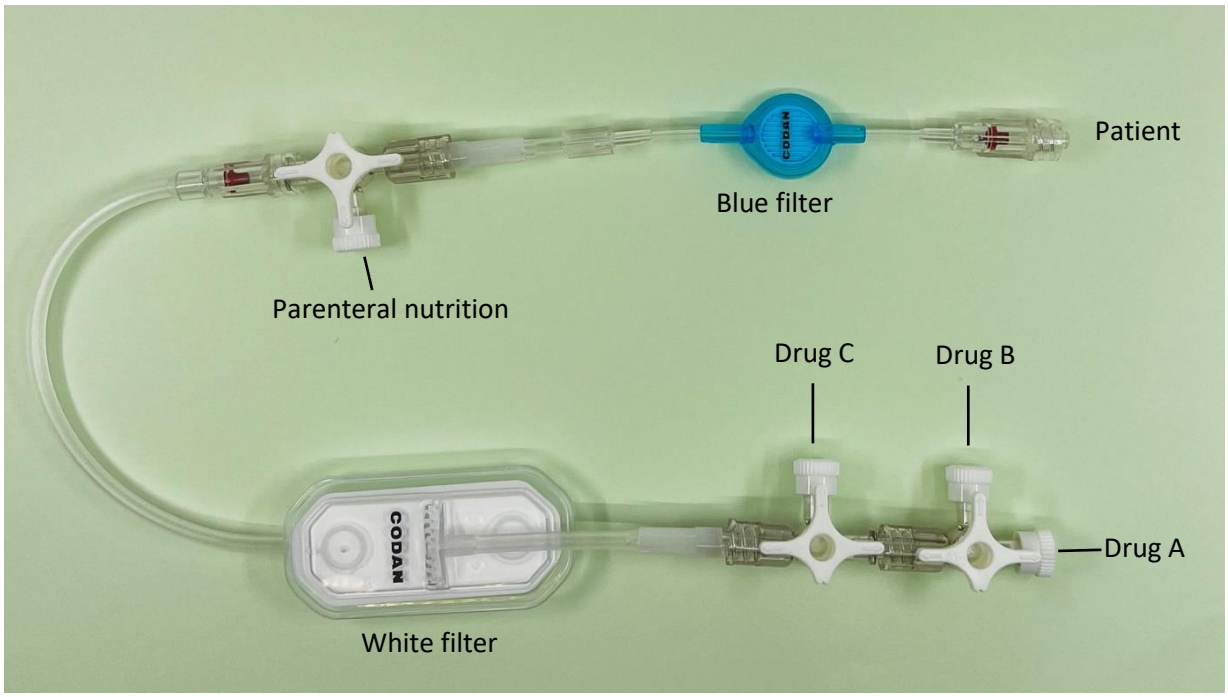


Figure 21. *Overview of an intravenous (i.v.) catheter with several three-way stopcock connectors and filters where white filter is for medications and fluids (0.2 µm) and blue filter is for emulsions (1.2µm) from NICU at OUH (Personal photography).*

6.3 Raman spectroscopy

In paper IV, the well-known precipitation of ceftriaxone-calcium was used as a model-system when to investigate the potential of using Raman spectroscopy in identifying a precipitate in a multi-drug mixture. After mixing calcium chloride dihydrate, ceftriaxone sodium, cefotaxime sodium, ampicillin sodium, metoclopramide and paracetamol the formed precipitate was referred as "unknown". The spectrum of the "unknown" was compared to the spectra of each of the medications and for ceftriaxone-calcium. Visually the "unknown" spectrum seemed to resemble the ceftriaxone-calcium spectra (Figure 22). In the PCA score-plot the "unknown" was clustered with ceftriaxone-calcium, which further contribute to the impression that the "unknown" precipitate had to be ceftriaxone-calcium (Figure 23). Ceftriaxone sodium was also located close by in the score plot, indicating similarities between the spectra. The SIMCA classified the "unknown" as ceftriaxone calcium and not ceftriaxone sodium.

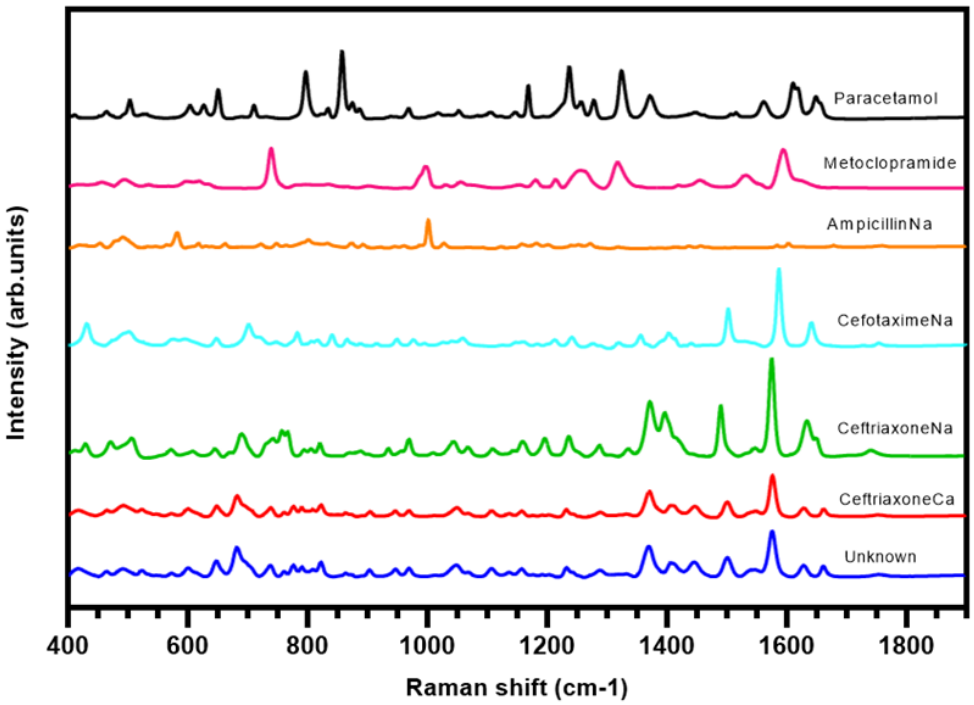


Figure 22. *Raman spectra of raw powder from ceftriaxone sodium (ceftriaxonNa), cefotaxime sodium (cefotaximNa), ceftriaxone calcium (ceftriaxonCa), paracetamol, metoclopramide, ampicillin (ampicillinNa) and the "unknown" precipitated from the combined drug mixture.*

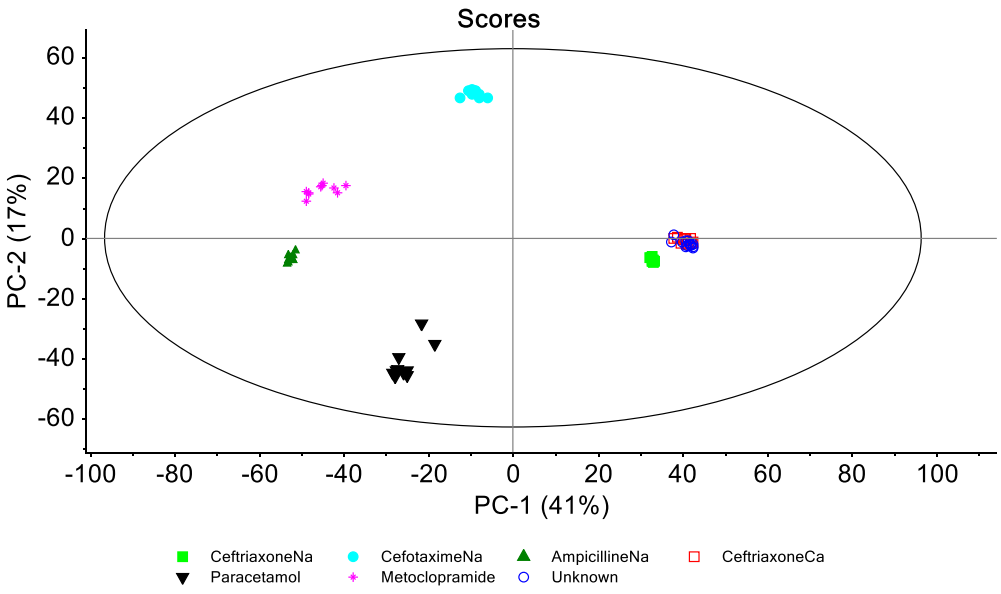


Figure 23. *Principal Component Analysis (PCA) of the Raman spectra (400-1900 cm^{-1}) of all solid form drugs and the "unknown" precipitate from the multi-drug mixture.*

Raman spectroscopy can provide detailed information about chemical structure and solid form, such as the two different salt forms of ceftriaxone. It can also differentiate between drugs with similar molecular structure. Ceftriaxone and cefotaxime have similar molecular structure (see Figure 14, page 48) but have clearly differences in their Raman spectra (Figure 22**).**

To use Raman spectroscopy in a clinical setting it would be relevant to also extract the probable origin of a precipitation, even when the solid form differs from the library (reference) spectra. To do this the normalised intensity spectra were compared using the Pearson's correlation coefficient, which measures the statistical relationship or association between two continuous variables. For ceftriaxone-calcium, ceftriaxone sodium and cefotaxime sodium the proportion of explained variance were 97 %, 48 % and 8 %, respectively. For the other three drugs (paracetamol, metoclopramide and ampicillin sodium) the explained variance were below 2 %. This shows with high significance that the "unknown" sample was ceftriaxoneCa. In addition, if a reference spectrum of ceftriaxoneCa had not been available, from the correlation of the "unknown" to ceftriaxoneNa (48 %), it could have been possible to predict that ceftriaxone was part of the precipitate, perhaps as a different salt or solid form. These results are promising for use of Raman spectroscopy in i.v. drug compatibility testing. Raman spectroscopy was found to be a powerful tool for proving the identity of the solid form precipitating in a multi-drug co-administration regimen and the origin may be traced even if the actual reference spectrum are not available.

Raman spectroscopy could be a quick and problem-solving tool when exploring which drug(s) have contributed to the precipitation. Various scenarios for the use of rapid Raman spectroscopy to solve clinically relevant compatibility issues can be envisioned. The easiest approach is to implement a test laboratory or a facility in the hospital or in a hospital pharmacy that runs tests on drugs that are about to be co-administered in order to declare safe combinations by eliminating the compound(s) that can potentially precipitate. The Point-of-care testing would be a patient centric scenario where a handheld Raman probe is implemented for studying potential in-line particle formation (Figure 24). The use of filters that are often attached at the end of the infusion line when the compatibility of i.v. drugs is not known could also be explored (Figure 24). Particles on such filters are expected to be suitable for Point-of-care analysis with a Raman instrument suitable for single particle analyses. Providing a rapid identity of this particulate material would be a valuable tool for

subsequent safe drug administration and treatment consideration. It should be noted that some precipitates can be amorphous with a low intensity Raman signal, or the compound of interest is simply a poor Raman scattering structure. This is underpinning the importance of development of robust Raman instruments with a possibility for using different laser sources with a different laser wavelength. It should be noted that some precipitates can be amorphous with a low intensity Raman signal, or the compound of interest is simply a poor Raman scattering structure. This is underpinning the importance of development of robust Raman instruments with a possibility for using different laser sources with a different laser wavelength.

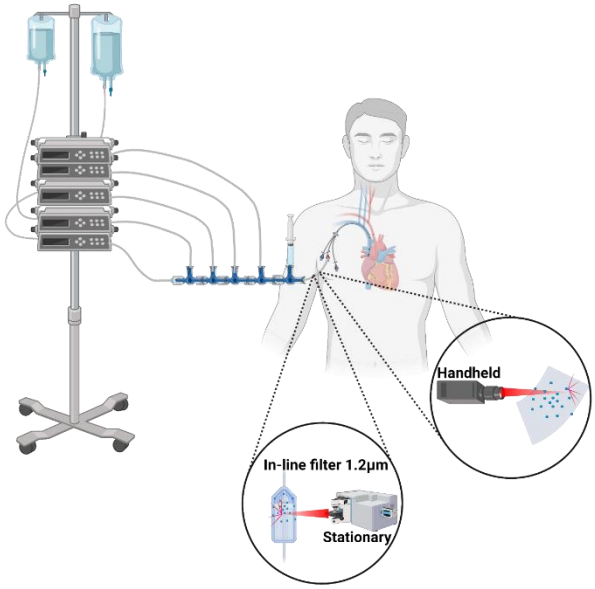


Figure 24. *Different possibilities to use Raman spectroscopy in the clinical setting. Handheld Raman instruments could be an alternative for the directly scan of the i.v. line and analyse for precipitates or analyse the i.v. filters that are often attached at the end of the infusion line.*

6.4 The clinical context and the contribution to the research field

6.4.1 Information on drug compatibility and problem solving

Most often, none or little information on drugs used by the neonatal or paediatric patient are to be found in recognised and available compatibility databases (12, 177) or from the PN manufactures (167, 178). Fernández-Peña *et al.* concluded that there was a gap of knowledge for drugs used in neonatal intensive care units (179). Oduyale *et al*. identified two main themes after interviewing nurses at intensive care units on their experiences with co-administration

of drugs (104). The nurses described that the patient often had insufficient i.v. access ports and that there is a lack of compatibility data on commonly used drugs.

Hospital pharmacists or clinical pharmacists are frequently contacted for solving drug compatibility issues. Almost one out of five questions directed to the on-call pharmacists in South-Eastern Norway Regional Health Authority were drug compatibility related (180). In the U.S., Belgado *et al.* found that 17 % of the questions directed to pharmacists were on compatibility issues (181). The pharmacist must possess adequate skills to identify potential incompatibilities of various i.v. drugs, and health care practitioners expect that the pharmacist is an expert on drug compatibility (92, 182). Roche even stated that the patients deserves nothing less (183). The Swedish medicines agency informed in 2008 that the hospital pharmacies have a responsibility to investigate and give advice when mixing compounds with i.v. fluids (184). All this shows that pharmacists need to be reliable and confident when handling compatibility issues. It is tempting to think that the questions on compatibility that is directed to the pharmacist are cases where nurses and physicians have tried to find out what is compatible or not, but not have found a good solution. PN or buffered fluids are often involved in compatibility issues since many neonatal and paediatric intensive care patients are in need of these during the hospital stay. As mentioned earlier, little information on compatibility for medications in concentrations and infusion rates used for the neonatal and paediatric patients is available. Often conflicting information on compatibility of PN and buffered solutions are a reality. Information from other PN admixtures could be valuable when there is no information on the specific PN, and is adding to the pool of information that can guide us in a direction of compatibility or incompatibility. However, it is never strait forward to extrapolate from one of these products to another and the exact composition and pH must always be considered upon extrapolation. PN admixtures are unstable formulations and could easily be disturbed when mixed with other drugs or used in other ways than intended from the manufacturer. For patient safety reasons, the health care professional who is responsible for administering the drugs correctly, and for time consuming reasons, it is preferable to have information on the actual combination of drugs that are to be judged for compatibility.

I.v. drug compatibility is one out of many things to handle and considers when treating patients. When the number of drugs outnumber access ports, health care professionals have to take action and decide which drugs are to be co-administered in the same i.v. line. This decision could be made based on for example practical reasons, knowledge or empirical reasons. The "five rights" of medication use: the right patient, the right drug, the right time, the right dose, and the right route—are a standard for safe medication practices (185). These five rights have shortcomings and suggestions of simply adding more 'rights' to the existing model, where checking for compatibility could be one assignment (186). How often health care professionals actually checks for drug compatibility is not known.

When attending at the neonatal- and paediatric intensive care units, the nurses and physicians clearly stated the need for more compatibility information on medication that are frequently used. Especially, the nurses expressed the stress they felt when not finding information on drug compatibility. This Thesis contributed with compatibility information on drugs and in combinations and in concentrations that are commonly used at NICU and PICU. Even findings on combinations that are safe to co-administer could help to ease the anxiety and pressure that the already heavily burden health care professionals have (187, 188).

6.5 Achievements in this Thesis

All aims, objectives and achievements of this Thesis, in addition to what can be improved or what to consider when planning new compatibility projects are summarised in Table 8 (next page).

Table 8. *Summary of objectives, achievements and considerations of the Thesis.*

Objectives	Achievements	Considerations
Investigate physical i.v. drug compatibility for the most vulnerable patients	Compatibility of PN designed for preterm was investigated with frequently used medications	Further studies should focus on the combination of PN with other sedatives and analgesics for example midazolam, catapressan, dexmedetomidine and ketamine
Investigate multi-drug compatibility when two medications are co-administered with PN	The following three components mixture, 1. Numeta G13E with dopamine and morphine 2. Numeta G23E with cefotaxime and morphine were both found to be compatible in concentrations and infusion rates suited for the preterm population	It is of interest to perform studies on more than two medications with PN since in the clinical neonatal and paediatric setting, many drugs are often co-administrated in the same i.v.- line
Investigate the compatibility of fluids with buffer properties with frequently used medications	Both Plasmalyte and Plasmalyte Glucos were investigated in a five-component mixture where two of the medications precipitated in both (midazolam) or one of the products (ketamine)	More compatibility studies with fluids with buffer properties together with other drugs should be investigated. When choosing drug candidates, drugs that are sensitive to pH-changes should be prioritised
To collaborate with PICU and find the cause to a five-component precipitation that was administrated to a patient	The result have been that the PICU have more focus on i.v. drug compatibility and the local clinical pharmacist is leading this task. Our contribution in how to handle a reported medication error is now used at Oslo university hospital as a learning example	To use the reporting system at hospitals more actively and reported precipitation even more in the hole process when investigating clinical relevant i.v.-drug compatibility issues
To develop compatibility analysis by exploring the possibility to prove identity of a precipitate from a multi drug mixture using innovative methods	Raman spectroscopy was found to be able to identify the solid for of the model ceftriaxone-calcium precipitate in a six-component drug mixture	Further development is necessary so that Raman spectroscopy can be implemented in the hospital setting for compatibility analysis

7. Conclusion

This Thesis was devoted to contribute with information regarding physical compatibility between drugs for the neonatal and paediatric patients. To have information on compatibility of combinations of drugs and in concentrations used at NICU and PICU are essential for administering i.v. drugs safely to these populations. This information will not only improve patient safety but also ease the already heavy burden of health care professionals. Since numerous drugs often are forced to be co-administered in the same i.v. line, this Thesis have investigated not only two-components mixtures but also three, five and six-components mixtures. Compatibility testing was performed using a panel of analytical methods, which included visual inspection (Tyndall effect), sub-visual particle count, turbidimetric analysis, pH-measurements, lipid droplet size distribution and mean fat droplet diameter and percentage of fat droplets with a diameter above 5 μm (PFAT5).

The PN used at the local NICU and designed for preterm neonates (Numeta G13E), was shown to be compatible with vancomycin, fentanyl, paracetamol, dopamine, cefotaxime and morphine, respectively in all mixing ratios. It was also compatible in three-component mixtures with morphine+dopamine and morphine+cefotaxime.

When replicating a case of incompatibility from the local PICU where fentanyl, ketamine, midazolam and potassium chloride had been co-administered with a buffered electrolyte solution (Plasmalyte), it was revealed that both midazolam and ketamine had precipitated. Midazolam also precipitated in the buffered electrolyte solution containing glucose (Plasmalyte Glucos) but to a less degree than in the buffered electrolyte solution without glucose. This could be explained by the pH of the buffered solution and the solubility of the drug in the mix. Both fentanyl and potassium chloride were found to be safe to co-administer with both buffered solutions.

When exploring new methods for improved detection and identification of precipitates in multi-drug mixtures, Raman spectroscopy was found to be a powerful tool able to detect and identify the ceftriaxone-calcium precipitate in a mixture of ampicillin sodium, calcium chloride dihydrate, cefotaxime sodium, ceftriaxone sodium, metoclopramide and

paracetamol. Even single sub-visual (25 μm) ceftriaxone-calcium particles could be analysed and identity proofed using Raman microscopy.

8. Future perspectives

There are still work to be envisioned in order to further improved safety for co-administration of i.v. drug in NICU and PICU. The following list of bullet points suggest where to continue.

- Expand compatibility testing on PN, with other 3-in-1 PN bags used to neonatal and paediatric patients. It would also be of interest to test compatibility of other drugs with focus on drugs with poor solubility at pH values of PN (approx. pH of 5.5-6.5). Medications that could be investigated are antiviral (ganciclovir, foscarnet), proton pump inhibitors (esomeprazole, pantoprazole), sedatives (midazolam, dexmedetomidine, ketamine), and antibiotics (meropenem).

- Test compatibility of PN with drugs in three-or more components mixtures.

- Test drugs in combination with other buffered electrolyte solutions since different solutions span over a broad pH-range but also because different buffered electrolyte solutions contains different electrolytes and in different amounts (for example Ca^{2+}, Mg^{2+}), which could affect the stability of both the solution itself but also some drugs could form poorly-soluble salts.

- In this Thesis physical compatibility has been studied but chemical compatibility should also be considered. In the ICU where drug is dosed to clinical effect, chemical degradation may be less relevant. However, degradation may form undesired degradation products and to gain knowledge on which drugs that potentially could affect other drugs upon co-administered could further increase patient safety.

- To further develop the use of Raman spectroscopy as a tool within drug compatibility analysis. Test more drugs that had precipitated in multi-drug mixture. It would be of great clinical relevance to analyse the precipitate in a liquid suspension. In the case where midazolam and ketamine precipitated when mixed with Plasmalyte (paper III) it should be of interested to investigate if Raman spectroscopy could be able to both identify and detect the origin of the precipitation.

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Appendix

***Table A1**. Summary of fluid and parenteral nutritional recommendations for preterm, neonatal and older children (35, 36, 144-147).*

	Preterm	Term neonate	1 month-3 years	3–11 years	12-18 years
Fluid (ml/kg/day)	140-160	140-170	*- the first 10 kg:* 100 *- weight between 10 and 20 kg:* 100+50 *- weight above 20 kg:* 100+50+25	*3-5 years:* 80-100 *6-12 years:* 60-80	50-70
Calories (kcal/kg/day)	110-120	90-100	75-90	*3-7 years:* 75-90 *7-12 years:* 60-75	30-60
Amino acids (g/kg/day)	1.5-3.5	1.5-3.0	1.0-2.5	1.0-2.0	1.0-2.0
Lipids (g/kg/day)	3- 4	3- 4	2-4	2-3	2-3
Carbohydrates (g/kg/day)	4-12	2.5-12	*1 month–10 kg:* 2.0-14 *11–30 kg:* *1.5*--8.6 *31-45 kg:* 1.0-5.8 *>45 kg:* 0.5-4.3	-	-
Calcium (mmol/kg/day)	*First days of life:* 0.8-2.0 *Growing premature:* 1.6-3.5	*0-6 months:* 0.8-1.5 *7-12 months:* 0.5	*1-18 years:* 0.25-0.4	-	-
Phosphorus (mmol/kg/day)	*First days of life:* 1.0-2.0 *Growing premature:* 1.6-3.5	*0-6 months:* 0.7-1.3 *7-12 months:* 0.5	*1-18 years:* 0.2-0.7	-	-
Magnesium (mmol/kg/day)	*First days of life:* 0.1-0.2 *Growing premature:* 0.2-0.3	*0-6 months:* 0.1-0.2 *7-12 months:* 0.15	*1-18 years:* 0.1	-	-

***Table A2**. Composition and physicochemical properties of the two buffered electrolyte products* (P = *Plasma-Lyte 148 and* PG = *Plasma-Lyte 148 with 5% Glucose, Baxter).*

Ingredients	**P**	**PG**
Glucose monohydrate (g/L)	-	55.00
Sodium chloride (g/L)	5.26	5.26
Potassium chloride (g/L)	0.37	0.37
Magnesium chloride hexahydrate (g/L)	0.30	0.30
Sodium acetate trihydrate (g/L)	3.68	3.68
Sodium gluconate (g/L)	5.02	5.02
Amounts		
Na^+ (mmol/L)	140	140
K^+ (mmol/L)	5.0	5.0
Mg^{++} (mmol/L)	1.5	1.5
Cl^- (mmol/L)	98	98
Acetate (mmol/L)	27	27
Gluconate (mmol/L)	23	23
Osmolarity (mosmol/L)	approx. 295	approx. 572
pH	approx. 7.4 (6.5-8.0)	4.0-6.0

***Table A3**. Composition of Numeta G13E after mixing and with all additives per L. aqNumetaG13E+ refers to the aqueous phase used in paper I and II for assessment of potential precipitation.*

Component (amount)	Numeta G13E+	*aq*Numeta G13E+
Olive oil (80%) (g)	17.4	-
Soy bean oil [a] (20%) (g)	13.0	-
Glucose monohydrate (g)	127.5	139.7
Alanine (g)	2.2	2.4
Arginine (g)	2.3	2.5
Aspartic acid (g)	1.6	1.8
Cysteine (g)	0.5	0.6
Glutamic acid (g)	2.7	3.0
Glycine (g)	1.1	1.2
Histidine (g)	1.0	1.1
Isoleucine (g)	1.8	2.0
Leucine (g)	2.7	3.0
Lysine (g)	3.0	3.3
Methionine (g)	0.6	0.7
Ornithine (g)	0.7	0.7
Phenylalanine (g)	1.1	1.2
Proline (g)	0.8	0.9
Serine(g)	1.1	1.2
Taurine (g)	0.2	0.2
Threonine (g)	1.0	1.1
Tryptophan (g)	0.6	0.6
Tyrosine (g)	0.2	0.2
Valine (g)	2.1	2.3
Sodium (mmol)	19.1	21.0
Potassium (mmol)	18.0	19.7
Magnesium (mmol)	1.4	1.5
Calcium [b] (mmol)	11.0	12.1
Phosphate [c] (mmol)	11.0	12.1
Acetate (mmol)	20.9	22.9
Chloride (mmol)	27.4	29.5
Malate (mmol)	9.3	10.2
Zinc (mg)	10.0	11.9
Copper (mg)	0.9	1.0
Manganese (mg)	0.04	0.05
Selenium (mg)	0.1	0.1
Fluoride (mg)	2.5	2.7
Iodide (mg)	0.04	0.05
Thiamine mononitrate (mg)	27.0	-
Riboflavin sodium phosphate (mg)	42.6	-
Nicotinamide (mg)	347.8	-
Pyridoxine hydrochloride (mg)	42.6	-
Sodium pantothenate (mg)	143.5	-
Sodium ascorbate (mg)	982.6	-
Biotin (mg)	0.5	-
Folic acid (mg)	3.5	-
Cyanocobalamine (mg)	0.04	-
α-Tocopherol (mg)	55.7	-
Retinol (mg)	6.0	-
Phytomenadione (mg)	1.7	-
Ergocholecalciferol (mg)	0.1	-

Table A4: *Overview of some intravenous infusion protocols from bedside observations at NICU, OUS. Bolus=injection over 5 minutes, Int.inf.=infusion over more than 15 minutes, Cont.=continuous infusion. (Unpublished data)*

No	Dosing weight	Type of drug and/or nutrition	Dosing rate	Dose comment	Infusion type
I	634 g	Fluconazole		1.8 mg each 3d	Int.inf.
		Caffeine citrate		6 mg x 1	Int.inf.
		Numeta G13E	3 mL/h	38 mL	Cont.
		Primene	0.28 mg/h	6.7 mL	Int.inf..
II	665 g	Dexamethasone		50 µg x 2	Bolus
		Numeta G13E	4.1 mL/h	73 mL	Cont.
		Vaminolac	0.42 mL/h	10 mL	Cont
		Caffeine citrate		5 mg x1	Int.inf.
III	780 g	Fentanyl 10 µg/mL	0.7 µg/kg/h		Cont.
		Numeta G13E	5.1 mL/h		Cont.
		Meropenem		14.5 mg x 3	Bolus
		Metronidazole		11 mg each 48h	Bolus
		Vancomycin		7.5 x 2	Int.inf.
IV	900 g	Insulin	0.035 E/kg/h	0.28 m/h	Cont.
		Glucose 50 mg/mL			Cont.
		Numeta G13E			Cont.
V	1076 g	Fentanyl 10 µg/mL	1.3 µg/kg/h		Cont.
		Glucose 50 mg/mL			Cont.
VI	1109 g	Fentanyl 10 µg/mL	0.5 µg/kg/h		Cont.
		NumetaG13E			Cont.
VII	1150 g	Fentanyl 10 µg/mL	2.5 µg/kg/h		Cont.
		Numeta G13E		115 mL	Cont.
		Vancomycin		7 mg each 18 h	Int.inf.
		Paracetamol		15 mg x 2	Int.inf.
VIII	1600 g	Cefotaxime		80 mg x 2	Bolus
		Vancomycin		18 mg x 2	Int.inf.
		Numeta G13E	1.6 mL/h	40 mL	Cont.
IX	1684 g	Cefotaxime		80 mg x 3	Bolus
		Vancomycin		18 mg x 2	Int.inf.
		Vitamine K		1 mg	Bolus
		Numeta G13E	2.75 mL	66 mL	Cont.
X	1750 g	Fentanyl 10 µg/mL		6.5 µg x 1	Bolus
		Cisatracurium		250 µg	Bolus
		Paracetamol		15 mg x3	Int.inf.
		Glucose 100 mg/mL + Heparin 2.5 IE/mL	0.4 mL/h		Cont.
		Glucose 100 mg/mL + NaCl + KCl	1 ml/h	19 mL	Cont.
		Glucose 125 mg/mL	7.45 ml/h	179 mL	
		Magnesium sulphate		0.25 mmol/L x 2	Int.inf.
		Primene	2.54 mL/h	60 mL	Int.inf..
		Termin lipid-free	12,5 mL/h		Cont.
		Omegaven	2.5 mL/h	60 mL	Cont.

Table A5: *Overview of some intravenous infusion protocols from bedside observations at PICU, OUS, Rikshospitalet. (Unpublished data)*

No	Dosing weight	Drug and nutrition	Dose	Concentration	Infusion rate	Dosing rate	Infusion duration
I	3.435 kg	Clonidine	150 µg	10 µg/mL	0.34 mL/h	1 µg/kg/h	43.65 h
		Fentanyl	1250 µg	25 µg/mL	0.421 mL/h	3 µg/kg/h	121.3 h
II	3.5 kg	Fentanyl	500 µg	10 µg/mL	0.35 mL/h	1 µg/kg/h	143 h
		Numeta G13E with	300 mL				
		- Soluvit	0,4 vial	0.0013 vial/mL			
		- Vitalipid infant	10 mL	0.0323 mL/mL			
		- Gluconat	8 mL				
		- Glycophos	2.4 mL				
III	5.88 kg	Morphine	5.88 mg	0.188 mg/mL	0.5 mL/h	10 µg/kg/h	100 h
		Propofol	5 mg	10 mg/mL			0.25 mL (bolus)
		Glucose with	500 mL		20 mL/h		25 h
		- NaCl	35 mmol	0.07 mmol/mL			
		- KCl	5 mmol	0.01 mMol			
IV	6.235 kg	Morphine	6.015 mg	0.120 mg/mL	2.5 mL/h	50 µg/kg/h	20 h
		Dexmedetomidine	0.2 mg	0.04 mg/mL	1.5038 mL/h	1 µg/kg/h	33.25 h
V	6.8 kg	Clonidine	150 µg	10 µg/mL	0.34 mL/h	0.5 µg/kg/h	44.2h
		Fentanyl	1250 µg	25 µg/mL	0.544 mL/h	2 µg/kg/h	91.2 h
VI	6.8 kg	Clonidine	150 µg	8.17 µg/mL	0.34 mL/h	0.5 µg/kg/h	54 h
		Fentanyl	1250 µg	25.53µg/mL	0.544 mL/h	2 µg/kg/h	91-92 h
		Numeta G16E			20 mL/h		
VII	6.8 kg	Paracetamol	100 mg	10 mg/mL	40 mL/h	58.82 mg/kg/h	15 min
		SMOFlipid with	25 mL		1.7 mL/h		20 h
		- Soluvit	0.6 vial	0.0171 vial/min			
		- Vitalipid infant	10 mL	0.2857 mL/mL			
		Vaminolac with	210 mL		10 mL/h		24.22 h
		- $CaCl_2$	3 mmol	1 mmol/mL			

		- KCl	10 mmol	1 mmol/mL			
		- $MgSO_4$	1.2 mmol	1 mmol/mL			
		- Peditrace	6 mL				
		- NaCl	12 mmol	1 mmol/mL			
VIII	9.6 kg	Fentanyl	1250 µg	25 µg/mL	0.384 mL/h	1 µg/kg/h	130.2 h
		Dexmedetomidine	0.5 mg	10 µg/mL	0.384 mg/h	0.1 µg/kg/h	130.2 h
IX	9.7 kg	Fentanyl	1000 µg	50 µg/mL	0.776 mL/h	4 µg/kg/h	25.77 h
		Dexmedetomidine	0.4 mg	4 µg/mL	0.485 mL/h	0.2 µg/kg/h	
X	17.5 kg	Ketamine	200 mg	10 mg/mL	0.35 mL/h	0.2 mg/kg/h	57.14 h
		Rehydrex with glucose 25 mg/mL	500 mL	25 mg/mL	45.45 mL/h		11 h
XI	26 kg	Fentanyl	25 µg	50 µg/mL	0.4 mL/h	1 µg/kg/h	50 t
		Midazolam	20 mg	1 mg/mL	0.6 mL/h	0.03 mg/kg/h	33.33 h
		Propofol	500 mg	10 mg/mL	5.2 mL/h	2 mg/kg/h	9.62 h
		Ringer acetate			15 mL/h		
		Glucose		100 mg/mL	10 mL/h		50 h
XII	27.1 kg	Metronidazole	540 mg	5 mg/mL	2.7 mg/min	13.5 mg/min	40 min
		Heparin	5 IE				flushing
		Morphine	27.1 mg	0.542 mg/ml	1 mL/h	20 µg/kg/h	50 h
XIII	33 kg	Fentanyl	1000 µg	50 µg/mL	0.66 mL/h	1 µg/kg/h	30.3 h
		Propofol	500 mg	10 mg/mL	6.6 mL/h	2 mg/kg/h	7.58 h
XIV	35 kg	Midazolam	20 mg	1 mg/mL	1.4 mg/h	0.04 mg/kg/h	14.29 h
		Propofol	1000 mg	20 mg/mL	3.5 mg/h	2 mg/kg/h	14.29 h
XV	35 kg	Midazolam	20 mg	1 mg/mL	1.4 mg/h	0.04 mg/kg/h	14.29 h
		Dexmedetomidine	0.5 mg	0.01 mg/mL	5.25 mL/h	1.5 µg/kg/h	9.25 h
XVI	70 kg	Fentanyl		50 µg/mL	1.4 mL/h	1 µg/kg/h	
		Propofol	1000 mg	20 mg/mL	7 mL/h	2 mg/kg/h	7.14 h
		Numeta G19E			42.4 mL/h		
		Glucose		100 mg/mL			

Paper I-IV

I

pharmaceutics

MDPI

Article

Y-Site Physical Compatibility of Numeta G13E with Drugs Frequently Used at Neonatal Intensive Care

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Abstract: Preterm neonates require parenteral nutrition (PN) in addition to intravenous drug therapy. Due to limited venous access, drugs are often co-administered with PN via the same lumen. If incompatible, precipitation and emulsion destabilization may occur with the consequent risk of embolism and hyper-immune reactions. Information on intravenous compatibility is scarce. Our aim was to analyse the compatibility of Numeta G13E with paracetamol, vancomycin and fentanyl because of the frequency of their use. A panel of methods was chosen to assess precipitation (sub-visual particle counting, turbidity measurement, Tyndall beam effect and pH measurement) and emulsion destabilization (mean droplet diameter measurement and sub-visual counting of oil droplets, followed by estimation of PFAT5 (percentage of fat residing in globules larger than 5 µm) and pH measurement). Samples in clinically relevant mixing ratios were tested immediately and after 4 h. All samples of drugs mixed with Numeta G13E were compared to unmixed controls. None of the tested drugs precipitated in contact with Numeta G13E, and we did not see any sign of emulsion destabilization when clinically relevant mixing ratios were applied. These results are reassuring. However, when contact time exceeds the established norm, caution in the form of filter utilisation and close inspection is advised.

Keywords: patient safety; parenteral nutrition PN; paracetamol; vancomycin; fentanyl; precipitation; emulsion stability; PFAT5; paediatrics; clinical pharmacy

1. Introduction

Uninterrupted nutrition is of paramount importance in all preterm neonates and infants in the neonatal intensive care unit (NICU). This is because inadequate nutrient supplies are associated with extra-uterine growth restriction, increased frequency and severity of postnatal medical complications resulting from impaired immunity, suppressed motor- and neurodevelopment and severe retinopathy of prematurity [1,2]. Due to temporary gut immaturity, most preterm neonates require parenteral nutrition (PN) as their main nutrient supply, particularly in the immediate postnatal period [3]. However, providing optimal nutrition to this patient group remains a challenge. NICU patients receive 20% less PN than assumed due to a very complex clinical environment [1]. Because of the complex nature of PN containing lipids, amino acids, carbohydrates and added vitamins, trace elements and electrolytes, physical stability is a delicate balance to maintain. It is desirable that PN is administered via a separate catheter lumen to achieve this. Most preterm neonates have a single-lumen central venous catheter (CVC), in the best of cases a three-lumen CVC, or peripherally inserted central catheter

(PICC), which then precludes the simultaneous administration of vital drugs, blood transfusions, blood sampling or central venous pressure monitoring [3]. Pausing the nutrient supply and flushing the i.v. lines prior to and after drug administration is undesirable due to hypervolemia and low fluid capacity. Therefore, drugs are often administered simultaneously with PN via the same catheter lumen after all. This must be regarded as off-label use as co-administration rarely is described in the summary of product characteristics (SmPCs). Co-administration is known to increase the risk of incompatibility reactions between the products [4]. The consequences of incompatibilities between PN and drugs include crystalline particle formation and lipid emulsion destabilization, which may in turn lead to lumen occlusion, oxidative stress, organ defects and, in worst case scenario, emboli formation [4–6]. A tragic example of incompatibility with a lethal outcome are neonatal deaths following concurrent intravenous administration of ceftriaxone and calcium [7]. Both the EMA (European Medicines Agency) and FDA (U.S. Food and Drug Agency) express specific concerns regarding co-administration and incompatibility in their guidelines for the development of drugs to neonates [8,9].

Unfortunately, information about which drugs may be compatible with PN during Y-site administration is very scarce. Staven et al. performed several compatibility tests, assessing both visual and sub-visual particles/fat droplets and found that ampicillin, fosphenytoin and furosemide precipitated when mixed with Olimel N5E, Numeta G16E and a locally compounded preterm mix from Fresenuis Kabi, whereas ceftazidime, clindamycin, dexamethasone, fluconazole, metronidazole, ondansetron and paracetamol were compatible with Olimel N5E and Numeta G16E [10]. However, for the preterm mix, an unexpected micro-precipitation, probably caused by an interaction between copper and cysteine, disturbed the analyses so that none of the drugs could be concluded as compatible [11]. No emulsion destabilization was noted [10,11]. Fox et al. found that caffeine citrate, clindamycin, enalaprilat, epinephrine, fluconazole, fosphenytoin, hydrocortisone, metoclopramide and midazolam were compatible with a locally compounded neonatal lipid-free PN solution for up to 3 h in a simulated Y-site injection. Amiodarone, pentobarbital, phenobarbital, and rifampin were not compatible with the neonatal PN solution. Of note is the fact that only visual examination was performed [12]. Greenhill et al. reported no incompatibilities when mixing calcium gluconate, adrenaline, vasopressin and milrinone with another locally compounded lipid-free PN solution; however, this study was also carried out using drugs and dosages most commonly used in older paediatric patients [13]. Veltri and Lee found that neonatal lipid-free PN solutions with added amino acids were compatible with several drugs, including frequently used antibiotics, such as cefotaxime, penicillin G, and metronidazole, but were incompatible with acyclovir and ampicillin [14]. Both visual examination and Tyndall effect were utilised. Watson analysed the compatibility of 28 antibiotics with a locally compounded lipid-free PN solution for slightly older children weighing between 5 and 30 kg. He measured pH changes and used visual inspection. He found that ampicillin, cefamandole, cephalothin, cephradine and oxacillin led to a pH change in the PN solution (an increase) and ampicillin and cephradine produced a visible precipitate of calcium phosphate [15]. None of these studies were able to examine emulsion destabiliazation, since they excluded the lipid-phase. The results from these studies, whilst contributing to the information pool, are either not updated with respect to the parenteral nutrition currently used at NICUs or not generalisable when combinations with locally compounded PN solutions were tested. In addition, only Staven et al. performed a battery of compatibility tests that would ensure the reliability and reproducibility of their results [10].

The aim of our study was to use the same battery of compatibility tests to analyse the Y-site compatibility of Numeta G13E, to the best of our knowledge the only universal three-in-one PN mixture for premature neonates, with three drugs frequently administered together with it via the same catheter lumen: paracetamol, vancomycin and fentanyl. No documented compatibility information is available for such co-administration.

2. Materials and Methods

2.1. Materials

An overview of Numeta G13E, additives and drug formulations, dilution media and concentrations is presented in Tables 1 and 2. European guidelines and information from products' SmPCs were applied for maximum shelf-life after first opening or reconstitution of drugs, dilution media and PN after mixing and addition of supplements [16].

Table 1. Overview of Numeta G13E and additives.

Product Type	Name	Manufacturer	Lot no.
Three-in-one PN admixture	Numeta G13E	Baxter	17E15N44 16K22N40
Trace elements	Peditrace	Fresenius Kabi	12LBL19 12LFL99
Vitamins—water soluble	Soluvit	Fresenius Kabi	10LF1840 10LK6141
Vitamins—lipid soluble	Vitalipid Infant	Fresenius Kabi	10LA5346 10LH3632

Table 2. Overview of drug formulations, dilution media, and their final concentrations.

Drug	Manufacturer	Lot no.	Dilution Medium	Concentration after Dilution
Paracetamol Excipients: mannitol, sodium citrate trihydrate, glacial acetic acid, aqua purificata pH: 4.5–5.5	B.Braun	17233450	Undiluted	10 mg/mL
Vancomycin Excipients: none pH: not stated	MIP	2725616	Glucose 50 mg/mL	5 mg/mL
Fentanyl Excipients: sodium chloride, hydrochloric acid/sodium hydroxide, aqua purificata pH: 5.0–7.5	Hameln	07400817A	Glucose 50 mg/mL	10 µg/mL

2.2. Selection of Test Materials

Numeta G13E is the three-in-one PN admixture used at our local NICU. An overview of the composition of Numeta G13E is shown in Table S1 in the Supplementary materials.

Bedside observations of drugs used at the local NICU formed the base for selection of drugs to be investigated. Frequently used drugs with an infusion time of 15 min or more combined with pH dependent solubility were regarded as relevant. The final selection was done after discussions with the clinicians, taking their priorities into consideration. Paracetamol, vancomycin and fentanyl were selected for analysis of Y-site infusion with Numeta G13E with additives (from now on referred to as Numeta G13E+ in this manuscript). Trace elements, water-soluble and fat-soluble vitamins are always added to PN admixtures in NICU, and maximum permissible amounts for prematurely born infants of Peditrace (10 mL), Soluvit (3 vials) and Vitalipid infant (30 mL) (as stated by the manufacturer) were added in order to represent the extreme case scenario during co-administration via an intravenous catheter [17]. The composition of each type of additive is shown in Tables S2–S4 in the Supplementary materials.

2.3. Study Design

To simulate the Y-site mixing ratios of Numeta G13E+ and the selected drugs, infusion rates of Numeta G13E and the selected drugs were utilised. The infusion rates for Numeta G13E were calculated based on the daily requirements using the ESPEN/ESPGHAN guidelines for paediatric parenteral nutrition [1], and the volume (mL/day), covering the nutritional requirements for several weight categories (kg) from 0.5 to 10 kg. It is unlikely that neonates in NICU should weigh 5.0 kg or more, but this weight class was included to represent a possible extreme. The 8 h and 24 h infusion regimens were used to calculate the infusion rates (mL/h); again, an 8 h infusion is too fast in the NICU-setting, but was necessary to represent an extreme situation. The drug doses were based on local guidelines and information in the British National Formulary (BNF) for children [18], the concentrations were chosen based on dialogue with clinicians to ensure clinical relevance and mL/kg was calculated for the same weight categories as for Numeta G13E. The infusion rates of the drugs were obtained by observation at NICU and when necessary calculated using the local guidelines at Oslo University Hospital that are based on recommendations in Neofax, BNF for children, Pediatric and Neonatal Dosage Handbook [18–20] and the Norwegian Medicines for Children Network's mixing tables [21]. To simulate a range of potential mixing ratios of Numeta G13E and paracetamol, vancomycin and fentanyl, respectively, that might occur in the infusion line, mixing ratios were calculated by dividing the infusion rate of each drug with the infusion rate of Numeta G13E for each weight category (Figure 1, page 5). The most extreme ratio of Numeta G13E+ > drug as well as the 1 + 1 ratio were chosen. In cases where no ratio of drug > Numeta G13E+ was identified, two mixing ratios with more Numeta G13E+ relative to the drug in question were chosen instead, as shown in Table 3.

Calculation of Numeta G13E infusion rates

1. Nutrients/ mL of Numeta G13E (SmPC)
   - Amount of glucose, lipids, amino acids, kcal, electrolytes, fluids per mL was estimated
2. ESPEN/ ESPGHAN recommended nutrient requirements per kg per day expressed as mL
   - For a 1.0 kg neonate, the corresponding volume/ kg/ day of Numeta G13E was
   - 120 mL/ kg/ day = 120 mL/ day
3. Infusion rates of Numeta G13E (mL/h) for the 8-h and 24-h infusion regimes
   - For a 1.0 kg neonate, 8-h infusion corresponds to 120 mL/ 8h = 15 mL/ h
   - For a 1.0 kg neonate, 24-h infusion corresponds to 120 mL/ 24h = 5 mL/ h

Calculation of vancomycin infusion rates

i. Drug dose per kg (local guidelines at Oslo University Hospital)
   - For a 1.0 kg neonate: 15 mg/ kg = 15 mg total dose

ii. Drug concentration to test (local guidelines at Oslo University Hospital)
   - 5 mg/ mL

iii. Total drug volume
   - For a 1.0 kg neonate: 15 mg/ 5 mg/ mL = 3 mL

iv. Drug infusion time (local guidelines at Oslo University Hospital)
   - 1 h

v. Infusion rate of drug (mL/ h)
   - 3 mL/ 1 h = 3 mL/ h

Estimation of mixing ratio Vancomycin and Numeta G13E

Infusion rate of vancomycin (from v.) / infusion rate of Numeta G13E (from 3.):

a. For the 8-h and 24-h infusion regime of Numeta G 13E:
   - 8-h regime: (3 mL/ h) / (15 mL/ h) = 0.2 ≈ 1 + 5
   - 24-h regime: (3 mL/ h) / (5 mL/ h) = 0.6 ≈ 1 + 2

b. All chosen weight categories were included in calculations
   - 0.5, 0.7, 0.8, 1.0, 2.0, 3.0, 5.0, 8.0, 10.0 kg

c. Resulting in several mixing ratios, and selection of which ones to test
   1 + 1, 1 + 2, 1 + 5

Figure 1. Example of the estimation of mixing ratios for Vancomycin and Numeta G13E.

Table 3. Overview of selected mixing ratios of drug and Numeta G13E+ for Y-site simulation.

Drug	Selected Mixing Ratio Drug + Numeta G13E+
Paracetamol	1 + 1, 1 + 10, 3 + 2
Vancomycin	1 + 1, 1 + 2, 1 + 5
Fentanyl	1 + 1, 1 + 10, 1 + 20

2.4. Sample Preparation

To enable potential precipitate detection, Milli-Q water was used to substitute the lipid constituent of Numeta G13E as suggested by Staven et al. [22]. This admixture will be referred to as *aq*Numeta G13E+ in this manuscript. Only trace elements were added to *aq*Numeta G13E and no vitamins because the water-soluble vitamins discolour the solution and may lead to analytical problems and lipid soluble vitamins are insoluble in water (Table 4).

Table 4. Composition of Numeta G13E after mixing and with all additives per L [23]. *aq*NumetaG13E+ refers to the aqueous phase used in tests for assessment of potential precipitation.

Component (Amount)	Numeta G13E+	*aq*Numeta G13E+
Olive oil (80%) (g)	17.4	-
Soy bean oil [a] (20%) (g)	13.0	-
Glucose monohydrate (g)	127.5	139.7
Alanine (g)	2.2	2.4
Arginine (g)	2.3	2.5
Aspartic acid (g)	1.6	1.8
Cysteine (g)	0.5	0.6
Glutamic acid (g)	2.7	3.0
Glycine (g)	1.1	1.2
Histidine (g)	1.0	1.1
Isoleucine (g)	1.8	2.0
Leucine (g)	2.7	3.0
Lysine (g)	3.0	3.3
Methionine (g)	0.6	0.7
Ornithine (g)	0.7	0.7
Phenylalanine (g)	1.1	1.2
Proline (g)	0.8	0.9
Serine(g)	1.1	1.2
Taurine (g)	0.2	0.2
Threonine (g)	1.0	1.1
Tryptophan (g)	0.6	0.6
Tyrosine (g)	0.2	0.2
Valine (g)	2.1	2.3
Sodium (mmol)	19.1	21.0
Potassium (mmol)	18.0	19.7
Magnesium (mmol)	1.4	1.5
Calcium [b] (mmol)	11.0	12.1
Phosphate [c] (mmol)	11.0	12.1
Acetate (mmol)	20.9	22.9
Chloride (mmol)	27.4	29.5
Malate (mmol)	9.3	10.2
Zinc (mg)	10.0	11.9
Copper (mg)	0.9	1.0
Manganese (mg)	0.04	0.05
Selenium (mg)	0.1	0.1
Fluoride (mg)	2.5	2.7
Iodide (mg)	0.04	0.05
Thiamine mononitrate (mg)	27.0	-

Table 4. *Cont.*

Component (Amount)	Numeta G13E+	*aq*Numeta G13E+
Riboflavin sodium phosphate (mg)	42.6	-
Nicotinamide (mg)	347.8	-
Pyridoxine hydrochloride (mg)	42.6	-
Sodium pantothenate (mg)	143.5	-
Sodium ascorbate (mg)	982.6	-
Biotin (mg)	0.5	-
Folic acid (mg)	3.5	-
Cyanocobalamine (mg)	0.04	-
α-Tocopherol (mg)	55.7	-
Retinol (mg)	6.0	-
Phytomenadione (mg)	1.7	-
Ergocholecalciferol (mg)	0.1	-

a: including soy bean oil from the addition of Vitalipid Infant, b: from calcium chloride dehydrate, c: from sodium glycerol phosphate and the lipid emulsion. Phosphate contribution from Vitalipid Infant is not known.

To test emulsion stability, Numeta G13E+ was mixed with both trace elements and vitamins. Maximum amounts were added according to manufacturers' recommendations to mirror the extreme case scenario in clinical practice (Table 4).

Samples for analysis were all prepared in a laminar airflow hood under ambient laboratory conditions by adding *aq*Numeta G13E+ or Numeta G13E+ to the drug in the three selected mixing ratios. Control samples (unmixed drug and PN) were also prepared in the laminar airflow hood. Samples for precipitate testing (where *aq*Numeta G13E+ was added to the drug) were mixed in 100 × 24 × 1.0 mm flat-bottomed glass tubes (Scherf Präzision Europa GmbH, Germany) for test of Tyndall effect and in sterile 50-mL polypropylene tubes (Corning, Mexico) for particle counting, turbidity and pH measurements. All solutions (not the emulsion) were filtered directly into the container through a sterile 0.22 µm syringe filter (VWR, Radnor, PA, USA) to reduce background particle pollution. In total, six parallels were prepared, each containing the calculated ratio of drug and *aq*Numeta G13E+: three for immediate testing and three for testing after 4 h. Since some of the analyses are destructive, the four-hour analyses could not be performed on the same sample as the immediate. Samples were kept at room temperature until they were analysed. Controls containing Milli-Q water, drug only and *aq*Numeta G13E+ only were also prepared. For turbidity measurements, the samples were transferred to the test glass and the outside was wiped with a glass wipe to remove dust that could influence the measurements.

Likewise, samples for emulsion stability testing were mixed in sterile 50-mL polypropylene tubes (Sigma-Aldrich Química, Toluca, Mexico). Three parallels containing each calculated ratio of drug and Numeta G13E+ (used for both immediate and four-hour analyses), as well as two controls containing Numeta G13E+ were used.

2.5. Analyses

A panel of quality-assured methods and established acceptance criteria was used to assess potential particle formation and emulsion destabilization [22]. Samples were tested immediately after mixing, which in practical terms means within 1 h, and 4 h after mixing. The late time point was added to check for incompatibility that might occur at long contact times due to low infusion rates. All samples were compared with controls described in the section above. With the exception of visual examination and emulsion testing, different samples were required because the tests were destructive.

2.5.1. Methods and Assessment Criteria for the Detection of Potential Particle Precipitation

Sub-visual particle counting was carried out by light obscuration (Accusizer Syringe Injection Sampler, Optical Particle Sizer, PSS NICOMP, Billerica, MA, USA) to find the total number of particles/mL

≥ 0.5 µm, 5 µm, 10 µm, and 25 µm. The sensor was used in summation mode. The accepted background count of Milli-Q-water and sampling tubes was set to be below 100 particles/mL ≥ 0.5 µm. A 15 mL sample was measured undiluted to avoid dissolution of potential precipitate. Criteria: not more than a total of 2000 particles/mL ≥ 0.5 µm [22], and larger particles not exceeding the limits for "large volume parenterals" (not more than 3 particles/mL ≥ 25 µm and 25 particles/mL ≥ 10 µm) [24]. Furthermore, the total number of particles ≥ 5 µm was counted because neonatal capillaries are of approximately that diameter [25].

Turbidity measurement (2100Qis Turbidimeter, Hach Lange GmbH, Duesseldorf, Germany). Criteria: the upper limits are 0.2–0.3 Formazine Nephelometry Units (FNU) [22].

Visual examination against a black background using the fiber optic Tyndall beam (Schott KL 1600 LED, Germany) and red laser pen (630–650 nm, P 3010 RoHS, Chongqing, China). Criteria: no visible signs of precipitation or Tyndall effect [22,26].

pH measurement was carried out using a pH meter (Seven Compact, Mettler Toledo, Greifensee, Switzerland). Criteria: a change in pH >1.0 pH unit could induce the risk of precipitation of a drug and pH > approximately 7.2 could induce the risk of calcium phosphate precipitation [27].

2.5.2. Methods and Assessment Criteria for the Evaluation of Emulsion Stability

The hydrodynamic diameter of the oil droplet and polydispersity index (PDI) of the droplet distribution were measured using dynamic light scattering (Zetasizer nano series, Malvern instruments, Malvern, UK). The Z-average mean size was used as a mean droplet diameter (MDD). Criteria: MDD < 500 nm [28]. Low PDI indicates narrow droplet size distribution; PDI below 0.2 may be regarded as representative of monodisperse samples.

Light obscuration was used to investigate potential droplet growth in the large diameter tail of the droplet sizes of the o/w emulsion. The instrument (Accusizer Syringe Injection Sampler, Optical Particle Sizer, PSS NICOMP, Billerica, MA, USA) was used in extinction mode and the lower detection threshold was 1.80 µm. Samples were diluted to ensure detection of single droplets one at the time. The counts of number of droplets with a diameter (D) > 2 µm, > 5 µm and > 10 µm was derived. The equivalent spherical volumes (ESV; cm^3) of the oil droplets was calculated according to Equation (1) and total spherical volume (TSV; cm^3) was calculated according to Equation (2). Finally, the weighted volume (WV) percentage of lipid droplets > 5 µm (PFAT5) [28], but also 2 µm (PFAT2) and 10 µm (PFAT10) were estimated from Equation (3).

$$ESV = \frac{\pi \times D^3}{6} \quad (1)$$

$$TSV = number\ of\ particles \times ESV \quad (2)$$

$$PFATX = \frac{\left[TSV\left(cm^3\right) \times Density\left(\frac{g}{mL}\right) \times Dilution\ factor\right]}{\left[sample\ volume\ (cm^3) \times Final\ oil\ composition\left(\frac{g}{mL}\right)\right]} \quad (3)$$

The density of oil used in the calculations was 0.92 g/mL, and the final oil composition was 0.030 g/mL (including oil from Vitalipid Infant). Criteria: PFAT5 < 0.40% [29].

pH measurement criteria: emulsion destabilization is more likely to occur at pH values < 5.5 [29].

3. Results

3.1. Detection of Potential Particle Precipitation

The physico-chemical characteristics of the controls used during particle precipitation detection are shown in Table 5. Sub-visual particle counts for *aq*Numeta G13E+ and paracetamol and vancomycin were low and not influenced by time. Tests of controls requiring large sample volume, such as particle counting (40 mL) and extended period of time (4 h), were not performed for fentanyl based on internal routines for drugs containing narcotic controlled substances. All control samples appeared clear upon

visual inspection apart from a weak Tyndall effect in *aq*Numeta G13E+ samples, which is an inherent phenomenon that has been described earlier [26], and a weak Tyndall effect in vancomycin samples. All controls showed low turbidity. pH was in the range given in the SmPC for paracetamol and *aq*Numeta G13E+, but somewhat lower for fentanyl (Table 2 and Table S1). For vancomycin powder for infusion no pH information after reconstitution was given in the SmPC, but after reconstitution in glucose 50 mg/mL (pH 3.5–5.5) the drug showed the most acidic pH at 3.2.

Table 5. Physico-chemical characteristics of unmixed controls of *aq*Numeta G13E+ and drugs (average values ± SD, $n = 3$).

Control	Particles/mL ≥ 0.5 µm		Turbidity (FNU)		Visible Particles or Tyndall Effect (+/−)		pH	
	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h
*aq*Numeta G13E+	12 ± 5	25 ± 32	0.02	0.05	+	+	5.40	5.39
Paracetamol	9 ± 2	12 ± 9	0.03	0.01	−	−	5.24	5.23
Vancomycin	15 ± 9	8 ± 3	0.02	0.06	+	+	3.20	3.19
Fentanyl	N/A [1]	N/A [1]	0.16	N/A [1]	−	−	4.81	N/A [1]

[1] N/A: Controls containing narcotic controlled substances are not routinely performed for large sample volumes or extended periods of time.

The results from precipitation testing are presented in Table 6. Deviations from acceptance criteria are shown in bold. Total particle counts above 0.5 µm, as well as counts for particles larger than 5 µm, 10 µm, and 25 µm, respectively (results not shown), were all low and within the acceptance criteria. The only drug that showed borderline signs of precipitation was fentanyl with FNU nearing 0.2 when *aq*Numeta G13E+ was present in abundance. However, these values did not differ greatly from the average FNU count found for the control samples containing only fentanyl. Other authors suggested using changes < 0.5 NTU (NTU is equivalent to FNU up to 40 NTU [30]) as acceptance limit [11]. All three drugs displayed occasionally weak Tyndall effects independent of mixing ratio with *aq*Numeta G13E+ and the time factor.

Table 6. Results from precipitation testing after mixing drug and *aq*Numeta G13E+ (bold font indicate values outside the acceptance criteria) (average ± SD; $n = 3$).

Drug	Mix Ratio	Particles/mL ≥ 0.5 µm		Turbidity (FNU)		Visible Particles or Tyndall Effect (+/−)		pH	
		0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h
Paracetamol	1 + 1	52 ± 26	78 ± 31	0.01 ± 0.01	0.01 ± 0.01	−	+	5.45 ± 0.02	5.47 ± 0.01
	1 + 10	18 ± 7	11 ± 2	0.08 ± 0.05	0.01 ± 0.02	+	−	5.45 ± 0.01	5.42 ± 0.01
	3 + 2	22 ± 4	9 ± 2	0.01 ± 0.01	0.05 ± 0.03	+	−	5.46 ± 0.04	5.45 ± 0.01
Vancomycin	1 + 1	36 ± 37	12 ± 5	0.00 ± 0.01	0.03 ± 0.03	+	+	5.41 ± 0.02	5.41 ± 0.01
	1 + 2	12 ± 2	12 ± 1	0.01 ± 0.02	0.02 ± 0.01	+	+	5.39 ± 0.01	5.39 ± 0.02
	1 + 5	18 ± 11	20 ± 10	0.01 ± 0.02	0.01 ± 0.01	−	−	5.45 ± 0.01	5.45 ± 0.01
Fentanyl	1 + 1	23 ± 3	16 ± 4	0.12 ± 0.00	0.14 ± 0.01	+	+	5.52 ± 0.01	5.50 ± 0.01
	1 + 10	20 ± 14	21 ± 5	**0.19 ± 0.02**	**0.17 ± 0.04**	−	−	5.39 ± 0.02	5.41 ± 0.01
	1 + 20	13 ± 3	18 ± 7	**0.17 ± 0.03**	0.14 ± 0.01	+	+	5.47 ± 0.02	5.44 ± 0.01

3.2. Evaluation of Emulsion Stability

The results from the emulsion stability analyses of mixed samples are presented in Table 7 with values exceeding the stipulated acceptance criteria or deviations shown in bold. The control sample of Numeta G13E+ showed mean droplet diameter below 270 nm with a narrow droplet distribution as indicated by low PDI. The PFAT5 of 0.2–0.3% confirmed that a low percentage of the lipid can be found in droplets with a diameter of 5 µm or larger.

Table 7. Results from emulsion stability analysis when drug was mixed with Numeta G13E+ (bold font indicate values outside the acceptance criteria) (average ± SD; $n = 3$).

Drug	Mix Ratio	Z-Average (nm)	PDI	%PFAT5		pH	
				0 h	4 h	0 h	4 h
Numeta G13E+	-	**266 ± 1**	0.12 ± 0.01	0.20 ± 0.09	0.28 ± 0.08	5.50	5.50
Paracetamol	1 + 1	236 ± 1	0.15 ± 0.02	0.24 ± 0.08	0.34 ± 0.00	5.56 ± 0.04	5.54 ± 0.02
	1 + 10	240 ± 3	0.12 ± 0.01	0.24 ± 0.21	**0.66 ± 0.36**	5.49 ± 0.03	5.50 ± 0.03
	3 + 2	239 ± 1	0.12 ± 0.01	**0.41 ± 0.18**	**0.57 ± 0.41**	5.50 ± 0.05	5.47 ± 0.04
Vancomycin	1 + 1	241 ± 2	0.12 ± 0.04	**0.49 ± 0.22**	0.29 ± 0.10	5.50 ± 0.02	5.49 ± 0.02
	1 + 2	238 ± 4	0.13 ± 0.02	0.16 ± 0.02	**0.46 ± 0.35**	5.48 ± 0.01	5.47 ± 0.02
	1 + 5	240 ± 1	0.12 ± 0.01	0.17 ± 0.03	0.25 ± 0.05	5.52 ± 0.01	5.50 ± 0.01
Fentanyl	1 + 1	241 ± 2	0.10 ± 0.00	0.16 ± 0.04	0.09 ± 0.04	5.58 ± 0.03	5.59 ± 0.05
	1 + 10	240 ± 1	0.10 ± 0.01	0.13 ± 0.04	0.12 ± 0.04	5.58 ± 0.10	5.54 ± 0.03
	1 + 20	221 ± 1	0.12 ± 0.03	0.23 ± 0.06	0.16 ± 0.03	5.53 ± 0.02	5.47 ± 0.02

For mixed samples, all mean droplet diameters (Z-average values) were slightly lower than the control sample and low PDIs suggested stable emulsions also after mixing with the respective drugs. For Numeta G13E+ with paracetamol, the PFAT5 values increased slightly both with increasing Numeta G13E+ volume in the mixing ratio and proportionately with contact time when Numeta G13E+ was in abundance: mixing ratio 1 + 10. When paracetamol and Numeta G13E+ were almost in equal proportions, in the ratio of 3 + 2, the values of PFAT5 were above the limit irrespective of time. Not surprisingly, the PFAT2 values (the percentage of lipid droplets > 2 µm: results not shown) displayed the same pattern and in the case of the 3 + 2 ratio, so did PFAT10 values (the percentage of lipid droplets > 10 µm: results not shown). pH of the mixed samples remained in the same range as the unmixed Numeta G13E+.

In the case of vancomycin, immediate contact with Numeta G13E+ in the mixing ratio 1 + 1 seemed to produce a value of PFAT5 above the stipulated acceptance criterium. This also occurred in the case of PFAT2 and PFAT10 values (results not shown). This was also noted when vancomycin was mixed with Numeta G13E+ in the ratio 1 + 2; however, here the time factor played a role because immediate contact did not yield PFAT5 values that deviated from the accepted normal. This was also the case for PFAT2 and PFAT10 values (results not shown). pH of the mixed samples was again in the same range as the unmixed Numeta G13E+, even though the vancomycin control sample showed a clearly more acidic pH (Table 5). Fentanyl was not found to have any effect on any of the PFAT values when mixed with Numeta G13E+. Again, no marked changes in pH values were noted for the mixed samples as compared to the unmixed Numeta G13E+ control.

4. Discussion

Our findings are reassuring for clinical use. In summary, none of the tested drugs precipitated when in contact with Numeta G13E, and we did not see any clear signs of emulsion destabilization when clinically relevant mixing ratios were applied.

No positive precipitation results were found when analysing sub-visual particle counts exceeding 0.5 µm/mL, turbidity and pH changes for paracetamol and vancomycin when these drugs were mixed with *aq*Numeta G13E+. These results are in accordance with those reported by Staven et al. [10] and Veltri and Lee [14]. A positive Tyndall effect was noted for paracetamol when mixed with *aq*Numeta G13E+ in equal amounts but only after 4 h contact time and as for the other mixing ratios, a Tyndall effect was seen immediately after mixing. Vancomycin also displayed a positive Tyndall effect when mixed with *aq*Numeta G13E+ both immediately and after 4 h in all but one mixing ratio where *aq*Numeta G13E+ was in abundance. However, the Tyndall effect was also observed in the unmixed controls of vancomycin (Table 5), and since the drug comes as a powder that requires reconstitution before use, the observed Tyndall effect might derive from dissolving the powder. The same has been observed for ampicillin in earlier studies [11]. All visual inspection methods are highly subjective so

data interpretation can be challenging and in general not reproducible, hence, these results need to be interpreted with caution [26]. It can be noted that Staven et al. tested several generic paracetamol products with different excipient compositions and the generic products behaved differently in Tyndall light, where some products showed no Tyndall effect, whereas others had an inherent Tyndall effect and even displayed turbidity results > 0.4 FNU [10,22]. The composition of the paracetamol product in the current study was not similar to any of the products tested in the above-mentioned studies.

Fentanyl's FNU counts came close to our acceptance limit of 0.2–0.3, but other researchers suggest a change between unmixed controls and the mixed samples < 0.5 as the acceptance limit. The fentanyl control (fentanyl not mixed with *aq*Numeta G13E+) displayed similar characteristics, and the effect of background noise must not be underestimated. The number of particles per mL exceeding 0.5 µm was low for all fentanyl mixed samples and well within the acceptance criteria, and no pH changes were noted when fentanyl was mixed with *aq*Numeta G13E+. No other studies performed turbidity measurements on fentanyl, so we lack a comparator. However, Veltri and Lee found fentanyl to be compatible with PN [14]. Furthermore the manufacturer provided test results on Numeta G13E mixed with fentanyl 3.6 µg/mL in the mixing ratio 1 + 10 (drug + PN) and found it compatible [17].

From a theoretical perspective, precipitation of paracetamol and fentanyl after mixing with the aqueous phase of the neonatal TPN mixture is unlikely. Both paracetamol and fentanyl have pKa-values on the basic side with 9.5 and 8.99, respectively [31,32], and the pH of 5.4 for *aq*Numeta G13E+ promotes solubility of both drugs. The pH of both drug products was measured to be close to that of *aq*Numeta G13E+, and since the amino acids provide buffer properties the pH of the mixed samples resembled that of the *aq*Numeta G13E+. Vancomycin is an amphoteric glycopeptide with several pKa-values (2.6, 7.2, 8.6, 9.6, 10.5 and 11.7), but it is freely soluble in water [31], and the particles detected are most likely due to slow dissolution of the powdered drug after reconstitution, as mentioned above.

There was no destabilization of the emulsion when paracetamol and Numeta G13E+ were mixed in equal amounts, neither immediately nor after 4 h. However, when Numeta G13E+ was in abundance, a common scenario in the clinical setting, the PFAT5 values exceeded the acceptance criterium after 4 h contact time. This is not of a great concern as infusions of paracetamol rarely exceed 4 h. This phenomenon was also observed when both paracetamol and Numeta G13E+ were mixed in a 3 + 2 ratio, seemingly irrespective of contact time; however, the PFAT5 value after immediate contact was borderline and only the value after 4 h contact time was obviously higher than the limit of 0.4%. It should be mentioned that the PFAT5 monograph of the USP is intended for injectable lipid emulsions and not for complex PN admixtures that contain electrolytes. The electrolytes carry charges, which may lead to temporary flocculation of the droplets (i.e., loosely connected droplet aggregates) and might explain somewhat large variations (SD) in PFAT5 for some of the drug+PN− samples. Since flocculates may be redispersed easily this is not as serious as the droplet growth that leads to coalescence and phase separation. The latter is not a reversible process.

In the case of vancomycin, immediate contact with Numeta G13E+, when in equal mixing ratios, resulted in an increased PFAT5 value. However, after 4 h, the PFAT5 value returned to normal. Whether this was a chance finding or the effect of time destabilizing the lipid emulsion is difficult to judge. Especially since the time factor seemed to play a role in the destabilization of the lipid emulsion when vancomycin and Numeta G13E+ were mixed in the 1 + 2 ratio. Just like in the case of paracetamol, vancomycin infusion time rarely exceeds 4 h so the deviating findings should not be of clinical significance. Fentanyl and Numeta G13E+ showed no signs of emulsion destabilization, irrespective of mixing ratio and time.

Since the solubility of the selected drugs is promoted by the pH of the neonatal TPN, the drugs may be regarded as low-risk for co-administration with Numeta G13E. Nevertheless, these drugs are frequently used in the NICU and it is important for the clinical environment to obtain documented information supporting their co-administration. Typical high-risk drugs for co-administration with the neonatal Numeta G13E would be drugs with low pKa-values that risk precipitating at the pH-value governed by the TPN. One well-known example is furosemide, which has pKa of 3.8 and has been

shown to precipitate with the Numeta G16E [10], which is indicated for term born neonates and children up to 2 years of age.

On account of there being no generally accepted golden standard to how compatibility between i.v. fluids should be investigated, and the fact that neither the EMA [8] nor the FDA guidelines [9] offer any recommendations, our study was conducted using state of the art, validated methods that have been applied in similar studies in literature [10,12,13,22]. Our results should be interpreted with the following limitations and strengths in mind: Only one person was carrying out the analyses. This may lead to bias, particularly when highly subjective methods such as visual examination and the Tyndall beam effect were used. Due to the static nature of the test set-up when mixing in test tubes (glass and polypropylene), the dynamic Y-site interaction between Numeta G13E and the tested drugs in a clinical setting using syringe pumps could not be recreated. We have therefore no way of certifying that the interaction in a test tube mirrors the one between two flowing liquids in the lumen of an i.v. catheter. On the other hand, we utilized a battery of quality assured test methods and established acceptance criteria in order to make as objective conclusions as possible. Furthermore, background noise was measured and thereby controlled for. Lastly, but by far not of least importance, we utilized clinically relevant drug and PN combinations in clinically relevant mixing ratios as well as extreme ratios in an attempt to cover as many paediatric patient scenarios as possible.

5. Conclusions

Our findings are reassuring. Neither paracetamol, vancomycin nor fentanyl precipitated when in contact with Numeta G13E and the emulsion remained stable when clinically relevant mixing ratios were utilized. Deviations from particle number and stability acceptance criteria did occur albeit on an insignificant scale. However, when contact time between paracetamol or vancomycin or fentanyl and Numeta G13E exceeds the established norm, caution in the form of filter utilization and close inspection is advisable.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4923/12/7/677/s1, Table S1: Composition of Numeta G13E per activated three-in-one 300 mL bag (SmPC, Baxter), Table S2: Composition of Peditrace (SmPC, Fresenius Kabi), Table S3: Composition of Soluvit (SmPC, Fresenius Kabi), Table S4: Composition of Vitalipid Infant (SmPC, Fresenius Kabi).

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ORIGINAL ARTICLE

Co-administration of drugs with parenteral nutrition in the neonatal intensive care unit—physical compatibility between three components

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Abstract

There is a lack of compatibility data for intravenous therapy to neonatal intensive care unit (NICU) patients, and the purpose of this study was to contribute with documented physical compatibility data to ensure safe co-administration. We selected Numeta G13E, the 3-in-1 parenteral nutrition (PN) used at our NICU, together with the frequently used drugs morphine, dopamine and cefotaxime in two- but also three-component combinations. Incompatibility may lead to particle formation (precipitation) and oil-droplet growth (emulsion destabilisation), both which are undesirable and pose a safety risk to already unstable patients. We assessed potential particle formation of three mixing ratios for each combination (always including 1 + 1 ratio) using light obscuration, turbidity and pH measurements combined with visual inspection by focused Tyndall beam. Potential droplet-growth and emulsion destabilisation was assessed by estimating PFAT5 from droplet size measurements and counts, mean droplet diameter and polydispersity index from dynamic light scattering, and pH measurements. Mixed samples were always compared to unmixed controls to capture changes as a result of mixing and samples were analysed directly after mixing and after 4 h to simulate long contact time. None of the samples showed any sign of precipitation, neither in the drug-drug nor in the two- or three-component mixture with PN. Neither did we detect any form of emulsion destabilisation.

Conclusion: Dopamine, morphine and cefotaxime were found to be compatible with NumetaG13E, and it is safe to co-administer these drugs together with this PN in NICU patients.

What is Known:
- *The need for co-administration of drugs and complex PN admixtures occurs frequently in NICU due to limited venous access.*
- *Available compatibility data are scarce and for combinations of more than two components non-existent.*

What is New:
- *Here we report physical compatibility data of two- as well as three-component combinations of frequently used NICU drugs and a 3-in-1 PN admixture.*
- *Co-administration of Numeta G13E with dopamine and morphine, but also with morphine and cefotaxime is safe in NICU.*

Keywords Numeta G13E · Morphine · Dopamine · Cefotaxime · Precipitation · Oil-droplet growth

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Abbreviations

*aq*Numeta G13E	Version of the PN product with lipid phase replaced with water
CVC	Central venous catheter
FNU	Formazine nephelometry units
MDD	Mean droplet diameter
Mw	Molecular weight
NICU	Neonatal intensive care unit
Numeta G13E +	Full version of the PN product with lipid phase and all additives
OUS	Oslo University Hospital
PDI	Polydispersity index
PFAT5	Percentage of fat residing in globules larger than 5 µm
PICC	Peripherally inserted central catheter
PN	Parenteral nutrition
SmPCs	Summary of product characteristics

Introduction

Preterm neonates hospitalised at the neonatal intensive care unit (NICU) need intravenous drug therapy and parenteral nutrition (PN) to ensure survival and proper growth and development. Due to their small size and narrow veins, most preterm neonates only tolerate the insertion of a single or double lumen central venous catheter (CVC) or peripherally inserted central catheter (PICC). Even though, the vascular access technology and culture has evolved, and jugular and brachiocephalic short and large catheters can be inserted under ultrasound guidance [1–3]. Limited venous access is a considerable challenge for the involved health care professionals when they have to administer several drugs, PN and blood transfusions intravenously and often together via the same catheter lumen [4]. Co-administration increases the risk of incompatibility reactions between the infused solutions because of differences in their physicochemical properties, composition and the complex nature of PN [4]. Consequences of incompatibilities between drugs and PN may result in formation of solid particles (precipitation) and oil-droplet expansion (lipid emulsion destabilisation). Resultant lumen occlusion, organ malfunction, oxidative stress and embolus formation have been reported [5–7]. Co-administration is off-label administration, as this practice is almost never described in the summary of product characteristics (SmPCs). This is in addition to the fact that most of the drugs used in NICU are off-label or unlicensed for other reasons [8]. Pausing infusions and flushing the intravenous lines prior to and after administration is a safety recommendation but might be undesirable in neonates due to hypervolemia and low fluid capacity. It is estimated that over 25% of co-administrations in NICU are incompatible and up to 75% are either incompatible or undocumented [4, 9].

Documented information about which drugs and PN may be compatible during Y-site administration is very scarce. There are a couple of retrospective studies reporting fatal embolism after infusion of incompatible drugs [10, 11]. However, compatibility studies cannot be performed in vivo due to ethical reasons; hence, there is a need for in vitro translational studies. Often even in vitro studies only describe combination of two components and the results are derived from analyses performed under predefined and not necessarily clinically relevant conditions [12]. Only a few original research studies on intravenous drug and PN compatibility in neonates have been published. Two studies analysed compatibilities with locally compounded PN, making their findings not generalisable [13, 14]. Nezvalova-Henriksen et al. found that paracetamol, vancomycin and fentanyl were all compatible with Numeta G13E at clinically relevant mixing ratios and infusion times [15]. Hammond et al. concluded that adrenaline, dobutamine, dopamine, morphine and milrinone were compatible with Plasma-Lyte 148 whereas furosemide and midazolam were not [16]. Staven et al. found that ampicillin, fosphenytoin and furosemide showed precipitation when mixed with Olimel N5E and Numeta G16E, whereas ceftazidime, clindamycin, dexamethasone, fluconazole, metronidazole, ondansetron and paracetamol were compatible [17, 18].

The results from these studies, whilst contributing to the information pool, are neither exhaustive nor generalisable and none reports on intravenous compatibility between more than two components at a time. In addition, only Hammond et al. [16], Nezvalova-Henriksen et al. [15] and Staven et al. [19] performed a battery of compatibility tests that would ensure the reliability and reproducibility of their results.

Our aim was to analyse the Y-site compatibility of dopamine, morphine, cefotaxime and Numeta G13E in a two- and three-component system. To the best of our knowledge, no documented compatibility information is available for such co-administration.

Materials and methods

Test materials

Our Neonatal Intensive Care Unit at Oslo University Hospital (OUS) utilises Numeta G13E® (Baxter) when preterm infants need PN. It is a 3-in-1 chamber bag that requires the addition of water-soluble vitamins (Soluvit®, Fresenius Kabi), lipid-soluble vitamins (Vitalipid infant®, Fresenius Kabi) and trace elements (Peditrace®, Fresenius Kabi) to be deemed complete or total. The detailed composition of Numeta G13E and additives used in this study are identical to those used by Nezvalova-Henriksen et al. [15]. In order to test the stability of Numeta G13E in

Table 1 Overview over drug formulations, excipients, dilution media and concentrations

Drug	Excipients	Dilution medium	Final concentration
Dopamine hydrochloride (Takeda) pH: 2.5–4.5 Lot.nr: 11,512,398	Sodium pyrosulphate, sodium chloride, water for injection	Undiluted	2 mg/ml
Morphine hydrochloride (Orion) pH: 3.0–5.0 Lot.nr: 41,210,619	Sodium chloride, hydrochloric acid, water for injection	Glucose 50 mg/ml	0.2 mg/ml
Cefotaxime (Villerton and MIP Pharma) pH: 5.0–7.5 (after dilution) Lot.nr: GNC2039	–	Glucose 50 mg/ml	40 mg/ml

extreme scenarios, the maximum amount of all additives was added according to manufacturer guidelines.

The test drugs, dopamine, morphine and cefotaxime, were selected based on the frequency of use at our NICU. An overview of dopamine, morphine and cefotaxime formulations, their dilution media and concentrations are presented in Table 1.

Study design

To replicate potential mixing ratios between the selected drugs and PN in the catheter, infusion rates were utilised as described by Nezvalova-Henriksen et al. [15]. The amount of PN was based on ESPGHAN nutrition requirements for neonatal and paediatric patients, and the estimates covered bodyweights from 0.5 to 10 kg [20]. Drug doses used to calculate infusion rates were based on national neonatal therapy guidelines and local syringe pump protocols as well as information from Kinderformularium [21]. Calculated mixing ratios selected for two- and three-component mixtures are presented in Table 2. Cefotaxime was mainly tested at high concentrations (40 mg/ml), but a lower concentration (10 mg/ml) was also evaluated in combination with morphine (1 + 1 and 30 + 1) and for the three-component mixture with morphine and PN (1 + 30 + 20, 1 + 9 + 1, 1 + 1 + 1). All mixing ratios are given in volumes of each component.

Preparation of samples

Because the lipid component of a 3-in-1 PN is an emulsion, which makes the admixture milky and opaque, the possibility to detect precipitation is lost. Therefore, the lipid compartment was replaced with Milli-Q water for studies of potential precipitation. This version of PN admixture will be referred to as *aq*Numeta G13E. No vitamins were added since water-soluble vitamins give a strong colour which might influence the analyses, and lipid-soluble vitamins make the solution cloudy or opaque. Only trace elements and electrolytes were added to *aq*Numeta G13E.

For the analysis of emulsion stability, all three chambers of Numeta G13E were mixed and maximum amounts of water-soluble vitamins (15 ml), lipid-soluble vitamins (25 ml), trace elements (15 ml), phosphate (2.5 mmol) and calcium gluconate (3.5 mmol) were added to the bag as specified by the manufacturer. This version will be referred to as Numeta G13E + .

All samples (i.e. mixing ratios of various volumes of drugs and/or PN) and controls were prepared at room temperature and filtered through a 0.22-µm syringe filter (VWR, Radnor, PA, USA), except for lipid containing admixtures. To check reproducibility, three replications of each mixing ratio of drug and PN were prepared and analysed, both for immediate and 4-h sample. Also, the unmixed controls were analysed in replications. All results are reported as mean and standard deviation (SD).

Analyses

In order to assess the physical compatibility, a number of well-established analysis methods were used [22]. Since all analytical methods have their strengths and weaknesses, and incompatibility reactions can present themselves differently, conclusion regarding drug compatibility should not be drawn based on one method alone but be based on supportive

Table 2 Overview of two- and three-component mixtures and mixing ratio of drug + PN, drug + drug and drug + drug + PN

Morphine + PN	Cefotaxime + PN	Dopamine + PN	Cefotaxime + morphine	Dopamine + morphine	Cefotaxime + morphine + PN	Dopamine + morphine + PN
1 + 1	1 + 1	1 + 1	1 + 1	1 + 1	1 + 1 + 1	1 + 1 + 1
1 + 7	9 + 1	1 + 6	1 + 2	1 + 8	1 + 2 + 20	1 + 1 + 10
1 + 39	1 + 20	1 + 56	9 + 1	40 + 1	9 + 1 + 2	1 + 4 + 10
						4 + 1 + 10

information drawn from several methods. All samples were tested immediately after mixing and after 4 h. Controls of unmixed drugs and/or PN admixtures were measured in all analyses and compared to the mixed samples.

Methods for detection of particle precipitation

Samples of drug + drug and drug + *aq*Numeta G13E two- and three-component combinations were analysed for possible particle formation. Sub-visual particle counting was carried out by light obscuration (Accusizer Syringe Injection Sampler, Optical Particle Sizer, PSSNICOMP, Billerica, MA, USA) to estimate the total number of particles/ml of sizes ≥ 0.5 µm, 5 µm, 10 µm and 25 µm, respectively. The acceptance criteria were not more than a total of 2000 particles/ml ≥ 0.5 µm [22], whilst larger particles were not to exceed the limits for "large volume parenterals" of the Pharmacopoeia (not more than 25 particles/ml ≥ 10 µm or not more than 3 particles/ml ≥ 25 µm) [23]. The total number of particles ≥ 5 µm was included because particles in this size range could potentially block capillaries. A limit of not more than 100 particles/ml ≥ 0.5 µm was employed as acceptable background of particles in Milli-Q water and sampling tubes.

Turbidity measurements (2100Qis Turbidimeter, Hach Lange GmbH, Duesseldorf, Germany) required samples to not exceed 0.2–0.3 formazine nephelometry units (FNU) higher than the unmixed control FNU values [22].

Visual examination was used to detect precipitation or colour changes utilising two different light sources. The sample, in flat-bottom tubes, was placed above a fiberoptic Tyndall beam (Schott KL 1600 LED, Germany) and inspected. The sample was also inspected with a red laser pen (630–650 nm, P 3010 RoHS, Chongqing, China) shining perpendicularly through it. A Tyndall effect (i.e. visible red line throughout the sample) was interpreted as identification of particles, even though particles could not be seen with the naked eye. Both analyses were carried out in a dark room against a black background [24].

pH measurements were carried out using a pH metre (Seven Compact, Mettler Toledo, Greifensee, Switzerland). A change of > 1.0 pH unit for mixed samples as compared with the unmixed controls was seen as alarming, and depending on the solubility of the drug, was considered to potentially induce precipitation. For samples with PN, a pH > approximately 7.2 was regarded as alarming, since this could induce the risk of forming poorly soluble calcium phosphate precipitate [25].

Methods for analysing emulsion stability

Two- and three-component mixtures of drug + Numeta G13E + were investigated. Initial signs of destabilisation of an emulsion can be seen as a growth in oil-droplet size detected in the large diameter tail of the droplet size distribution. This was evaluated by droplet counting using light obscuration in extinction mode (Accusizer Syringe Injection Sampler, Optical Particle Sizer, PSS NICOMP, Billerica, MA, USA) and calculating the fraction of the large diameter oil-droplets (PFAT5: percentage of fat residing in globules larger than 5 µm). For details regarding preparation, instrument settings and calculation of PFAT5, please refer to previous papers [15, 18].

Later in the destabilisation process, the mean hydrodynamic diameter of the oil-droplet and polydispersity index (PDI) of the droplet size distribution will increase; therefore, these parameters were measured using dynamic light scattering (Zetasizer nano series, Malvern instruments, Malvern, UK). The Z-average mean size was used as a mean droplet diameter (MDD). According to USP, MDD of injectable emulsions should be < 500 nm [26]. A PDI below 0.2 was regarded as a monodisperse size distribution and hence a stable sample.

Again, pH of the mixed samples was compared to the unmixed controls. pH values below 5.5 reduce droplet repulsion forces and increase the probability of droplet coalescence and thereby emulsion destabilisation [27].

Statistical evaluation

Average and SD were calculated for all results. Compatibility was evaluated based on the overall results from several methods including stated acceptance criteria and controls combined with theoretical assessments based on pH and physico-chemical properties of drugs and TPN. An overall assessment of these factors was considered more appropriate than isolated statistical analysis.

Results

Analyses of potential particle precipitation

In all controls, samples with drug + drug combinations and drug(s) + *aq*Numeta G13E + combinations, both two- or three-component, low sub-visual particle counts were seen immediately after mixing and also after 4 h (Table 3). In the three-component mixture of cefotaxime (40 mg/ml), morphine and *aq*Numeta G13E +, the total sub-visual particle count was slightly increased for all mixing ratios yet well within the acceptance criteria of 2000 particle/ml > 0.5 µm. Importantly, larger particle counts (> 5, 10 and 25 µm) were also well within the limits (data not shown). Of note is that the controls from the same test set also had relatively high sub-visual particle counts.

Table 3 Results from precipitation testing after mixing cefotaxime 40 mg/ml, dopamine 2 mg/ml, morphine 0.2 mg/ml and aqNumeta G13E + in different mixing ratios (bold font indicates values outside acceptance criteria) (average ± SD; $n = 3$)

Drug		Mix ratio	Particles/ml ≥ 0.5 µm		Turbidity (FNU)		pH	
			0 h	4 h	0 h	4 h	0 h	4 h
aqNumeta G13E +		Control	149 ± 114	102 ± 61	0.17 ± 0.06	0.15 ± 0.05	5.71 ± 0.23	5.72 ± 0.20
Morphine		Control	124 ± 78	78 ± 21	0.13 ± 0.02	0.14 ± 0.01	4.55 ± 0.18	4.48 ± 0.09
Dopamine		Control	121 ± 32	69 ± 29	0.13 ± 0.01	0.14 ± 0.01	3.84 ± 0.08	3.87 ± 0.09
Cefotaxime		Control	65 ± 23	93 ± 11	0.17 ± 0.01	0.17 ± 0.01	5.40 ± 0.06	5.36 ± 0.06
Two-component analysis (drug + PN)	Morphine + aqNumeta G13E +	1 + 1	696 ± 92	358 ± 143[a]	0.14 ± 0.01	0.12 ± 0.01	5.92 ± 0.00	6.01 ± 0.04
		1 + 7	528 ± 183	243 ± 110	0.13 ± 0.02	0.12 ± 0.01	5.76 ± 0.07	5.69 ± 0.04
		1 + 39	248 ± 82	282 ± 55	0.13 ± 0.00	0.12 ± 0.02	5.84 ± 0.01	5.87 ± 0.02
	Cefotaxime + aqNumeta G13E +	1 + 1	135 ± 27	94 ± 9	0.30 ± 0.07	0.18 ± 0.02	5.79 ± 0.01	5.76 ± 0.01
		9 + 1	74 ± 25	152 ± 26	0.22 ± 0.02	0.17 ± 0.02	5.77 ± 0.03	5.66 ± 0.03
		1 + 20	191 ± 156	56 ± 13	0.15 ± 0.01	0.14 ± 0.04	5.86 ± 0.01	5.87 ± 0.01
	Dopamine + aqNumeta G13E +	1 + 1	585 ± 236	401 ± 94	0.14 ± 0.04	0.12 ± 0.01	5.67 ± 0.01	5.69 ± 0.01
		1 + 6	724 ± 228	400 ± 155	0.13 ± 0.02	0.13 ± 0.02	5.77 ± 0.02	5.78 ± 0.01
		1 + 56	434 ± 97	252 ± 90	0.13 ± 0.02	0.13 ± 0.02	5.81 ± 0.01	5.81 ± 0.01
Two-component analysis (drug + drug)	Cefotaxime + morphine	1 + 1	38 ± 18	51 ± 5	0.14 ± 0.03	0.13 ± 0.04	5.20 ± 0.02	5.16 ± 0.06
		1 + 2	42 ± 36	59 ± 50	0.18 ± 0.04	0.20 ± 0.02	4.88 ± 0.33	4.90 ± 0.20
		9 + 1	103 ± 32	190 ± 59	0.13 ± 0.03	0.14 ± 0.01	5.29 ± 0.01	5.07 ± 0.03
	Dopamine + morphine	1 + 1	59 ± 68	88 ± 35	0.13 ± 0.01	0.13 ± 0.01	4.08 ± 0.02	4.04 ± 0.01
		1 + 8	126 ± 29	68 ± 13	0.13 ± 0.01	0.13 ± 0.01	4.31 ± 0.01	4.29 ± 0.01
		40 + 1	58 ± 21	113 ± 65	0.14 ± 0.00	0.14 ± 0.00	3.82 ± 0.04	3.77 ± 0.04
Three-component analysis (drug + morphine + PN)	Dopamine + morphine + aqNumeta G13E +	1 + 1 + 1	10 ± 3	14 ± 2	0.13 ± 0.01	0.13 ± 0.03	5.87 ± 0.03	5.95 ± 0.01
		1 + 1 + 10	127 ± 51	102 ± 32	0.18 ± 0.03	0.17 ± 0.03	5.68 ± 0.02	5.66 ± 0.01
		1 + 4 + 10	172 ± 32	134 ± 62	0.15 ± 0.01	0.14 ± 0.01	5.85 ± 0.01	5.85 ± 0.01
		4 + 1 + 10	114 ± 63	77 ± 58	0.17 ± 0.03	0.16 ± 0.03	5.80 ± 0.01	5.80 ± 0.01
	Cefotaxime + morphine + aqNumeta G13E +	1 + 1 + 1	241 ± 143	440 ± 156	0.09 ± 0.02	0.11 ± 0.03	5.74 ± 0.01	5.71 ± 0.02
		1 + 2 + 20	362 ± 87	719 ± 282	0.16 ± 0.01	0.21 ± 0.07	5.81 ± 0.03	5.77 ± 0.01
		9 + 1 + 2	1120 ± 662	678 ± 183	0.13 ± 0.04	0.15 ± 0.03	5.61 ± 0.01	5.60 ± 0.00

[a]Result is based on two parallels

All controls and mixed samples showed low turbidity (Table 3). Slightly elevated turbidity in samples of cefotaxime with morphine were detected, but the values were within the acceptance criteria.

Upon visual inspection, none of the samples showed any signs of precipitation. However, *aq*Numeta G13E + itself (control) showed signs of a weak inherent Tyndall effect, which could also be seen in mixtures with the drugs. Reconstituted cefotaxime (control) had a weak yellow colour and gave rise to a weak Tyndall effect which could be traced to some samples when mixed with *aq*Numeta G13E + .

When it comes to pH, no alarming changes were seen for any of the mixed samples during the analysis time range of 4 h, and the pH values of the mixtures were found to mirror the unmixed controls (Table 3).

In addition to the main test design, cefotaxime was analysed using a lower drug concentration (10 mg/ml) in a two-component combination of cefotaxime with morphine and in a three-component combination with morphine and aqNumeta G13E + . All these samples were stable and within acceptance criteria in all analyses for both two- and three-component mixtures (data not shown).

Analyses of potential emulsion destabilisation

PFAT5 values are presented in Table 4, and in most combinations PFAT5 was below the recommended limit for parenteral nutrition (PFAT5 < 0.4%) [27]. Only two mixing ratios showed slightly increased PFAT5 results but only slightly above the threshold. This was in a sample of dopamine and Numeta G13E + (1 + 56) at both time points and in a sample of cefotaxime and Numeta G13E + (1 + 1) after 4 h.

All mixed combinations of two as well as three components showed low and stable mean droplet diameter in the range of 240 to 280 nm (Z-average) and small polydispersity indexes. The variations observed can be traced back to differences between the PN bags (batches) used in the test set. The pH values of mixed samples were similar to the unmixed control of Numeta G13E + (Table 4).

Table 4 Results from emulsion stability analyses when drug was mixed with Numeta G13E + (average ± SD; $n = 3$)

Drug		Mix ratio	Z-average (nm)	PDI	%PFAT5		pH	
					0 h	4 h	0 h	4 h
Numeta G13E +		Control	248 ± 2	0.13 ± 0.02	0.23	0.12	5.80 ± 0.07	5.79 ± 0.05
Two-component analysis (drug + PN)	Morphine + Numeta G13E +	1 + 1	249 ± 1	0.13 ± 0.02	0.19 ± 0.03	0.19 ± 0.01[a]	5.92 ± 0.01	5.90 ± 0.01
		1 + 7	249 ± 2	0.13 ± 0.01	0.29 ± 0.05	0.20 ± 0.05	5.81 ± 0.02	5.84 ± 0.03
		1 + 39	247 ± 2	0.15 ± 0.03	0.31 ± 0.08	0.25 ± 0.05	5.79 ± 0.01	5.79 ± 0.01
	Cefotaxime + Numeta G13E +	1 + 1	252 ± 2	0.13 ± 0.02	0.28 ± 0.04	0.41 ± 0.05*	5.71 ± 0.01	5.66 ± 0.02
		9 + 1	248 ± 2	0.14 ± 0.03	0.24 ± 0.09	0.15 ± 0.15	5.84 ± 0.01	5.86 ± 0.01
		1 + 20	249 ± 2	0.12 ± 0.01	0.39 ± 0.04	0.24 ± 0.02	5.84 ± 0.02	5.83 ± 0.02
	Dopamine + Numeta G13E +	1 + 1	248 ± 1	0.13 ± 0.02	0.24 ± 0.03	0.23 ± 0.05	5.86 ± 0.01	5.89 ± 0.01
		1 + 6	247 ± 2	0.15 ± 0.02	0.25 ± 0.02	0.22 ± 0.03	5.67 ± 0.01	5.66 ± 0.03
		1 + 56	248 ± 2	0.13 ± 0.02	0.42 ± 0.09*	0.46 ± 0.06*	5.81 ± 0.02	5.83 ± 0.03
Three-component analysis (drug + morphine + PN)	Dopamine + morphine + Numeta G13E +	1 + 1 + 1	248 ± 2	0.14 ± 0.02	0.05 ± 0.01	0.16 ± 0.03	5.90 ± 0.01	5.87 ± 0.01
		1 + 1 + 10	248 ± 3	0.13 ± 0.02	0.07 ± 0.01	0.16 ± 0.01	5.81 ± 0.01	5.81 ± 0.01
		1 + 4 + 10	249 ± 2	0.12 ± 0.01	0.12 ± 0.04	0.20 ± 0.08	5.82 ± 0.01	5.86 ± 0.01
		4 + 1 + 10	249 ± 3	0.12 ± 0.01	0.08 ± 0.03	0.19 ± 0.06	5.82 ± 0.03	5.80 ± 0.01
	Cefotaxime + morphine + Numeta G13E +	1 + 1 + 1	240 ± 2	0.09 ± 0.03	0.05 ± 0.01	0.03 ± 0.01	5.69 ± 0.02	5.66 ± 0.01
		1 + 2 + 20	272 ± 31	0.20 ± 0.07	0.19 ± 0.04	0.15 ± 0.06	5.81 ± 0.03	5.81 ± 0.03
		9 + 1 + 2	279 ± 2	0.12 ± 0.01	0.06 ± 0.01	0.03 ± 0.00	5.45 ± 0.02	5.44 ± 0.01

[a]One parallel/sample was contaminated and was excluded

*Values outside the acceptance criteria

Discussion

We can conclude that there were no signs of particle precipitation nor emulsion destabilisation in simulated co-administration of dopamine, morphine and cefotaxime with Numeta G13E, either in drug + drug combination or in a two- or three-component mixture with Numeta G13E in our study.

Most compatibility studies involving morphine have been done using morphine sulphate [28]. However, morphine products available in the local NICU (as in the rest of Scandinavia) are morphine hydrochloride. Morphine (Mw 285.3 g/mol) has a pKa of 8.21 [29], and the main difference between morphine sulphate (Mw 668.8 g/mol) and morphine hydrochloride (Mw 321.8 g/mol) is the different aqueous solubility (1:15.5 and 1:17.5, respectively). To the best of our knowledge, no other studies have investigated the compatibility of morphine hydrochloride with dopamine or cefotaxime nor with 3-in-1 PN admixtures. Trissel et al. studied the physical compatibility of morphine sulphate 15 mg/ml and 1 mg/ml, and found that the high concentration was incompatible with nine parenteral nutrition formulations (emulsion destabilisation) whereas morphine sulphate 1 mg/ml was compatible with all PN in their study [30]. As the current study addresses neonates, a clinically relevant morphine concentration of 0.2 mg/ml was used. The finding that low concentration of morphine hydrochloride is compatible with Numeta G13E supports the hypothesis that morphine could have a concentration dependent emulsion destabilisation effect [30]. When it comes to potential precipitation, Trissel et al. used a test setup where the lipid components were removed by centrifugation [30]. However, Staven et al. has shown that a similar setup left traces of lipids and surfactants in the aqueous phase which interfered with light obscuration and turbidity measurements [22]. Therefore, Staven's and our assessments of potential precipitation were performed after substituting the liquid volume of the lipid phase with water. Neither Staven's nor our study showed signs of precipitation.

Samples of dopamine 2 mg/ml mixed with *aq*Numeta G13E were compatible and showed low turbidity, low subvisual particle count and stable pH. Trissel et al. on the other hand found dopamine 3.2 mg/ml to be incompatible with two of the central line PN formulations whereas seven other PN formulations were found compatible [30]. It is difficult to make direct comparisons since a different test setup was used. The three-component mixture of dopamine, morphine and *aq*Numeta G13E did not reveal any surprises after finding the two-component mixtures compatible; this was also compatible. When it comes to emulsion stability, there was one mixing ratio (1 + 56) of dopamine and Numeta G13E + that showed slightly elevated PFAT5 values. Strictly interpreted, this would be an indication of droplet growth and the beginning of emulsion destabilisation. However, the average values observed for these samples were very close to the acceptance limit of 0.4% suggested by Driscoll et al. [27]. Moreover, the

three-component mixture of morphine, dopamine and Numeta G13E was found to be compatible in all mixing ratios, which suggests that the slight increase in PFAT5 in the one mixing ratio of the two-component combination could be a reversible aggregation of droplets rather than droplet coalescence [18]. Baptista et al. analysed emulsion stability by visual observation and did not see any disruption of the emulsion after mixing dopamine and PN [31].

Cefotaxime was tested in two concentrations, 40 mg/ml and 10 mg/ml, since both are frequently used in the NICU. With a battery of methods, all measurements were found to be within acceptance limits; thus, we concluded that cefotaxime is compatible with *aq*Numeta G13. This finding is in line with the conclusion of Trissel et al. who tested cefotaxime 20 mg/ml with nine different parenteral nutrition bags included in their study [30]. Cefotaxime possessed a slight, inherent Tyndall effect after reconstitution, even though the solutions were filtered 0.22 µm as part of preparation. The same weak Tyndall effect could also be seen when cefotaxime was mixed with morphine and *aq*Numeta G13E. Since cefotaxime did not reveal any signs of incompatibility with *aq*Numeta G13E in the other analyses performed in this study, it was assumed to be an effect of colour disturbance. When it comes to emulsion stability, a slightly elevated PFAT5 was found for the 4-h sample of one mixing ratio for cefotaxime with Numeta G13E + . Again, the three-component mixture did not show any increases in PFAT5, and therefore, this was not assumed to be a sign of destabilisation upon mixing.

An interesting study analysed retrospective and prospective data on drug administration of drugs and evaluated the compatibility of frequent combinations in the PICU of an Indonesian hospital [32]. Hanifah et al. explored the compatibility by looking at the single time of administration (STA) approach where bolus and intermittent drugs are given consecutively, but also together via three-way connector, through the single lumen peripheral catheter. They found that three infusions typically met sequentially and have the potential to interact. The most frequent combinations identified included some of the drugs in the current study, namely, triple combinations with morphine and dobutamine, where we studied dopamine. Moreover, Hanifah has rebuilt the infusion model with the tubing and connectors used in the clinic area in the laboratory and monitored what came out [33]. This interesting setup should be further employed.

Our results showed that the studied combinations were compatible for the specific drug products when using drug concentrations and infusion rates clinically used in the neonatal patient. It should be kept in mind that drug products from different manufacturers can have different formulations and excipients, and that both factors can influence compatibility [19]. Altogether, our results indicate that the emulsion of Numeta G13E is stable upon contact with morphine, dopamine and cefotaxime up to 4 h and no formation of precipitate should be expected; hence, co-administration of two- or three-component combinations of these drugs and PN should be safe.

Our results should be interpreted with the following limitations in mind. Only one person performed the analyses of each test set, which could, especially in the case of visual examination, have been subjective. All samples were prepared, stored and analysed at room temperature, but in the neonatal intensive care setting the drugs could be exposed to higher temperature within the neonatal ward and because of the incubators that are keeping the newborn body temperature stable. This could affect the stability of the drugs negatively, e.g. precipitation of poorly soluble calcium phosphate may increase with increased temperature [34]. Effects of incubator temperatures are not captured in the current study. The simulated Y-site compatibility analysis was performed in test tubes whereas the drugs and PN are in reality co-infused and meet in the catheter line. The liquid dynamics could introduce effects that are not accounted for in test tubes. However, since several mixing ratios were evaluated using several different analysis methods that support the same conclusions, the findings account for considerable variation and are assumed to be robust.

Conclusions

The results of this study indicate that Numeta G13E should be compatible in co-infusion with morphine, dopamine and cefotaxime, respectively, but also in three-component infusions together with morphine + dopamine and morphine + cefotaxime. In addition, the drug + drug combinations of morphine + dopamine and morphine + cefotaxime were compatible. These findings are reassuring and contribute to safe and effective administration of drugs in the same catheter line as Numeta G13E in the neonatal intensive care patient.

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Authors' Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Niklas Nilsson and Ingebjørg Storesund. The first draft of the manuscript was written by Niklas Nilsson and Katerina Nezvalova-Henriksen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval This is an in vitro experimental study involving no human or animal subjects. The study is performed according to ethical standards for laboratory experiments.

Consent to participate Not applicable.

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Conflict of interest The authors have no relevant financial or non-financial interests to disclose. The Hospital Pharmacy Enterprise had no impact on the design and results reported in this study. Niklas Nilsson and Dr. Katerina Nezvalova-Henriksen work as clinical pharmacists at Oslo University Hospital, Rikshospitalet, consulting in questions regarding medication of neonatal and paediatric intensive care patients.

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RESEARCH REPORT

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Exploring a case of incompatibility in a complex regimen containing Plasma-Lyte 148 in the pediatric intensive care

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Abstract

Background: In the local pediatric intensive care unit, precipitation was observed in the intravenous catheter upon co-administration of four drugs together with the buffered electrolyte solution (Plasma-Lyte 148, Baxter). Co-infusion of incompatible combinations represents a safety concern.

Aims: To reproduce the clinical case of precipitation. To further explore and understand the risk of precipitation, different combinations of the components as well as the corresponding electrolyte solution with 5% glucose (Plasma-Lyte 148 with 5% glucose) should be investigated.

Methods: Physical compatibility of fentanyl, ketamine, midazolam, and potassium chloride was tested in combination with the buffered electrolyte solutions. The concentrations and infusion rates representative of children 10–40 kg were used to estimate mixing ratios. Analyses detecting visual particles (Tyndall beam) and sub-visual particles (light obscuration technology) were undertaken. Measured turbidity and pH in mixed samples were compared with unmixed controls.

Results: Both midazolam and ketamine showed formation of visual and sub-visual particles upon mixing with Plasma-Lyte 148, respectively. Particle formation was confirmed by increased turbidity and a distinct Tyndall effect. pH in mixed samples mirrored the pH of the buffered electrolyte, suggesting that the solubility limits of midazolam, and in some ratios also ketamine, were exceeded. Midazolam also precipitated in combination with the glucose-containing product that held a lower pH, more favorable for keeping midazolam dissolved.

Conclusions: Replication of the case revealed that both midazolam and ketamine contributed to the precipitation. Midazolam and ketamine were both evaluated as incompatible with the buffered electrolyte solution and midazolam also with the buffered electrolyte-glucose solution and should not be co-administered in the same i.v.-catheter line. Fentanyl and potassium chloride were interpreted as compatible with both buffered electrolytes.

KEYWORDS
fentanyl, ketamine, midazolam, pH, plasmalyte, precipitate

1 | INTRODUCTION

Intravenous crystalloid fluids are widely used in the treatment of the pediatric intensive care patients for the replenishment of intravascular volume and restoration of hemodynamic stability.[1] The buffered electrolyte solution (Plasma-Lyte 148, Baxter) and the buffered electrolyte-glucose solution (Plasma-Lyte 148 with 5% Glucose, Baxter) are two relatively new crystalloids that have recently been introduced in our local pediatric intensive care unit (PICU). These balanced fluids have high buffer capacity and have similar composition regarding electrolytes (Table 1), but only the one without glucose is isotonic with similar osmolarity as in extracellular compartment fluids.[2] The two buffered electrolytes also differ in product pH (Table 1). Buffered electrolyte solutions are used to provide daily needs of water, glucose and essential electrolytes and have been reported to reduce the incidence of hyperchloremia and metabolic acidosis compared with normal saline.[3] In buffered electrolyte solutions, some of the chloride anions have been replaced with buffers to reduce the acid–base balance disturbances. This is achieved by a lower chloride content that more closely matches that of human plasma through the substitution of the chloride ion with an anion, such as lactate, acetate, or gluconate.[4] These buffers are rapidly metabolized and excreted.

Pediatric intensive care patients are often in need of numerous intravenous (i.v.) drugs and the number of i.v. drugs and fluids often outnumbers the number of i.v. access ports. Co-infusion of incompatible drugs and fluids via the same catheter line and/or lumen may result in the formation of solid particles, that is, drug precipitates.[5,6] These precipitates may clot the i.v.-catheter line and find their way into organs.[7–9] There have been reports of fatal outcome in children due to infusion of solid particles.[10,11] Documented compatibility information is scarce, especially for the pediatric population, and most studies only investigate the stability of two drugs in 1 + 1 ratio.[12] To the best of our knowledge, no compatibility studies involving buffered electrolytes in a complex therapy regime are available.

We present here a case of precipitation observed in the infusion line of a critically ill patient, with a body weight of 16 kg, who received a complex infusion regime containing the buffered electrolyte solution Plasma-Lyte 148. The buffered electrolyte solution (10.83 ml/h) was co-administered in the same i.v.-catheter line (22 Gauge) with fentanyl (50 μg/ml, 0.64 ml/h), ketamine (10 mg/ml, 4.8 ml/h), potassium chloride (1 mmol/ml, 0.2 ml/h), and midazolam (5 mg/ml, 3.2 ml/h). The nurses noted that the syringe pump was alarming due to high pressure, and visual inspection of the i.v.-catheter line clearly showed signs of precipitation. Different actions were undertaken; first, the ketamine-infusion was transferred to one of the other lumen of the patient's i.v.-catheter lines (22 Gauge), but still a white precipitate could be observed in the catheter. It was first when the buffered electrolyte solution was stopped, that no precipitation could be seen in the catheter-line. In order to keep rehydrating the patient, the fluid was changed to the corresponding product with 5% glucose.

The aim of the current study was to investigate the precipitating factors in our clinical case and to explore and understand the risk of precipitation using buffered electrolytes in a complex therapy regime. Other clinically relevant mixing ratios and scenarios were included in the study to warrant safe use of both buffered electrolyte solutions with and without 5% glucose.

What is already known about the topic

Plasma-Lyte 148 and Plasma-Lyte 148 with 5% glucose are commonly used in pediatric intensive care, often co-administered in the same intravenous catheter as drugs and other fluids. Information on their compatibility is very limited, especially for use in a complex therapy regime.

What new information this study adds

Midazolam and ketamine were found to be incompatible with Plasma-Lyte 148 and midazolam also with Plasma-Lyte 148 with 5% glucose. This information contributes to preventing precipitation in the catheter and thereby prohibit possible occlusion and infusion of large particles.

2 | METHODS

The buffered electrolytes investigated were Plasma-Lyte 148 (Baxter, Oslo, Norway) and the corresponding product Plasma-Lyte 148 with 5% glucose (Table 1). An overview of the drug products used in this study, their composition and physico-chemical properties is presented in Table 2.

Prior to simulating the case, each of the drugs was tested separately with the buffered electrolyte to identify if any of the drugs were incompatible with the fluid in a simple system (Table 3). Since drugs and fluids in the clinical setting are given at different infusion rates based on the required dose (per kg body weight) and the concentration of the drugs, different ratios of drugs and fluids could potentially meet in the i.v.-catheter line. In order to test clinically relevant pediatric mixing ratios (children with body weights from 10 to 40 kg), doses and infusion rates were calculated for each drug and fluid using information from UpToDate[13] and Koble.[14] Mixing ratios were calculated as earlier described by Nezvalova-Henriksen et al.[5] Table 3 summarizes the mixing ratios tested. In two-component drug+fluid combinations, equal parts (1 + 1) were tested in addition to one ratio where the fluid was in excess. None of the calculations resulted in mixing ratios containing more drug than fluid.

As midazolam was identified early as incompatible, two-component mixtures (same mixing ratios) were also tested with the buffered electrolyte-glucose solution. Midazolam was further investigated in a three-component mix where sterile water was used to mimic the volume of the other drugs in a simplified simulation of the case. These simulations were done for both types of buffered electrolytes. To replicate the case, all involved drugs were mixed in concentrations and volumes that were an exact representation of the ratio

TABLE 1 Composition and physicochemical properties of the two buffered electrolyte products (P = Plasma-Lyte 148 and PG = Plasma-Lyte 148 with 5% glucose, Baxter)

Ingredients	P	PG
Glucose monohydrate (g/L)	–	55.00
Sodium chloride (g/L)	5.26	5.26
Potassium chloride (g/L)	0.37	0.37
Magnesium chloride hexahydrate (g/L)	0.30	0.30
Sodium acetate trihydrate (g/L)	3.68	3.68
Sodium gluconate (g/L)	5.02	5.02
Amounts		
Na^+ (mmol/L)	140	140
K^+ (mmol/L)	5.0	5.0
Mg^{++} (mmol/L)	1.5	1.5
Cl^- (mmol/L)	98	98
Acetate (mmol/L)	27	27
Gluconate (mmol/L)	23	23
Osmolarity (mosmol/L)	approx. 295	approx. 572
pH	approx. 7.4 (6.5–8.0)	4.0–6.0

administered to the patient (Table 3). The mix of five components was studied for Plasma-Lyte 148, the corresponding product with glucose, and finally, also one mix where the midazolam was replaced with sterile water. The intention with the two latter was to check if the formation of a precipitate could be pH-mediated and to clarify if midazolam alone was responsible for the precipitation in the complex regime.

2.1 | Sample preparation

All products were used as they were received from the pharmacy. Mixed samples were prepared by extracting the desired volume of each product and filtering the solution through a 0.2 μm sterile syringe filter (VWR) into sample tubes (15 ml; Coning). Aliquots of unmixed products were used as controls. All samples and controls were analyzed in triplicates, except controls of narcotics, which were only measured in one parallel. Samples were analyzed immediately and 4 h after mixing. The time points were chosen to detect the immediate formation of precipitation whereas the 4-h time point was chosen to study whether the potential precipitate formed increased or was dissolved covering situations with very slow infusion rates. All samples were prepared, stored, and analyzed at ambient temperature.

2.2 | Analysis of potential particle formation (precipitation)

A number of well-established methods was used to scrutinize for any sign of particle formation in the mixed samples.[5,6,15] The smallest capillaries in the body have a diameter of approximately 5 μm. Particles in this range are sub-visible and cannot be detected by visual examination. Sub-visible particle counts were analyzed using light obscuration (Accusizer Optical Particle Sizer with Syringe Injection Sampler, PSSNICOMP, Billerica, MA, USA). The total number of particles/ml was assessed. The particle counts were divided in particles/ml for particle sizes >0.5, >5, >10, and >25 μm. Particles with a size of 5 μm and above were of main concern. The two upper limits were included since these are used by the USP where large-volume parenterals (i.e., infusions) should not contain more than 25 particles/ml larger than 10 μm and not more than 3 particles/ml larger than 25 μm.[16] In addition, a high number of very small particles may also be alarming since they can grow in size over time; therefore, an acceptance criteria in this study was set to contain no more than 2000 particles/ml larger than 0.5 μm.[15]

Increased turbidity or haze could be a sign of microprecipitation. Turbidity was measured for all mixed samples and all controls using the portable 2100Qis Turbidimeter (Hach Lange GmbH). The acceptance criteria were that mixed samples should not deviate by more than 0.2–0.3 Formazine Nephelometry Units (FNU) from the turbidity of the unmixed control samples.[15]

Since the drugs are salts of weak bases, their solubility will depend on pKa of the parent base and the pH of the sample. The fact that the electrolyte solution also contains a buffer can challenge the theoretical assessment of solubility; hence, pH measurements (Seven Compact pH-meter, Mettler Toledo) of mixed samples compared to unmixed controls is a very useful tool. A pH-shift of more than 1.0 pH unit in the mixed sample as compared to unmixed control should be regarded as alarming.

Large particles (>50 μm) can be captured by visual examination. Also, microprecipitates can be observed with the naked eye if one uses a focused Tyndall beam. Visual observation was performed in two ways, firstly, by shining a focused light beam (Schott KL 1600 LED, Germany) through the mixed samples, comparing them with the unmixed controls, and, secondly, by passing a 630–650 nm laser beam (P 3010 RoHS, Chongqing, China) through the samples and controls. The mixed sample should be free from visually observed particles and with no Tyndall effect (visible coherent laser line through the sample).

3 | RESULTS

3.1 | Compatibility with buffered electrolyte solution (Plasma-Lyte 148)

In the case of midazolam, all analyzes indicated precipitation with values exceeding acceptance criteria. The detector of the particle counter was overloaded and could not detect each particle individually without dilution of the sample (which could not be performed due to the scope of the test as this would dissolve the particles). Precipitation was observed with the naked eye and high turbidity values were recorded. The pH in the mix shifted from approx. 3.5 in midazolam (unmixed) to around 5.1 for the mixing ratio 1 + 1.

Ketamine showed a low particle count for particles of all sizes immediately after mixing. However, 2 h (data not shown) and 4 h

TABLE 2 Overview of drug product information (manufacturer information) and physico-chemical information

Drug product (manufacture)	Active ingredient[a]	Excipients[a]	pH product[a]	Active ingredient[b]	
				pKa	Solubility
Fentanyl 50 µg/ml (Hameln)	Fentanyl citrate	Sodium chloride, hydrochloric acid or sodium hydroxide, water for injection	5.0–7.5	8.43	0.74 mg/ml
Potassium chloride 1 mmol/ml (B. Braun)	Potassium chloride	Water for injection	4.5–7.5	–	>100 mg/ml
Ketalar 10 mg/ml (Pfizer)	Ketamine hydrochloride	Benzethonium chloride, sodium chloride, water for injection	3.5–5.5	7.5	0.046 mg/ml
Midazolam 5 mg/ml (B. Braun)	Midazolam hydrochloride	Sodium chloride, hydrochloric acid 10%, water for injection	2.9–3.7	6.6	0.1 mg/ml
Sterile water (Fresenius Kabi)	Water for injection	–	6–7	–	–

[a]Summary of Product Characteristics.

[b]Parent compound (weak acid or base) obtained from PubChem.

TABLE 3 Overview of controls and tested mixing ratios for drug(s) + fluid(s)

Drugs and fluids	Concentration	Control
Fentanyl (F)	50 µg/ml	x
Ketamine (K)	10 mg/ml	x
Midazolam (M)	5 mg/ml	x
Plasma-Lyte 148 (P)	–	x
Plasma-Lyte 148 with 5% Glucose (PG)	–	x
Potassium chloride (KCl)	1 mmol/ml	x
Sterile water for injection (SW)	–	x

Drug + Fluid(s)	Components mixed	Mixing ratios
F + P	2	1 + 1 and 1 + 20
K + P	2	1 + 1 and 1 + 3
M + P	2	1 + 1 and 1 + 19
M + PG	2	1 + 1 and 1 + 19
KCl + P	2	1 + 1 and 1 + 12
M + SW + P	3[a]	1 + 2 + 3
M + SW + PG	3[a]	1 + 2 + 3

Drug(s) + Fluid(s)	Components mixed	Mixing ratios
F + KCl + K + M + P	5[b]	3 + 1 + 24 + 16 + 55
F + KCl + K + M + SW	5[b]	3 + 1 + 24 + 16 + 55
F + KCl + K + M + PG	5[b]	3 + 1 + 24 + 16 + 55

[a]Simplified simulation of clinical case.

[b]Replication of exact ratios from the clinical case.

after mixing, the detector was overloaded for the 1 + 1 ratio, and single-particle number could not be reported. However, there did not seem to be larger particles >5 µm in the mix. Elevated turbidity values (over the acceptance criteria) and clear signs of particles could be detected in the visual examination with Tyndall beam. The pH in the mixed samples was around 6.0 whereas that of the ketamine control was 4.6. In the ketamine mixing ratio of 1 + 3 with the buffered electrolyte, the total particle count was also elevated after 4 h and the turbidity exceeded the acceptance level in the 2 h sample (not shown) and 4 h after mixing. In this mixing ratio, the pH changed to 6.3.

Neither fentanyl nor potassium chloride precipitated when mixed with the buffered electrolyte solution (Table 4).

Finally, in the complex mix of five components mimicking the case, precipitation was observed, with a high total number of particles >0.5 µm immediately after mixing, which developed into detector overload after 4 h. The number of large particles (>10 µm) exceeded the acceptance limit after 4 h. The turbidity was high, and Tyndall effect could be observed. pH in the mix was around 5.6. When replacing midazolam with sterile water, fewer particles were observed, but still all analysis methods used in this study indicated that a precipitation occurred.

3.2 | Compatibility with buffered electrolyte-glucose solution (Plasma-Lyte 148 with 5% glucose)

The buffered electrolyte-glucose solution had a pH of approximately 5.2 whereas the one without had a pH of around 7.0. To elucidate which effect the pH would have on the precipitation on the complex regime, the analyses focused around midazolam.

First, in the two-component mix consisting of equal parts of midazolam and the buffered electrolyte-glucose solution the total number of particles >0.5 µm was slightly increased but most importantly the number of particles with a diameter >10 µm was over the acceptance level and showed an increasing trend over time (Table 4).

Replicating the full case by mixing fentanyl, ketamine, midazolam and potassium chloride with the glucose-containing buffered electrolytes, particles developed with time and after 4 h, there was detector overload. Also, turbidity measurements indicated particle precipitation and the pH values were above acceptance criteria.

4 | DISCUSSION

The physical compatibility of the buffered electrolyte solution and the buffered electrolyte-glucose solution has never been studied in a complex mixture of several intravenous drugs that are often administered at PICUs simultaneously. Since the use of these buffered electrolyte products offers the advantage of avoiding hyperchloremic metabolic acidosis that tends to occur with the use of 0.9% NaCl, it is of benefit to study the impact its intravenous co-administration might have on the physical stability of drugs given simultaneously via the same catheter. To our best knowledge, this study is the first to analyze the physical stability of a multi-drug mixture with this type of products.

By replicating the case where precipitation occurred during co-administration of fentanyl, ketamine, midazolam, and potassium chloride in the same i.v.-catheter line as Plasma-Lyte 148 using the mixing volumes arising from the infusion rates, our study confirmed that a precipitation was formed and identified the problematic drugs in the mix to be midazolam and ketamine.

Three studies investigating the intravenous compatibility of the same buffered electrolyte solution and the buffered electrolyte-glucose solution with one-drug-at-a-time have been published. These studies reported concentration dependent compatibility between midazolam and the two buffered electrolytes, irrespective of whether glucose was present.[17–19] Hammond et al.[17] investigated physical compatibility by visual inspection and reported that precipitation formed immediately when mixing three parts of midazolam 3 mg/ml with two parts of the buffered electrolyte solution or the buffered electrolyte-glucose solution. Dawson et al.[18] did not see any precipitation when investigating compatibility of midazolam 0.25 mg/ml with the buffered electrolyte solution. On the contrary, the manufacturer Baxter Medical reported that midazolam 1 mg/ml was compatible with the buffered electrolyte solution.[19] The concentration of midazolam in our case was 5 mg/ml, which caused precipitation and resonates well with the literature confirming that there is a concentration dependent solubility challenge of midazolam when the pH in the mix deviates from the pH of the drug product, a rather acidic pH of 3.5. It is well-known that water solubility of midazolam is pH-dependent exhibiting a drastic decrease with pH values exceeding 4.[20,21] Midazolam base has a pKa of 6.2 (Table 2), which means that when the pH of its environment, in this case the mixture with the buffered electrolyte (and for the case also other drugs) reaches the pKa-value of midazolam, more than 50% of midazolam molecules will be deprotonated and less soluble. It is important to mention that the structure of midazolam changes with increased pH. Upon deprotonation a ring-structure is formed in the molecule (Figure 1), which affects the physico-chemical properties of midazolam from being water-soluble at low pH to more lipid-soluble with increased pH.[22] This explains the precipitation. Our analyses suggest that at a pH between 4.7–5.7 the dilution of midazolam 5 mg/ml is not sufficient, neither in 1+1 ratio (=2.5 mg/ml) nor in the simulated case 1+5 ratio (=0.83 mg/ml), to keep the drug in solution. This is in agreement with the low solubility of midazolam of 0.1 mg/ml at neutral pH (Table 2).[21] At a mixing ratio 1+19, the dilution will be higher (=0.25 mg/ml), which should still indicate too low solubility. This mixture shows a higher pH and is more influenced by the buffer of the electrolyte fluid. These factors will also have an impact on the degree of dissociation and solubility, illustrating how complex buffered systems are. This complicates quick theoretical estimation of mixed pH in a clinical setting.

Dawson et al. studied the compatibility of ketamine 0.2 mg/ml (1+30) with the buffered electrolyte solution and concluded that the mix was physically compatible in the mixing ratio of 1+30.[18] Baxter Medical concluded that equal parts of ketamine 2 mg/ml and the buffered electrolyte solution to be physically compatible.[19] It should be noted that the ketamine concentration in the mixtures of both these studies were lower than in our studies (=6.5 μg/ml and 1 mg/ml, respectively), compared with our clinical case and study using 10 mg/ml for 1+1 (=5 mg/ml) and 1+3 (=2.5 mg/ml). Even though we could not find the exact solubility of ketamine base, only the theoretically predicted one of 46 μg/ml from DrugBank, it is reasonable to assume that it would be below 2.5 mg/ml. Again, ketamine is a weak base, and the solubility depends on the pH of the surroundings and the pKa of the drug. However, the buffering electrolytes will also have an impact on the ionization and solubility. As discussed earlier, the buffered electrolyte governs the pH of the mixture of ketamine (pH control of 4.6) and the buffered electrolyte solution (pH control approx. 7) and keeps it at 6–6.3. Since ketamine is a weak base with a pKa of 7.5 (the strongest base), a higher proportion of the drug will be deprotonated and can precipitate.

Fentanyl is also a weak base with a pKa on the basic side (8.77). It is used as the citrate salt in the product, which showed a pH of 6.0 in the unmixed control (Table 4). The solubility of fentanyl base was reported to be 0.74 mg/ml. However, fentanyl is a very potent opioid, and in our hospital, the clinical concentration used is 50 μg/ml. Hence, in our studies, the mixing ratios 1+1, 1+20 and 1+32 (the latter represents the case), all represented drug concentrations well below the solubility limit (25 μg/ml, 2.4 and 1.5 μg/ml, respectively), and the precipitation could therefore not be traced to fentanyl. Baxter Medical have concluded 10 μg/ml fentanyl, in mixing ratio 1+1, to be compatible with the buffered electrolyte solution,[19] and Dawson et al.[18] tested 30 μg/ml of fentanyl in mixing ratio of 1.2+1 and concluded it to be compatible with both the buffered electrolyte solution and the buffered electrolyte-glucose solution. All concentrations were below solubility limits and confirmed our findings. It can be concluded that fentanyl is safe to co-administer with either one of the two buffered electrolytes.

Potassium chloride (KCl) has a very high solubility (>100 mg/ml, Table 2), and there was no reason to expect salting out or precipitation of KCl. Neither of the ions form poorly soluble salts or complexes with any of the other constituents, also not with any of the excipients from the various drug products (Table 2). Baxter Medical supplied information of compatibility of 0.5 mmol/ml potassium chloride with both buffered electrolytes,[19] also supporting the findings in the current study.

When replicating the case but replacing midazolam with sterile water in order to see which of the components, midazolam or

TABLE 4 Results from analyses of precipitation after mixing fentanyl (F), ketamine (K), midazolam (M), sterile water (SW), and potassium chloride (KCl) with the two buffered electrolyte solutions in different combinations and mixing ratios (bold font indicates values outside acceptance criteria) (average ± SD; $n = 3$)

		Particles/ml											
		≥0.5 μm		≥5 μm		≥10 μm		≥25 μm		Turbidity (FNU)		pH	
Drug	Mix ratio	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h
Fentanyl (F)	Control	263[a]	99[a]	1[a]	1[a]	0[a]	1[a]	0[a]	0[a]	0.12[a]	0.07[a]	6.02[a]	6.05[a]
Ketamine (K)	Control	330[a]	260[a]	5[a]	4[a]	4[a]	4[a]	2[a]	2[a]	0.16[a]	0.23[a]	4.63[a]	4.59[a]
Midazolam (M)	Control	204 ± 32	218 ± 80	4 ± 2	4 ± 4	1 ± 0	2 ± 3	0 ± 1	1 ± 2	0.12 ± 0.03	0.23 ± 0.16	3.47 ± 0.21	3.52 ± 0.27
Plasma-Lyte 148 (P)	Control	274 ± 29	196 ± 84	11 ± 2	10 ± 4	2 ± 1	4 ± 2	0 ± 0	0 ± 1	0.21 ± 0.08	0.16 ± 0.01	7.02 ± 0.07	6.85 ± 0.14
Plasma-Lyte 148–5%-Glucose (PG)	Control	193 ± 64	120 ± 37	3 ± 2	1 ± 0	1 ± 1	0 ± 1	0 ± 0	0 ± 0	0.13 ± 0.02	0.12 ± 0.03	5.22 ± 0.04	5.21 ± 0.05
Potassium chloride (KCl)	Control	221 ± 20	165 ± 1	1 ± 1	6 ± 4	1 ± 1	3 ± 3	1 ± 1	1 ± 1	0.07 ± 0.01	0.08 ± 0.00	5.84 ± 0.00	5.82 ± 0.01
Potassium chloride (KCl)	Control	221 ± 20	165 ± 1	1 ± 1	6 ± 4	1 ± 1	3 ± 3	1 ± 1	1 ± 1	0.07 ± 0.01	0.08 ± 0.00	5.84 ± 0.00	5.82 ± 0.01
Drug (s) + Plasma-Lyte 148													
F + P	1 + 1	185 ± 6	356 ± 53	2 ± 1	5 ± 2	1 ± 1	2 ± 2	0 ± 0	0 ± 1	0.07 ± 0.02	0.09 ± 0.02	6.78 ± 0.06	6.81 ± 0.01
	1 + 20	147 ± 29	187 ± 51	6 ± 3	11 ± 5	2 ± 1	4 ± 2	0 ± 0	0 ± 0	0.11 ± 0.03	0.11 ± 0.01	7.04 ± 0.01	7.06 ± 0.01
K + P	1 + 1	224 ± 76	**OL**[b]	3 ± 1	2 ± 1	2 ± 1	1 ± 2	1 ± 0	0 ± 1	**0.45 ± 0.09**	**0.85 ± 0.07**	**6.05 ± 0.01**	**6.04 ± 0.03**
	1 + 3	538 ± 43	**1594 ± 1316**	1 ± 1	3 ± 1	0 ± 1	1 ± 1	0 ± 0	0 ± 0	0.26 ± 0.05	**0.38 ± 0.10**	**6.30 ± 0.03**	**6.30 ± 0.03**
M + P	1 + 1	**OL**[b]	**OL**[b]	**OL**[b]	**OL**[b]	**OL**[b]	**OL**[b]	**636 ± 41**	**273 ± 34**	**313 ± 11.0**	**122 ± 42.6**	**5.09 ± 0.19**	**5.10 ± 0.22**
	1 + 19	423 ± 202	293 ± 202	7 ± 6	2 ± 6	4 ± 2	0 ± 1	0 ± 1	0 ± 0	0.25 ± 0.20	0.08 ± 0.01	**6.18 ± 0.02**	**6.21 ± 0.02**
KCl + P	1 + 1	155 ± 50	290 ± 25	3 ± 2	12 ± 6	2 ± 1	5 ± 4	0 ± 0	1 ± 2	0.10 ± 0.04	0.11 ± 0.01	6.87 ± 0.05	6.60 ± 0.36
	1 + 12	264 ± 95	272 ± 94	11 ± 9	14 ± 5	3 ± 2	6 ± 2	0 ± 0	1 ± 1	0.10 ± 0.01	0.11 ± 0.02	7.00 ± 0.01	7.00 ± 0.03
M + SW + P	1 + 2 + 3	853 ± 217	943 ± 450	57 ± 12	56 ± 16	**31 ± 7**	**30 ± 8**	**5 ± 3**	**5 ± 1**	**1.41 ± 0.45**	**13.5 ± 15.1**	**5.33 ± 0.28**	**5.57 ± 0.14**
F + KCl + K + M + P	3 + 1 + 24 + 16 + 55	**3883 ± 528**	**OL**[b]	32 ± 7	86 ± 26	15 ± 3	**35 ± 12**	2 ± 1	3 ± 2	**0.70 ± 0.17**	**1.00 ± 0.10**	**5.63 ± 0.03**	**5.64 ± 0.01**
F + KCl + K + SW + P	3 + 1 + 24 + 16 + 55	496 ± 209	**OL**[b]	2 ± 1	3 ± 1	0 ± 1	1 ± 1	0 ± 0	0 ± 0	**0.33 ± 0.07**	**0.48 ± 0.09**	**6.24 ± 0.02**	**6.26 ± 0.03**
Drug(s) + Plasma-Lyte 148 - 5%													
M + PD	1 + 1	516 ± 98	884 ± 455	60 ± 85	49 ± 76	**18 ± 26**	**43 ± 72**	0 ± 0	0 ± 0	**1.99 ± 1.40**	**79.9 ± 53.1**	**4.77 ± 0.09**	**4.74 ± 0.09**
	1 + 19	120 ± 33	127 ± 29	0 ± 1	1 ± 2	0 ± 0	1 ± 1	0 ± 0	0 ± 1	0.08 ± 0.01	0.11 ± 0.03	**5.23 ± 0.03**	**5.25 ± 0.02**
M + SW + PD	1 + 2 + 3	220 ± 25	265 ± 52	5 ± 3	3 ± 1	1 ± 1	1 ± 0	0 ± 0	0 ± 0	0.25 ± 0.20	0.08 ± 0.01	**5.07 ± 0.03**	**5.06 ± 0.02**
F + KCl + K + M + PD	3 + 1 + 24 + 16 + 55	1016 ± 86	**OL**[a]	4 ± 1	3 ± 1	2 ± 2	1 ± 0	0 ± 0	0 ± 0	**0.54 ± 0.14**	**0.76 ± 0.21**	**5.07 ± 0.02**	**5.06 ± 0.02**

[a]Over the detector limit (OL), >9000 particles/mL.

[b]Only one parallel for the narcotics (local laboratory guidelines).

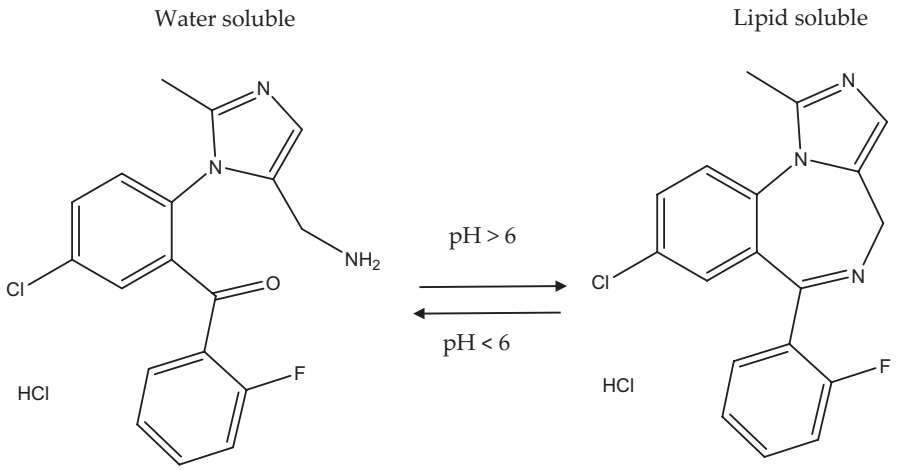

FIGURE 1 Schematic representation of pH dependent ring-formation in midazolam structure

ketamine, contributed to precipitation, it was clear that precipitation occurred also without midazolam for the buffered electrolyte. The particle levels were lower in the admixture with sterile water, due to lack of midazolam, emphasizing that ketamine alone also led to an increase in turbidity. It would be interesting to conduct the same study and replace also ketamine with sterile water, however, this was not done.

Comparing the results of the complex regime for the two buffered electrolytes with and without glucose, midazolam showed a higher degree of particle formation when mixed with the buffered electrolyte solution than with the buffered electrolyte-glucose solution. Nevertheless, the latter also showed signs of particle formation and pH-values of the mix that should be alarming. Still, the pH of the buffered electrolyte-glucose solution (pH approx. 5.2) is more favorable for keeping midazolam in solution than the corresponding product without glucose (pH approx. 7).[21] The acidic pH-range of heat-sterilized glucose (5% Glucose approx. 3.5–6.5[23]) is described to be a result of glucose decomposition into levulinic and formic acids at temperatures in the autoclave.[24] Since the glucose content is the only difference between the two buffered electrolyte solutions, this is the probable cause of the more acidic product pH in the glucose-containing product. For midazolam, the acidic pH of the glucose-containing product was beneficial. Nevertheless, our studies emphasize that a pH difference of approximately 2 pH units between two corresponding products is not trivial and switching between the two product types in a clinical scenario should cause extra attention if co-administration with drugs comes into question.

The experimental setup in this study, the mixing volumes of drugs in tubes to simulate Y-site co-administration of i.v.-drugs, does not replicate the true clinical scenario and our results should therefore be interpreted with this in mind. However, we have performed both visual and sub-visual particle analysis and analyzed clinically relevant concentrations and mixing ratios combined with theoretical evaluations, which makes our conclusions sufficiently robust. This is in contrast to many, especially older published studies that only evaluate 1+1 mixing ratios and rely on visual examination alone. We maintain that when testing for drug compatibility, it is important to use several analytical methods and not only perform visual inspections since it is shown to be subjective and will not capture sub-visual particles. Since drugs are given in ever-changing infusions rates depending on the need of the patient it is advised to analyze mixed samples in at least three different mixing ratios. Given the variability in clinical practice where different buffered electrolyte solutions are used an extra safety precaution is to use in-line filter, which could help to prevent infusion of precipitated particles into the bloodstream of the patient.[25,26] Last, but not least, it should be emphasized that the exact composition and pH of buffered electrolytes might be product specific, and caution should be taken if and when extrapolating the findings to other products.

5 | CONCLUSION

Our case-based analysis of a five component mixture identified midazolam 5 mg/ml and ketamine 10 mg/ml as the causative agents of the precipitation when co-infused with the buffered electrolyte solution Plasma-Lyte 148. Midazolam was found to be physically incompatible in a two-component mix with the buffered electrolyte solution but also with the corresponding product containing 5% glucose and should not be co-administered in the same i.v.-catheter line. Ketamine also showed signs of incompatibility when mixed with the buffered electrolyte solution and co-administration should also be avoided. Fentanyl 50 µg/ml and potassium chloride were found to be compatible with both buffered electrolytes.

AUTHOR CONTRIBUTIONS

N.N., K.N-H, and I.T. conceptualized the study. N.N., K.N-H., and I.T. performed methodology. V.N. and N.N performed formal analysis. N.N. performed writing—original draft preparation. K.N-H., V.N., and I.T performed writing—review and editing. K.N-H., N.N., and I.T. supervised the study. K.N-H. and I.T involved in project administration. K.N-H performed funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest. The funding authority had no involvement in study design, collection, analysis, and interpretation of data, and writing manuscript or decision to submit manuscript for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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