

Article

Stability Assessment of Intravenous Iron–Carbohydrate Complexes in Commercial All-in-One Parenteral Nutrition: Potential for Therapeutic Iron Dose Admixing

Valentina V. Huwiler ^{1,2,3,*}, Peter J. Neyer ^{1,†}, Christoph Saxer ¹, Katja A. Schönenberger ^{1,2,3}, Angelika Hammerer-Lercher ¹, Zeno Stanga ³ and Stefan Mühlebach ²

- ¹ Institute of Laboratory Medicine, Cantonal Hospital Aarau, Tellstrasse 25, 5001 Aarau, Switzerland; peter.neyer@ksa.ch (P.J.N.); christoph.saxer@ksa.ch (C.S.); k.schonenberger.science@gmail.com (K.A.S.)
² Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, Spitalstrasse 26, 4056 Basel, Switzerland; stefan.muehlebach@unibas.ch
³ Department of Diabetes, Endocrinology, Nutritional Medicine and Metabolism (UDEM), Inselspital, Bern University Hospital, University of Bern, Freiburgstrasse 15, 3010 Bern, Switzerland
* Correspondence: vvhuwiler@gmail.com
† These authors contributed equally to this work.

Abstract

Background/Objectives: Iron deficiency and associated iron deficiency anaemia represent a major global health burden. Parenteral nutrition (PN) patients are at increased risk of iron deficiency due to inadequate iron supplementation. Currently, iron is added to all-in-one (AIO) PN mostly as low-dose ferric chloride in trace element solutions, limited to 1–2 mg in adults, to ensure emulsion stability and prevent lipid peroxidation. The objective of this study was to evaluate the compatibility and stability of selected, widely used complex-bound iron products added to AIO PN over a 48 h period. **Methods:** Ferric carboxymaltose and iron sucrose were added as non-biological complex intravenous iron oxide carbohydrate products to two different commercial AIO PN admixtures for adults. The iron concentrations used were 100 and 400 mg/L (1.79 and 7.16 mmol/L), corresponding to approximately 200 mg (3.58 mmol) of iron dose per PN bag. Free and complex-bound iron were separated using 100 kDa dialysis tubes. Free and complex-bound iron were assessed at 4, 24, and 48 h after admixing. pH was measured before and at 0, 4, 24, and 48 h after admixture. Iron quantification was performed by inductively coupled plasma mass spectrometry (ICP-MS). **Results:** No significant changes in complex-bound iron concentration were observed over the 48 h incubation period (p -value = 0.449; estimate 0.060 mg/L per h, 95% CI –0.089, 0.201 mg/L per h). The concentration of free iron was very low and increased only slightly over time. Iron recovery ranged from 95.8% to 103.9%. The addition of the alkaline iron sucrose significantly increased the pH of the AIO admixture (p -value = 0.033), whereas the addition of ferric carboxymaltose did not affect the pH (p -value = 0.351). After the initial increase, the pH of all conditions remained stable over the 48 h incubation period (p -value = 0.07). **Conclusions:** Ferric carboxymaltose demonstrated stable intravenous iron admixtures within the PN formulations tested. Before the clinical application of these findings, further studies should specifically evaluate the lipid peroxidation and stability of the lipid emulsions, the most sensitive and important PN compatibility and safety characteristics of AIO PN.

Keywords: parenteral nutrition; ferric carboxymaltose; Ferinject®; in vitro stability; dialysis tubes; ICP-MS

Academic Editors: Amir Fahmi,
Franciska Erdő and Romána Zelkó

Received: 20 January 2026
Revised: 13 February 2026
Accepted: 16 February 2026
Published: 18 February 2026

Copyright: © 2026 by the authors.
Licensee MDPI, Basel, Switzerland.
This article is an open access article
distributed under the terms and
conditions of the [Creative Commons
Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Iron deficiency and the associated iron deficiency anaemia (IDA) constitute a leading global health burden, with a prevalence of more than 1.2 billion people. In 2016, IDA ranked among the top five causes of years lived with disability worldwide, affecting key physiological functions such as oxygen transport, cognition, mitochondrial activity, neuromuscular performance, and cardiopulmonary health [1,2]. Its clinical presentation includes characteristic signs and symptoms such as skin and conjunctival pallor, hair loss, and hypoxia-related complaints such as fatigue, vertigo, syncope, and headache [3].

Patients requiring long-term parenteral nutrition (PN) are particularly susceptible to iron deficiency due to inadequate iron supplementation, absorption, and metabolism (e.g., reduced iron intake, intestinal malabsorption due to intestinal failure, inflammation with increased hepcidin). Furthermore, iron deficiency may be aggravated by concomitant blood loss, e.g., from gastrointestinal bleeding in these often multimorbid patients. This condition is common, affecting approximately 30% of such patients [4]. Additionally, pre-term infants, who start life with limited nutrient stores, have increased iron requirements [5]. In cases where oral iron supplementation proves ineffective or intolerable, intravenous iron infusion becomes essential [6].

Recognised clinical nutrition and metabolism societies in Europe, Australia, and New Zealand recommend intravenous iron administration for parenterally fed patients when indicated [7,8]. Currently, iron is routinely added to trace element solutions for adults, but not for paediatric patients. The main form is ferric chloride, which must be infused together with PN. However, these daily doses are limited to 1–2 mg, which is insufficient for individuals with increased iron requirements. Caution is warranted when introducing higher doses of iron, particularly with regard to free iron. Free polyvalent iron is redox active and can lead to the formation of toxic radicals and lipid peroxidation, but it can also cause physicochemical incompatibilities with all-in-one (AIO) PN, affecting emulsion stability [9–13]. AIO PN provides all the necessary macronutrients, i.e., amino acids, glucose, and lipids, in combination with electrolytes, and complete with the necessary micronutrients in a convenient but highly complex and reactive system [14,15].

The use of selected intravenous iron–carbohydrate complex medical products could be promising to overcome compatibility issues while allowing for higher iron doses. In the current landscape of intravenous iron products, various complex-bound iron alternatives such as ferric carboxymaltose (FCM), iron sucrose (ISC), and ferric derisomaltose are widely approved and implemented in clinical practice [6,16]. The combination of PN and intravenous iron administration has the potential to streamline patient care and convenience, reduce healthcare provider workload, minimise the need for frequent separate intravenous iron substitution via an additional access line, and effectively provide patients with adequate amounts of iron. However, the safety, efficacy, and quality (compatibility) of this combination require rigorous evaluation, in particular by demonstrating the exclusion of reactive free iron (Fe^{n+}) in a representative practical setting and by using a highly sensitive analytical method. This may prevent adverse reactions and physicochemical deterioration of the AIO PN system by maintaining the integrity of the colloidal iron [14], which is also a prerequisite for the iron uptake into the reticuloendothelial system and the iron stores [17]. However, there is a lack of data on the compatibility and stability of higher iron doses in AIO PN admixtures from the most commonly used intravenous iron–carbohydrate complex preparations (non-biological complex drugs) [18].

Previous studies have raised concerns about the stability of ISC because Venofer® (Ven-ISC), the first globally authorised iron–carbohydrate complex, caused the formation of (toxic) degradation products such as hydroxyalkenals and dialdehydes due to lipid peroxidation by free iron [12]. The next generation representative of intravenous iron–carbohydrate complex products, FCM (e.g., Ferinject® [Fer-FCM]), has the potential for

improved stability. FCM is characterised by a polynuclear ferric hydroxide core surrounded by a polymeric carbohydrate complex. It forms particles comparable to ferritin, the physiological intracellular Fe^{3+} protein store [17]. In the absence of a gold-standard method to assess the stability of iron–carbohydrate complexes in a compatible setting and because of the lack of a complete characterisation of such complex-bound iron products, our study modified a novel approach using dialysis tubes. This method was described by Hira et al. [19], who investigated the stability of Fesin[®], a similar intravenous ISC. We complemented the method by inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive method for the quantification of multiple elements [20].

The aim of this study was to assess the compatibility of Fer-FCM with commercial AIO PN admixtures using a nanoparticle integrity and size dialysis technique, together with pH stability investigation. Furthermore, we partially compared these results with those of the older, less stable, but still widely used Ven-ISC, building on the work of Neiser et al. [21]. We investigated the stability of therapeutic daily iron doses of approximately 200 mg over a 48 h period, beyond the usual AIO PN administration time of 24 h, to ensure the required stability range of this drug–PN admixture. Thus, we could expand the previously presented compatibility and stability of hydrophilic and solubilised lipophilic drugs admixed with AIO PN [22,23] by assessing the new class of pharmaceuticals represented by iron–carbohydrate complexes.

2. Materials and Methods

2.1. Materials

The commercial three-chamber AIO PN bags commonly used in Europe were kindly provided by the manufacturers: Omegaflex[®] special (OM) 625 mL (Lot 223518231, Expiration 07/2024) and 1875 mL (Lot 224418231, Expiration 09/2024) from B. Braun Medical AG, (Sempach, Switzerland) and SmofKabiven[®] (SK) 986 mL (Lot 10SA7857, Expiration 12/2024) and 1477 mL (Lot 10SB8729, Expiration 01/2025) from Fresenius Kabi (Schweiz) AG (Kriens, Switzerland). The composition of the investigated three-chamber bags is specified in Table 1. We used the glucose content of the respective chamber of the OM 1875 mL bag when glucose solution was needed ($\approx$ glucose 40%). We obtained Ferinject[®] (Fer-FCM) vials of 500 mg/10 mL (Lot 116112A, Expiration 11/2024) for injection and Venofer[®] (Ven-ISC) vials of 100 mg/5 mL (Lot 1050126AA, Expiration 10/2024) for injection from Vifor (International) Inc. (St. Gallen, Switzerland). We purchased 1 mL Float-A-Lyzer G2[®] Dialysis Devices (100 kD) from Repligen Corporation (Rancho Dominguez, CA, USA). We obtained 120 mL BD Vacutainer[®] urine collection containers from Becton Dickinson (Allschwil, Switzerland). Ultrapure water ($>18.2 \text{ M}\Omega$) was prepared using a Purelaba flex 3 integrated water purification system (Labtec, Villmergen, Switzerland). We purchased saline injection solution (0.9% NaCl) from B. Braun Medical AG (Sempach, Switzerland), ethylenediaminetetraacetic acid (EDTA) from Sigma Aldrich (Buchs, Switzerland), sodium hydroxide (3 mol/L) from the local hospital pharmacy, nitric acid (69%) from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), and 2-propanol from Honeywell Specialty Chemicals Seelze GmbH (Seelze, Germany). We purchased certified standard solutions of iron nitrate from PerkinElmer Inc. (Waltham, MA, USA). All reagents used were of analytical grade. pH was measured using a silver-referenced glass electrode (744 pH Meter, Metrohm, Herisau, Switzerland).

Table 1. Composition of tested all-in-one parenteral admixtures.

	Omegaflex® Special 625 mL	Omegaflex® Special 1875 mL	SmofKabiven® 986 mL	SmofKabiven® 1477 mL
Volume glucose solution [mL]	250	750	298	446
Glucose monohydrate [g]	99.0	297.0	414.1	620.3
Volume fat emulsion [mL]	125	375	188	281
Volume amino acid solution [mL]	250	750	500	750
Amino acid content [g]	35	105.1	50	75
Nitrogen content [g]	5	15	8	12
Carbohydrate content [g]	90	270	125	188
Lipid content [g]	25	75	37	56
Non-protein energy [kJ (kcal)]	2505 (600)	7510 (1795)	3800 (900)	5400 (1300)
Total energy [kJ (kcal)]	3090 (740)	9260 (2215)	4600 (1100)	6700 (1600)
Osmolarity [mOsm/kg]	2115	2115	1500	1500
pH	5.0–6.0	5.0–6.0	5.6	5.6
Complex-bound iron added concentration [mg iron/mL]	Fer-FCM, 0.4; Ven-ISC, 0.4	Fer-FCM, 0.1	Fer-FCM, 0.25	Fer-FCM, 0.1

Fer-FCM = ferric carboxymaltose as Ferinject®; Ven-ISC = iron sucrose as Venofer®.

2.2. Methods

2.2.1. Iron, Electrolyte, and Mineral Quantification Using ICP-MS

We quantified the total amount of iron in the samples by ICP-MS (NexION 2000; PerkinElmer Inc., Waltham, USA) using an autosampler (ESI, Elemental Scientific Inc., Omaha, NE, USA). For iron, the ^{56}Fe isotope was measured for determination in the analysis because of its predominance and the highest precision of the counts among the iron isotopes. The isotopes ^{54}Fe , ^{57}Fe , and ^{58}Fe were used for quality control. For sample preparation, we diluted 80 μL of test sample in 1920 μL of diluent in disposable polypropylene sample cups and homogenised it by pipetting, aspirating, and dispensing it three times. The diluent was prepared from 500 mL ultrapure water, 10 μL rhodium nitrate solution (1000 $\mu\text{g/mL}$), 5 mL 2-propanol, and 5 mL nitric acid solution (69%). Samples with an expected analyte concentration >250 mg/L were prediluted in the diluent. Signals were adjusted for background by subtracting the signals of a blank sample. Calibration was performed using a six-point calibration for iron (10, 100, 1000, 10,000, 100,000, and 250,000 $\mu\text{g/L}$) in duplicate. The counts of the calibration standards were fitted linearly through the zero value. Table 2 shows the final settings for the ICP-MS analysis.

Table 2. Developed and optimised settings for inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Mass/Analyte	^{103}Rh (IS)	^{54}Fe ^{56}Fe ^{57}Fe ^{58}Fe
Dwell time	50.0 ms	20.0 ms
RPq	0.25	0.35
Mode		KED
He flow rate		4.9
Auxiliary gas (Ar) flow rate		1.2 mL/min
Nebulizer gas (Ar) flow rate		0.84 mL/min
Plasma gas (Ar) flow rate		15 L/min
Sample introduction gas		None
RPa		0
Replicates		3

Readings/replicate	1
Sample uptake rate	0.43 mL/min
Cones	Nickel
Scan mode	Peak hopping
Sweeps/reading	15
Dilution factor	1:10

Fe = iron; IS = internal standard; KED = kinetic energy discrimination; RPa = retarding potential analyser; RPq = retarding potential quadrupole.

2.2.2. Separation of Complex-Bound and Free Iron

To separate intact complexes from free iron, we adapted the technique previously described by Hira et al. [19] using the Float-A-Lyzer G2[®] Dialysis Device. We used ultrapure water, glucose solution, saline injection, and PN admixture as dialysis separation fluids. The biotech-grade cellulose ester membrane had a pore size corresponding to 100 kDa in order to prevent diffusion of Fer-FCM with a molecular weight of around 150 kDa [24]. The dialysis tubes were prepared and filled according to supplier instructions. In brief, to prepare the membrane, we filled the dialysis tube with 20% 2-propanol and immersed it in the same solution for 20 min. We aspirated the 2-propanol from the dialysis tube and rinsed it three times with ultrapure water. We filled the dialysis tube with ultrapure water and immersed the membrane in it for 20 min. Dialysis tubes were removed and, while the membrane of the dialysis tube was still wet, we filled the tube with 2 mL of sample. In contrast to Hira et al. [19], saturation of the surface of the tube with iron standard solution was not necessary. We placed the dialysis tube in the polyethylene float ring and then immersed it into a urine collection cup filled with 70 mL of the appropriate solution (PN/sodium chloride/glucose), which served as the base for the sample. The urine collection container was shaken on a Titramax 1000 (Heidolph Instruments GmbH, Schwabach, Germany) at a speed level of 4 (corresponding to approximately 500 rounds per minute) at room temperature (25 °C). ICP-MS was then used to quantify the iron concentration inside the dialysis tubes, determined as complex-bound iron, and the iron concentration outside the dialysis tubes, determined as free iron.

2.2.3. Proof of Concept of the Dialysis Method

Hira et al. established a method to separate free (ionic) iron from complex-bound (encapsulated) iron (a generic colloidal saccharated iron oxide injection product) in 0.9% NaCl solution [18,19]. To assess the suitability of this method for AIO PN admixtures, we added 0.4 mg of iron as Fer-FCM colloidal iron or ionic iron standard solution into 0.9% NaCl solution or PN solution and studied diffusion over up to 28 h. Only a 0.04 mg dose of ionic iron standard solution could be added to the PN, as lipid emulsion stability deterioration occurred at higher concentrations due to the low pH.

In a second step, we aimed to destabilise the Fer-FCM complex-bound iron to release the iron content by addition of EDTA to PN (0.5 mg/mL) or in glucose solution and compared it with the diffusion characteristics of Fer-FCM in AIO PN without additives. Additionally, an identical colloidal iron dose of Fer-IS was added to PN to investigate the diffusion characteristics.

Separation of free from complex-bound iron was performed as described above for a maximum of 28 h, to ensure sufficient time for completion (Section 2.2.2). The manufacturer suggests a typical time of up to 20 h. All samples for the proof-of-concept experiments were prepared in 2 mL Eppendorf tubes and transferred directly into the dialysis tube without prior incubation of the sample.

Assessment of physicochemical stability of iron–carbohydrate complexes in the main investigation was performed as described below.

In total, five different conditions were tested with iron doses of approximately 200 mg. Details of the conditions studied are listed in Table 3. The doses were adjusted to obtain comparable iron concentrations, as bag sizes were given by the summary of product characteristics (SmPCs) from the authorised medicinal product.

Table 3. Assessed samples and their characteristics in the main investigation.

	OM + Fer-FCM 400 mg/L	SK + Fer-FCM 250 mg/L	OM + Fer-FCM 100 mg/L	SK + Fer-FCM 100 mg/L	OM + Ven-ISC 400 mg/L
Total bag volume [mL]	625	986	1875	1477	625
AIO PN solution	OM	SK	OM	SK	OM
Iron concentration [mg/L]	400	250	100	100	400
Iron quantity [mg]	250	246.5	187.5	197.0	250
Iron volume added [mL]	5.00	4.93	3.75	2.95	12.50
Iron form	FCM	FCM	FCM	FCM	ISC
Iron product	Fer-FCM	Fer-FCM	Fer-FCM	Fer-FCM	Ven-ISC
Start pH	5.53	5.50	5.53	5.50	5.53

AIO = all-in-one; FCM = ferric carboxymaltose; Fer-FCM = Ferinject®; ISC = iron sucrose; OM = Omegaflex special®; SK = SmofKabiven®; PN = parenteral nutrition; Ven-ISC = iron sucrose as Venofer®.

An overview of all assessments and their corresponding timepoints is summarised in Figure 1. We measured pH at 0, 4, 12, 24, and 48 h after addition of Fer-FCM in ready-to-use prepared AIO PN admixture bags. Ready-to-use admixture preparation was done by mechanically breaking the chamber seals and subsequent manual mixing of the components in the closed bag system according to the manufacturer's instructions. The respective colloidal iron dose was added by a 5 ml syringe injection through the injection port and then manually mixed. Complex-bound and free iron was assessed as described above at 4, 24, and 48 h of incubation and a dialysis duration of 5, 7, and 9 h (Section 2.2.2).

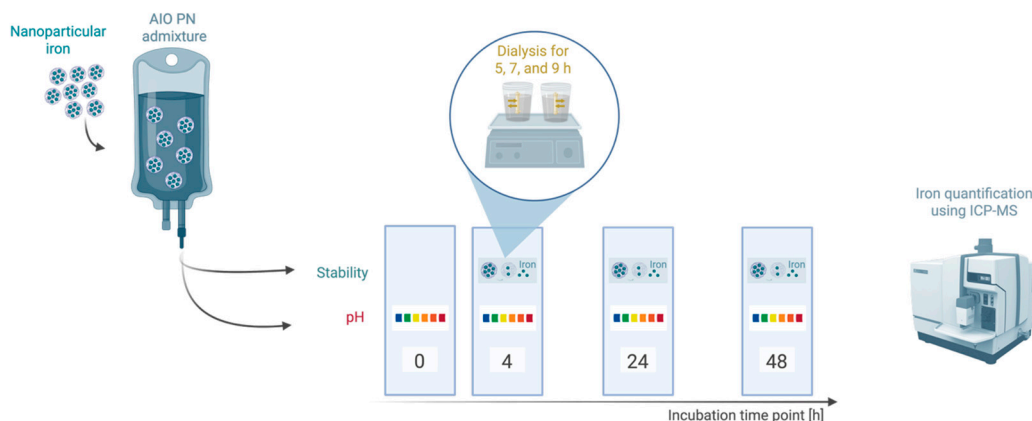


Figure 1. Overview of the sampling and assessments at the different timepoints for the main investigation. Iron–carbohydrate complexes (ferric carboxymaltose as Ferinject® [Fer-FCM] and iron sucrose as Venofer® [Ven-ISC]) were admixed to two different commercial all-in-one parenteral nutrition admixtures (Smofkabiven® and Omegaflex® special). At 0, 4, 24, and 48 h, a sample of each all-in-one parenteral nutrition admixture bag was taken to assess stability and/or pH. For the stability assessment, samples were incubated in individual dialysis tubes in duplicates on a shaker to separate free ionic iron from complex-bound iron. After 5, 7, and 9 h samples were taken from inside (= colloidal iron) and outside (= free ionic iron) of the dialysis tube. Final iron concentrations were measured using inductively coupled plasma mass spectrometry. AIO = all-in-one; ICP-MS = inductively coupled plasma mass spectrometry; PN = parenteral nutrition. This scheme was created using BioRender.com accessed on 13 February 2026.

2.2.4. Statistical Analysis

Stability was calculated as median $\pm$ standard deviation (SD) of the two runs, each with independent duplicates ($n = 4$). Confidence intervals (CI) and p -values were estimated by fitting linear mixed-effects models. Initial iron concentration was assumed as a fixed effect and the complex-bound and free iron as the random effects.

The pH results were expressed as means $\pm$ SD of two separate runs ($n = 2$). We analysed the pH increase before and after adding colloidal iron using a paired two-sided t -test. Linear models were fitted for pH over time and p -values calculated.

Recovery was calculated as (mean of total iron measured)/(added iron) $\times$ 100. Precision was assessed using the SD of all samples of the same condition and timepoint ($n = 4$). We considered p -values < 0.05 as significant. All statistical analyses were performed using R version 4.2.2 [25].

3. Results

3.1. Proof of Concept of Complex-Bound Iron Stability Assessment Using Dialysis Tubes

The sample used to assess the stability of the iron–carbohydrate complexes in the proof-of-concept experiments were investigated directly in the dialysis tube without prior incubation of the sample. Upon Fer-FCM admixing to NaCl, 99.7% of the iron was in the complex-bound form and this was the case for 90.1% when admixing to PN. When ionic standard iron solution was added (i.e., free iron), 2.8% and 3.6% remained in the dialysis tube for NaCl and PN, respectively. The overall recovery rates of these four settings ranged from 78.8% to 102.5%, with a lower recovery rate in the sample with a lower iron concentration (i.e., 0.04 mg/L instead of 0.4 mg/L). For the iron standard condition, an iron concentration of 0.04 mg/L was used due to emulsion failure in previous experiments when 0.4 mg/L or more were used (Table S1 and Figure S1).

When Fer-FCM was added to PN without any additives in the destabilisation experiment (control), 91.09% of the total iron remained in the dialysis tube. Meanwhile, when Fer-FCM was added to PN plus EDTA or glucose, 90.8% and 74.3%, respectively, remained in the dialysis tube. When Ven-ISC was added to PN, 71.1% of the total iron diffused out of the dialysis tube (Figure S2). The dialysis timepoints of 5, 7, and 9 h were selected based on a proof-of-concept experiment showing that equilibrium was reached after around 5 h and free and complex-bound iron concentration remained stable until 20 h (Figure S2).

3.2. Sample Stability in the Main Investigation Mimicking the Clinical Setting

We found no significant change in complex-bound iron concentration over the 48 h incubation period when Fer-FCM was present in the AIO PN admixtures (p -value = 0.449; estimate 0.060 mg/L per h, 95% CI -0.089 , 0.201 mg/L per h). However, the concentration of free iron increased significantly over time (p -value = 0.003; estimate 0.011 mg/L per h, 95% CI 0.004, 0.018 mg/L per h). The highest increase was observed in the sample with an iron dose of 400 mg/L, from a mean free iron concentration of 0.643 and 0.631 mg/L at 4 and 24 h, respectively, to a free iron concentration of 2.162 mg/L at 48 h (Figure 2). Overall recovery of the added iron ranged from 95.8% to 103.9%. The imprecision of each replicate ($n = 4$) ranged from 3.37 to 17.70 mg/L for colloidal iron and 0.05 to 2.78 mg/L for free iron per condition and timepoint (Figure 2, Table 4). The 7 h dialysis timepoint was chosen for the final analysis because of the low occurrence of outliers, and because this was the earliest timepoint at which equilibrium was reached, with no further changes observed in the concentrations of free and complex-bound iron (Figure S3). Sample stability when Ven-ISC was present in the AIO PN admixtures was not assessed.

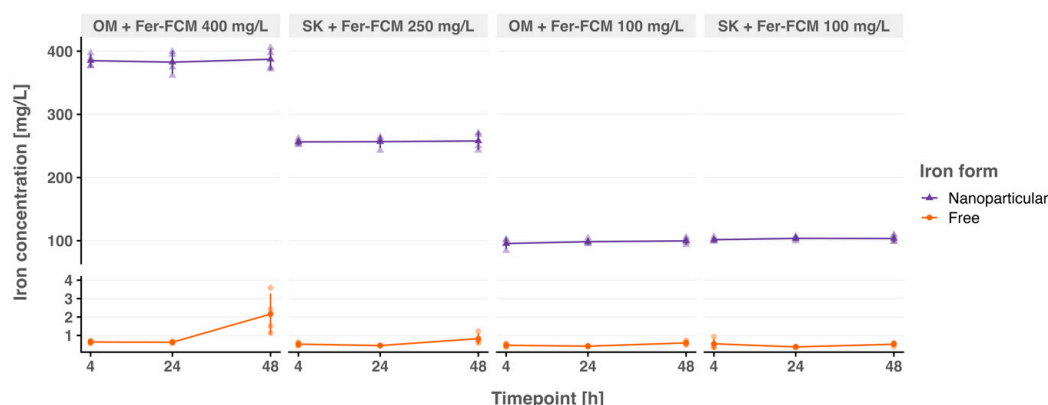


Figure 2. Concentration of complex-bound (ferric carboxymaltose as Ferinject®, Fer-FCM) and free iron (^{56}Fe) over the 48 h incubation period, measured after 7 h dialysis. Faded symbols correspond to single measurement. Solid symbols represent the mean and the solid vertical line the standard deviation ($n = 4$). Complex-bound iron concentrations shown in purple triangles and free iron concentration in orange circles. FCM = ferric carboxymaltose; OM = Omegaflex special®; SK = SmofKabiven®.

Table 4. Recovery of total iron and precision of complex-bound and free iron measurement of the main stability investigation.

Formulation + Additive	Added Iron Concentration [mg/L]	Total Iron Recovery [%] ^a	Precision Complex-Bound Iron [SD; mg/L] ^b	Precision Free Iron [SD; mg/L] ^b
OM + Fer-FCM 400 mg/L	400	95.8–97.3	9.96–17.70	0.05–1.10
SK + Fer-FCM 250 mg/L	250	102.8–103.5	4.72–13.50	0.07–2.78
OM + Fer-FCM 100 mg/L	100	96.0–100.3	4.16–8.14	0.20–1.12
SK + Fer-FCM 100 mg/L	100	102.1–103.9	3.37–5.63	0.27–2.68

^a Range of total iron recovery per condition and timepoint; ^b range of standard deviations per condition and each timepoint ($n = 4$). Fer-FCM = Ferric carboxymaltose as Ferinject®; OM = Omegaflex special®; SD = standard deviation; SK = SmofKabiven®.

The initial pH of the AIO PN admixtures was 5.53 and 5.50 for OM and SK, respectively. The intravenous iron products had a pH between 5.0 and 7.0 (Fer-FCM) and 10.5 and 11.1 (Ven-ISC). The addition of Ven-ISC significantly raised the pH from 5.53 to a pH of 5.63 ± 0.01 (p -value = 0.033) after admixing. The addition of Fer-FCM did not affect pH (p -value = 0.351), resulting in a pH of 5.52 ± 0.00 after admixing. During the 48 h incubation period, the pH did not change significantly (p -value = 0.07), regardless of the intravenous iron products used (Figure S4).

4. Discussion

In this study on the preparation of a new class of Fe drugs admixed to AIO PN admixtures, we demonstrated the stability of the iron–carbohydrate Fer-FCM complexes in two commonly used commercial AIO PN admixtures in the absence of added micronutrients. Furthermore, pH after admixing the iron–carbohydrate complexes changed depending on the medicinal product used but then remained stable. The addition of Ven-ISC increased the pH upon admixing, but no sedimentation of the measured minerals was observed. The dialysis approach for Ven-ISC, previously described by Hira et al. using a similar ISC, was adapted as a method to assess the stability for Fer-FCM in AIO PN admixtures [19]. The recovery and precision, prerequisites for a reliable and sensitive method, were high.

Two studies have previously assessed iron–carbohydrate admixing to PN. Both studies investigated Ven-ISC colloidal iron. MacKay et al. demonstrated the chemical stability of Ven-ISC in a non-lipid PN solution for patients weighing >20 kg [26]. However, the solutions were physically unstable for all iron doses measured from 10 to 100 mg/L, with visible particulate matter and crystal formation ($\geq 25 \mu\text{m}$) of iron with calcium and phosphorus within 24 h. Another study by Grand, Jalabert, Mercier, Florent, Hansel-Esteller, Cambonie, Steghens and Picaud [12] indicated instability when 1.5 mg of Ven-ISC was added to PN by measuring significantly increased malondialdehyde levels, a secondary decomposition product of lipid peroxidation. This effect was enhanced by exposure to light. These results indicate that the lipid emulsion, as well as the amino acid and glucose solutions, are sensitive to the addition of Ven-ISC. Lipid peroxidation may occur in lipid emulsions upon exposure to trace elements during storage and exposure to light [27]. Redox-sensitive ions (polycations), like iron and other metallic cations, trigger lipid peroxidation and catalyse further degradation to lipid radicals [11]. Importantly, previous studies by Grand et al. and MacKay et al. indirectly evaluated the stability of colloidal iron through the measurement of lipid peroxidation products such as hydroxypentenol, hydroxynonenol, and malondialdehyde and visual inspection combined with particle size quantification, respectively [12,26]. However, the absence of evidence does not guarantee the absence of other potential byproducts that were not included in the assessment, like radicals or peroxides. Therefore, conducting direct measurement of iron–carbohydrate complex stability with absence of free iron is crucial to ensure product safety.

The admixing or Y-site administration of drugs in or with AIO PN shows a risk of incompatibility because of the more than 50 reactive components present in that convenient PN–drug regimen. These incompatibilities require physicochemical laboratory testing for a wide range of physicochemical reactions, including PN stability and drug bioavailability, which cannot be addressed by extrapolation of bibliographic data [14,15]. The most important lipid stability upon admixture of drugs or electrolytes to an AIO PN carrier was investigated with a comparable approach for a hydrophilic drug [22] and a lipophilic representative [22,23]. In this study, we extend these *in vitro* investigations to a new class of pharmaceuticals. This is crucial as they are highly complex drugs with distinct characteristics [28]. The second-generation intravenous polymeric iron, Fer-FCM, with its specific carbohydrate ligands, appears to be a more stable *in vitro* and *in vivo* than previous first-generation intravenous iron–carbohydrate products, Ven-ISC or iron gluconate [17,29]. Unlike Ven-ISC, which has an alkaline pH that can destabilise the electrostatic repulsion of lipid droplets, Fer-FCM has a near-neutral pH. This preserves the negative surface charge (zeta potential) of the fat droplets and therefore the stability of the lipid emulsion in AIO PN admixtures [30,31]. Our findings of the proof-of-concept experiment support the stability of FCM formulations appearing to be superior to ISC products. However, in our experiments higher absolute iron doses and, considering the limited vehicle volume, higher concentrations such as 400 mg/L showed a rise in free iron content of 2 mg/L after 48 h and, therefore, exceed limits known to catalyse degradation of polyunsaturated fatty acids (0.56 mg/L equal to approx. 10 mM) according to Mozuraityte et al. [32]. Therefore, the current *in vitro* data suggest that an AIO PN bag containing FCM should be used within 24 h to avoid oxidative stress in a clinical situation.

Increased stability of the iron–carbohydrate complexes reduces the release of Fe^{3+} , a highly reactive iron species, and may therefore prevent redox reactions with toxic radical formation or Fe^{3+} precipitation. Our proof-of-concept experiments confirmed that a higher dose of free iron (0.4 mg) and a highly acidic pH led to emulsion failure. A recent review even postulates that Fer-FCM remains intact within macrophages in the blood circulation and iron is only metabolised or transferred to iron stores (bioavailability) after macrophagic digestion [24]. This would also serve as an argument in favour of administering

Fer-FCM as a single dose with PN upon requirement. Our study provides evidence that these Fer-FCM complexes remain intact in AIO PN admixtures, suggesting the prevention of lipid peroxidation and lipid destabilisation and, most importantly, preserving the complexes for their uptake into the reticuloendothelial system upon administration and admixture with AIO PN.

A key strength of the study was that we were able to assess the stability of the colloidal solution in a setting mimicking practical use. Complex-bound iron was added directly to the ready-to-use prepared AIO PN bags, stored at room temperature and without light protection. Our analyses were carried out over a time period of 48 h, which exceeds the recommendation for a maximal use period of 24 h [15] and may only exceptionally be extended to 36 (and 48) h for AIO PN administration, as stated in SmPC. Even within this time period and based on our measurements, no significant decrease in complex-bound iron was detected. We were able to adapt the novel stability assessment technique with high precision and recovery. Furthermore, we were able to eliminate with the step of saturating the tube surface with an ionic iron standard solution. We did not observe significant absorption of iron ions on the surface of the cellulose dialysis tube, as previously described by Hira, Suzuki, Kono, Shimokawa, Matsuoka, Hasumoto, Kawahara, Onoue, Fujita, Okano and Kakumoto [19], as indicated by the recovery rate of 95.8% to 103.9%, which falls within the range of the ICP-MS measurement error. Another strength is the quantification of the elements using ICP-MS, allowing for highly sensitive sample analysis, the possibility of multi-element measurements [20], and adaptation of the method for PN samples.

The limitations of our study were that the dialysis method tested was not suitable for Ven-ISC, which has a smaller molecular weight, at between 34 and 60 kDa, and particle diameter, 8 µm, compared to Fer-FCM (150 kDa and 23 µm) [24]. Dialysis tubes with a smaller pore size of 20 kDa showed recoveries of less than 60% in our experiments and were therefore not included in this investigation. This may be due to possible clogging of the pores by the lipid emulsion. The dialysis tube approach itself has limitations due to the time delay for the system to reach equilibrium. We minimised the dialysis time by selecting the shortest timepoint at which the iron concentration inside and outside the dialysis tube remained unchanged. Another limitation was that our analyses were restricted to adult AIO PN admixtures. However, most of the substrates used in compounding are common to both paediatric and adult PN admixtures, although the volumes and required doses of components may differ. However, the effect is expected to be small [33].

Caution is warranted when frequently administering high doses of iron to patients, as excess of iron can lead to iron toxicity, potentially resulting in iron accumulation in critical body organs like the liver, heart, and endocrine glands. Iron overload has serious clinical consequences, including death [34]. Patients receiving PN may be at increased risk of developing functional iron deficiency in the presence of inflammation. In such cases, iron supplementation may have adverse effects [35]. However, when patients have absolute iron deficiency with subclinical inflammation or without inflammation, intravenous iron supplementation has been shown to be both safe and effective in replenishing iron pools without being associated with serious adverse events [2].

The current research highlights the potential of colloidal iron to increase daily iron doses in patients with PN. However, the data do not provide sufficient evidence for implementation in clinical practice and cannot be extrapolated beyond the tested intravenous iron-carbohydrate complexes without additional specific testing. A small amount of free iron was still detected and a small increase of 0.004 to 0.018 mg/L per h was estimated. Future experiments should assess the stability of the lipid emulsion by measuring the size of individual lipid droplets in the upper range of between 2 and 5 (to 15) µm, which is

critical for occlusion of small vessels [36]. Quantification of lipid peroxidation would be appropriate for such iron–carbohydrate admixtures to AIO PN.

5. Conclusions

We conclude that the presented proof of concept and method allow for the estimation of iron carbohydrate stability in complex matrices such as AIO PN and may assist in the evaluation of further complex formulations where admixture of iron–carbohydrate complexes would be desirable. Further evidence of the safety of iron supplementation with AIO PN without an additional venous access line looks promising in light of the present *in vitro* investigation as a way to meet a clinical and patient care-related need. However, before clinical application, further *in vitro* studies on the stability of the emulsion are prerequisites.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/pharmaceutics18020255/s1>, Figure S1: Proof of concept for the complex-bound iron stability assessment; Figure S2: Destabilising ferric carboxymaltose in the form of Ferinject® (Fer-FCM) with glucose and parenteral nutrition containing EDTA compared to control with only parenteral nutrition solution; Figure S3: Concentration of complex-bound and free iron over 48 h measured after 5, 7, and 9 h dialysis; Figure S4: Change in pH over 48 h incubation period; Table S1: Proportion of complex-bound iron and overall iron recovery of the proof-of-concept experiment.

Author Contributions: Conceptualisation, V.V.H., P.J.N., C.S., and S.M.; methodology, V.V.H., P.J.N., C.S., and K.A.S.; validation, V.V.H., P.J.N., and C.S.; formal analysis, V.V.H. and P.J.N.; investigation, V.V.H., P.J.N., C.S., and K.A.S.; resources, V.V.H., C.S., and A.H.-L.; data curation, V.V.H. and P.J.N.; writing—original draft preparation, V.V.H.; writing—review and editing, V.V.H., P.J.N., C.S., K.A.S., A.H.-L., Z.S., and S.M.; visualisation, V.V.H. and P.J.N.; supervision, Z.S., A.H.-L., and S.M.; project administration, V.V.H. and C.S.; funding acquisition, S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a third-party grant of the Division of Clinical Pharmacy and Epidemiology, University of Basel [grant number FO119900]. Article processing charges (APC) were funded by the Forschungsrat, Kantonsspital Aarau.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author, VH.

Acknowledgments: This study was possible thanks to the valuable support of the laboratory staff of the Institute of Laboratory Medicine of the Cantonal Hospital Aarau and thanks to the statistical advice of the Department of Clinical Research at the University of Basel. Schemes were created using BioRender.com accessed 13 February 2026.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AIO	All-in-one
CI	Confidence interval
FCM	Ferric carboxymaltose
Fej-FCM	Ferinject®

ICP-MS	Inductively coupled plasma mass spectrometry
IDA	Iron deficiency anaemia
IS	Internal standard
ISC	Iron sucrose
KED	Kinetic energy discrimination
OM	Omegaflex special®
MΩ	Megaohms
PN	Parenteral nutrition
Rh	Rhodium
RPa	Retarding potential analyser
RPq	Retarding potential quadrupole
SD	Standard deviation
SmPC	Summary of product characteristics
SK	Smofkabiven®
Ven-ISC	Venofer®

References

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **2017**, *390*, 1211–1259. [https://doi.org/10.1016/s0140-6736\(17\)32154-2](https://doi.org/10.1016/s0140-6736(17)32154-2).
2. Pasricha, S.R.; Tye-Din, J.; Muckenthaler, M.U.; Swinkels, D.W. Iron deficiency. *Lancet* **2021**, *397*, 233–248. [https://doi.org/10.1016/s0140-6736\(20\)32594-0](https://doi.org/10.1016/s0140-6736(20)32594-0).
3. Lopez, A.; Cacoub, P.; Macdougall, I.C.; Peyrin-Biroulet, L. Iron deficiency anaemia. *Lancet* **2016**, *387*, 907–916. [https://doi.org/10.1016/s0140-6736\(15\)60865-0](https://doi.org/10.1016/s0140-6736(15)60865-0).
4. Hwa, Y.L.; Rashtak, S.; Kelly, D.G.; Murray, J.A. Iron Deficiency in Long-Term Parenteral Nutrition Therapy. *J. Parenter. Enter. Nutr.* **2016**, *40*, 869–876. <https://doi.org/10.1177/0148607115587329>.
5. Rao, R.; Georgieff, M.K. Iron therapy for preterm infants. *Clin. Perinatol.* **2009**, *36*, 27–42. <https://doi.org/10.1016/j.clp.2008.09.013>.
6. Schaefer, B.; Meindl, E.; Wagner, S.; Tilg, H.; Zoller, H. Intravenous iron supplementation therapy. *Mol. Asp. Med.* **2020**, *75*, 100862. <https://doi.org/10.1016/j.mam.2020.100862>.
7. Osland, E.J.; Ali, A.; Isenring, E.; Ball, P.; Davis, M.; Gillanders, L. Australasian Society for Parenteral and Enteral Nutrition guidelines for supplementation of trace elements during parenteral nutrition. *Asia Pac. J. Clin. Nutr.* **2014**, *23*, 545–554. <https://doi.org/10.6133/apjcn.2014.23.4.21>.
8. Pironi, L.; Arends, J.; Bozzetti, F.; Cuerda, C.; Gillanders, L.; Jeppesen, P.B.; Joly, F.; Kelly, D.; Lal, S.; Staun, M.; et al. Corrigendum to “ESPEN guidelines on chronic intestinal failure in adults” [Clin Nutr 35 (2) (2016) 247–307]. *Clin. Nutr.* **2017**, *36*, 619. <https://doi.org/10.1016/j.clnu.2016.12.008>.
9. Forbes, A. Iron and parenteral nutrition. *Gastroenterology* **2009**, *137*, S47–S54. <https://doi.org/10.1053/j.gastro.2009.08.013>.
10. Forbes, A. Corrected: Iron and parenteral nutrition. *Gastroenterology* **2010**, *139*, 360. <https://doi.org/10.1053/j.gastro.2010.06.001>.
11. Steger, P.J.; Mühlebach, S.F. Lipid peroxidation of intravenous lipid emulsions and all-in-one admixtures in total parenteral nutrition bags: The influence of trace elements. *J. Parenter. Enter. Nutr.* **2000**, *24*, 37–41. <https://doi.org/10.1177/014860710002400137>.
12. Grand, A.; Jalabert, A.; Mercier, G.; Florent, M.; Hansel-Esteller, S.; Cambonie, G.; Steghens, J.P.; Picaud, J.C. Influence of vitamins, trace elements, and iron on lipid peroxidation reactions in all-in-one admixtures for neonatal parenteral nutrition. *J. Parenter. Enter. Nutr.* **2011**, *35*, 505–510. <https://doi.org/10.1177/0148607110381768>.
13. Sobotka, L. *Basics in Clinical Nutrition*; GALEN: Tucson, AZ, USA, 2020.
14. Mühlebach, S. Basics in clinical nutrition: Drugs and nutritional admixtures. *e-SPEN Eur. E-J. Clin. Nutr. Metab.* **2009**, *3*, e134–e136.
15. Pironi, L.; Boeykens, K.; Bozzetti, F.; Joly, F.; Klek, S.; Lal, S.; Lichota, M.; Mühlebach, S.; Van Gossum, A.; Wanten, G.; et al. ESPEN practical guideline: Home parenteral nutrition. *Clin. Nutr.* **2023**, *42*, 411–430. <https://doi.org/10.1016/j.clnu.2022.12.003>.
16. Mühlebach, S.; Vulto, A.; de Vlieger, J.S.; Weinstein, V.; Flühmann, B.; Shah, V.P. The authorization of non-biological complex drugs (NBCDs) follow-on versions: Specific regulatory and interchangeability rules ahead. *GaBI J.* **2013**, *2*, 204–207.
17. Funk, F.; Flühmann, B.; Barton, A.E. Correction: Funk et al. Criticality of Surface Characteristics of Intravenous Iron-Carbohydrate Nanoparticle Complexes: Implications for Pharmacokinetics and Pharmacodynamics. *Int. J. Mol. Sci.* **2022**, *23*, 2140. *Int. J. Mol. Sci.* **2022**, *23*, 10230. <https://doi.org/10.3390/ijms231810230>.

18. Mühlebach, S.; Flühmann, B. Iron carbohydrate complexes: Characteristics and regulatory challenges. In *Non-Biological Complex Drugs: The Science and the Regulatory Landscape*; Springer: Cham, Switzerland, 2015; pp. 149–170.
19. Hira, D.; Suzuki, A.; Kono, Y.; Shimokawa, K.; Matsuoka, S.; Hasumoto, K.Y.; Kawahara, H.; Onoue, M.; Fujita, T.; Okano, T.; et al. Pharmaceutical stability of colloidal saccharated iron oxide injection in normal saline. *J. Pharm. Health Care Sci.* **2018**, *4*, 21. <https://doi.org/10.1186/s40780-018-0116-0>.
20. Wilschefschi, S.C.; Baxter, M.R. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *Clin. Biochem. Rev.* **2019**, *40*, 115–133. <https://doi.org/10.33176/aacb-19-00024>.
21. Neiser, S.; Rentsch, D.; Dippon, U.; Kappler, A.; Weidler, P.G.; Göttlicher, J.; Steininger, R.; Wilhelm, M.; Braitsch, M.; Funk, F.; et al. Physico-chemical properties of the new generation IV iron preparations ferumoxytol, iron isomaltoside 1000 and ferric carboxymaltose. *Biometals* **2015**, *28*, 615–635. <https://doi.org/10.1007/s10534-015-9845-9>.
22. Aeberhard, C.; Steuer, C.; Saxer, C.; Huber, A.; Stanga, Z.; Mühlebach, S. Physicochemical stability and compatibility testing of levetiracetam in all-in-one parenteral nutrition admixtures in daily practice. *Eur. J. Pharm. Sci.* **2017**, *96*, 449–455. <https://doi.org/10.1016/j.ejps.2016.10.015>.
23. Reber, E.; Neyer, P.; Schönenberger, K.A.; Saxer, C.; Bernasconi, L.; Stanga, Z.; Huber, A.; Hammerer-Lercher, A.; Mühlebach, S. Physicochemical Stability and Compatibility Testing of Voriconazole in All-in-One Parenteral Nutrition Admixtures. *Pharmaceutics* **2021**, *13*, 1447. <https://doi.org/10.3390/pharmaceutics13091447>.
24. Alston, A.B.; Digigow, R.; Flühmann, B.; Wacker, M.G. Putting square pegs in round holes: Why traditional pharmacokinetic principles cannot universally be applied to iron-carbohydrate complexes. *Eur. J. Pharm. Biopharm.* **2023**, *188*, 6–14. <https://doi.org/10.1016/j.ejpb.2023.04.025>.
25. R Core Team. R: A Language and Environment for Statistical Computing; R Core Team: Vienna, Austria, 2023.
26. MacKay, M.; Rusho, W.; Jackson, D.; McMillin, G.; Winther, B. Physical and chemical stability of iron sucrose in parenteral nutrition. *Nutr. Clin. Pr.* **2009**, *24*, 733–737. <https://doi.org/10.1177/0884533609351528>.
27. Hardy, G.; Austin, P.D.; Davis, M.R.; Manzanares, W.; Mühlebach, S.F.; Pietka, M.; Allwood, M.C. Photoprotection of parenteral nutrition: An international perspective. *Nutr. Clin. Pr.* **2021**, *36*, 921–925.
28. Mühlebach, S. How to Deal with Complexity of Nanomedicine in Practice. *Acta Pharm. Hung.* **2021**, *91*, 114–116.
29. Krupnik, L.; Joshi, P.; Kappler, A.; Flühmann, B.; Alston, A.B.; Digigow, R.; Wick, P.; Neels, A. Critical nanomaterial attributes of iron-carbohydrate nanoparticles: Leveraging orthogonal methods to resolve the 3-dimensional structure. *Eur. J. Pharm. Sci.* **2023**, *188*, 106521. <https://doi.org/10.1016/j.ejps.2023.106521>.
30. Geisser, P.; Burckhardt, S. The pharmacokinetics and pharmacodynamics of iron preparations. *Pharmaceutics* **2011**, *3*, 12–33. <https://doi.org/10.3390/pharmaceutics3010012>.
31. Heurtault, B.; Saulnier, P.; Pech, B.; Proust, J.E.; Benoit, J.P. Physico-chemical stability of colloidal lipid particles. *Biomaterials* **2003**, *24*, 4283–4300. [https://doi.org/10.1016/s0142-9612\(03\)00331-4](https://doi.org/10.1016/s0142-9612(03)00331-4).
32. Mozuraityte, R.; Rustad, T.; Storror, I. The role of iron in peroxidation of polyunsaturated fatty acids in liposomes. *J. Agric. Food Chem.* **2008**, *56*, 537–543. <https://doi.org/10.1021/jf0716073>.
33. Phillips, S.K. Pediatric parenteral nutrition: Differences in practice from adult care. *J. Infus. Nurs.* **2004**, *27*, 166–170. <https://doi.org/10.1097/00129804-200405000-00005>.
34. Kohgo, Y.; Ikuta, K.; Ohtake, T.; Torimoto, Y.; Kato, J. Body iron metabolism and pathophysiology of iron overload. *Int. J. Hematol.* **2008**, *88*, 7–15. <https://doi.org/10.1007/s12185-008-0120-5>.
35. Lanser, L.; Fuchs, D.; Kurz, K.; Weiss, G. Physiology and Inflammation Driven Pathophysiology of Iron Homeostasis-Mechanistic Insights into Anemia of Inflammation and Its Treatment. *Nutrients* **2021**, *13*, 3732. <https://doi.org/10.3390/nu13113732>.
36. Driscoll, D.F. Globule-size distribution in injectable 20% lipid emulsions: Compliance with USP requirements. *Am. J. Health Syst. Pharm.* **2007**, *64*, 2032–2036. <https://doi.org/10.2146/ajhp070097>.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.