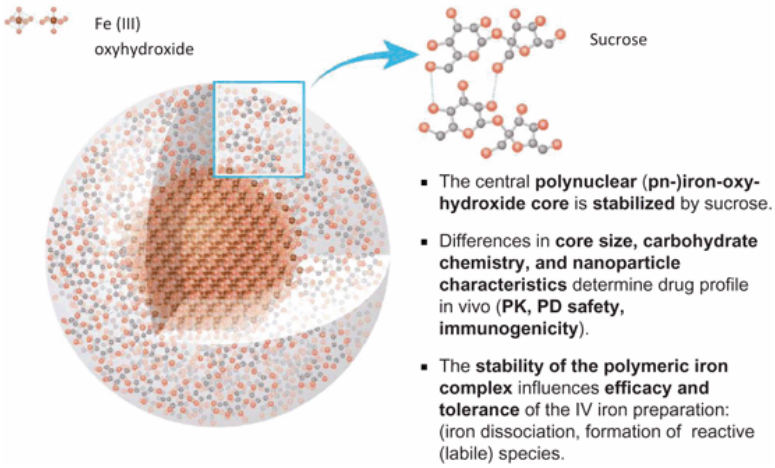
INTRODUCTION (Product Summary on final page)

The intravenous (IV) iron agents are colloids that consist of spheroidal iron-carbohydrate nanoparticles. At the core of each particle is an iron-oxyhydroxide gel and the core is surrounded by a shell of carbohydrate that stabilizes the iron-oxyhydroxide (the main function of the ligand is to stabilize the complex and to protect it against further polynuclearization).



Iron carbohydrate complexes behave as prodrugs, since the iron has to be released from the iron(III)-hydroxide core. According to the proposed mechanism, after administration, the stable (Type 1) complexes such as ferric carboxymaltose and iron dextran are taken up by endocytosis by macrophages of the reticuloendothelial system[1] (RES).

In the case of less stable iron(III)-carbohydrates (Type 2), significant amounts of labile iron from the complex can be released and lead to saturation of transferrin and, thus, to significant amounts of non-transferrin bound iron (NTBI), particularly if high doses are administered. This weakly bound Fe3+ is readily taken up in an unregulated way by cells and can induce oxidative stress.[2]

There are five types of injectable iron-carbohydrate products currently approved by the FDA:

Iron Complex Type	Type 1 Iron Complexes			Type 2 Iron Complex	Type 3/4 Iron Complex
Trade Name/ (Chemical Name)	INFeD®/ Dexferrum® (Iron dextran)	Ferahem® (ferumoxytol)	Injectafer® (ferric carboxymaltose)	Venofer® (Iron sucrose)	Ferrlecit® (Sodium ferric gluconate complex)
FDA Approval	1974	2009	2013 (submitted 2006)	2000 (marketed in EU since 1950's)	1999 (marketed in EU since 1950's)
Population	Adults and Pediatrics	Adults	Adults	Adults	Adults and Pediatrics
Company	Allergan/ Vifor Pharma	AMAG Pharmaceuticals	Luitpold Pharmaceuticals	Vifor Pharma	Sanofi; Watson
Other (non-US)	Cosmofer®; Imferon® (withdrawn) Dexlron/Infufer (Canada)		Jectofer® (withdrawn)	Sucrofer®(UK); Fermed®, Ferion®, Ferrologic®(EU) and FER® (France)	

[1] Danielson, J. Structure, chemistry, and pharmacokinetics of intravenous iron agents. Am. Soc. Nephrol. 2004, 15, S93-S98.

[2] Evans, R.W.; Rafique, R.; Zarea, A.; Rapisarda, C.; Cammack, R.; Evans, P.J.; Porter, J.B.; Hider, R.C. Nature of non-transferrin-bound iron: studies on iron citrate complexes and the thalassemic era. J. Biol. Inorg. Chem. 2008, 13, 57-74.

ANALYTICAL WORK

In this analytical work (performed at EAG), three samples of Injectable Iron/Sugars were tested at EAG in this work: Renibus FeS Iron Sucrose, Venofer Iron Sucrose and INFeD Iron Dextran as a comparison (to show Type 1 vs Type 2 properties).

Iron Complex	**Type 2 Iron Complex**		**Type 1 Iron Complex**
Sample #	S1	S2	S3
Description	Renibus FeS Sterile liquid	Venofer® (Iron Sucrose USP) 20mg/mL; elemental iron 20mg/mL	INFeD (Iron Dextran USP) 50mg/mL; elemental iron 50mg/mL
Lot #	AK2087	9043 exp Feb '21	18W11A exp Oct '21
Shorthand	Renibus	Venofer	INFeD

The Type 1 and Type 2 complexes have different pharmacokinetics, which are driven by their physiochemical properties:

Complex	Type 1	Type 2	Type 3 (Type 4 are mixtures)
Characteristics	Robust Strong	Semi-robust Moderately Strong	Labile Weak
Molecular weight (Daltons)	> 100,000	30,000-100,000	< 50,000 (~18,000)
In vitro degradation kinetics (k x 10^3/min at theta=0.5)	15-50	50-100	>100
LD_{50} (mg iron/kg)	1,013	359	155

Overall molecular weight affects two biologic characteristics of IV iron agents:

- **Iron release in-vivo:** Rate of release of iron from the ferric hydroxide core; Iron release in vitro is related to total molecular weight in an inverse log-log manner.
- **Rate of clearance of agent from the plasma**: The clearance rate of IV iron agents from plasma ranges depends on the molecular weight. In general, the lower the overall molecular weight, the more rapid the clearance of agent from plasma after an IV dose.

The FDA has indicated[3] the following physiochemical aspects are important for the biological activity of an Iron Sucrose drug:

Particle Size Parameters to measure: *D_{10}, D_{50}, D_{90}*

Bioequivalence based on: *D50 and SPAN [i.e. $(D_{90}-D_{10})/D_{50}$] or polydispersity index using the population bioequivalence statistical approach.* ***Special Considerations:*** *The proposed parenteral drug product should be qualitatively (Q1) and quantitatively (Q2) the same as the RLD. Equivalence in the stoichiometric ratios of iron, sucrose, and other relevant components need to be established. Sameness in physicochemical properties needs to be established. These in vitro characterizations should be conducted on at least three batches of the ANDA and RLD. Attributes that should be included in the characterization are:*

- *Iron core characterizations including but not limited to core size determination, iron oxide crystalline structure and iron environment.*
- *Composition of carbohydrate shell and surface properties.*
- *Particle morphology.*

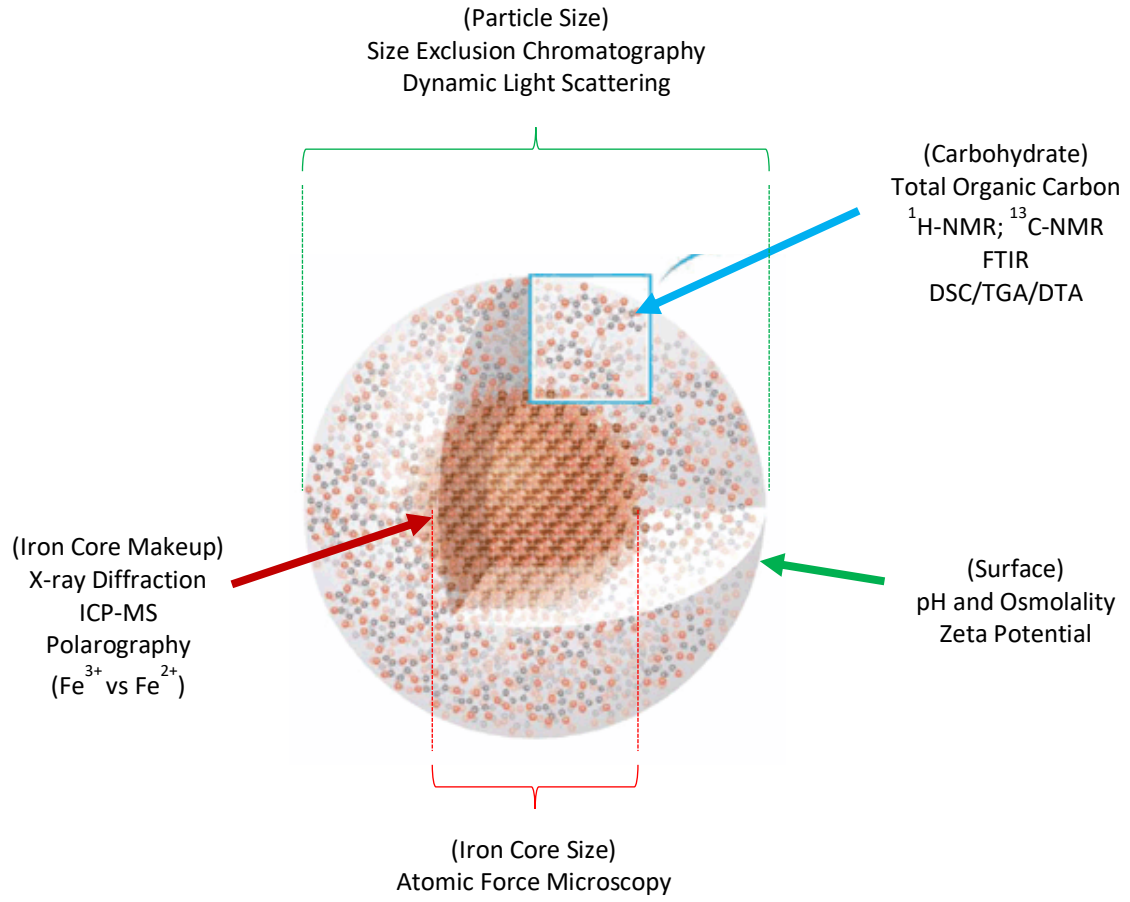
Other physiochemical properties can affect the biologic activity and a comprehensive physiochemical analysis of the Renibus Iron Sucrose vs Venofer was executed, with a comparison against a Type 1 Iron-carbohydrate complex (Iron-dextran) also shown.

[3] FDA Draft Guidance on Iron Sucrose Bioequivalence; 2012. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM297630.pdf

Testing of Iron Sucrose Particle (summary)

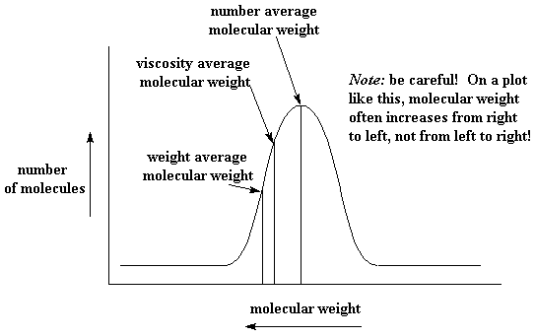
The key testing involved can be broken down in the following categories that affect iron delivery *in-vivo*:

Size: Range and mean size of inner core, Size of Overall particle, Mw of iron-sugar complex
Surface: Charge, pH
Iron Core: Iron oxidation state, molecular weight, crystalline structure, free iron, impurities
Sugar Coating: Iron:sugar ratio, chemical structure, complexation strength



TEST RESULTS

Molecular weight by Gel Permeation Chromatography (GPC) and Particle size by dynamic light scattering (DLS)



ANALYSIS		S1 Renibus	S2 VENOFER	S3 INFED
GPC	Mp (molecular weight of biggest peak)	29,239	35,709	83,090
	Mw (weight average molecular weight)	34,355	50,855	92,838
	Mn (number average molecular weight)	23,881	31,345	70,640
	PDI (polydispersity)	1.44	1.62	1.31
DLS	average	15.30 nm	15.41 nm	16.88 nm

Mw and Mn: The weight average and number average molecular weight determine the (a) iron release rate and (b) clearance rate. The Renibus material has is on the low end of class 2 iron-carbohydrate complexes.

In practical terms, this means the Renibus material with lower Mn and Mw, will have a *faster* uptake of iron into the reticuloendothelial system than Venofer.

Polydispersity: A polydispersity of 1.00 is when all the complex molecular weights are identical and the higher polydispersity, the higher the variability in the molecular weights. Renibus material polydispersity is lower than Venofer (1.44 vs 1.62), which means the iron-sucrose complexes of the Renibus material have less variability than Venofer.

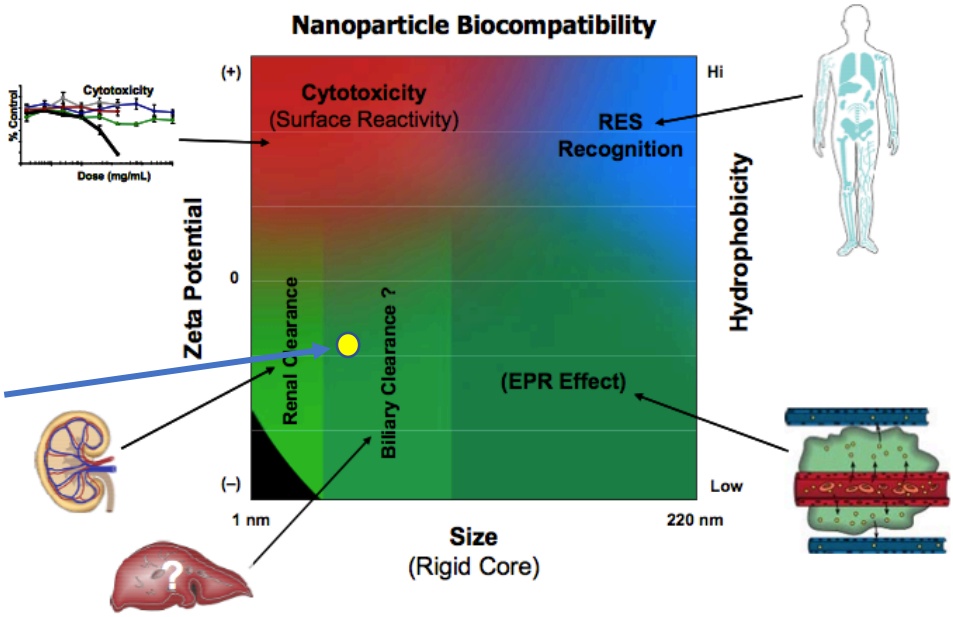
What is interesting is that the Renibus particle is lower molecular weight complex, but the nearly the same size particle as Venofer (15.30 vs 15.41nm). This implies a lower density/lower crystallinity of the Renibus Iron-sucrose, which is supported by the XRD testing (see later test results).

Surface Charge (Zeta Potential)

ANALYSIS	S1	S2	S3
	Renibus	VENOFER	INFED
Particle size by DLS (average)	15.30 nm	15.41 nm	16.88 nm
Zeta Potential	-10.16 mV	n/a	-2.61 mV
Zeta Potential Temp.	25.0 °C	n/a	25.0 °C
pH	10.70	n/a	10.23

EAG was unable to get a zeta potential readout on the Venofer sample due to a very weak signal. The literature[4] zeta potential value of Venofer at high pH (11) is around -28mV.

On the Renibus sample, the zeta potential was -10.16mV, and although we can't compare directly against the Venofer in this sample run (head to head), the experiment does confirm that there will be little surface toxicity/cytotoxicity generated by the surface potential of the Renibus iron sucrose as its zeta potential is negative (see below overview on nanoparticle biocompatibility and zeta potential).



Since the Renibus Iron Sucrose and the Venofer are the same particle size, the non-RES clearance rates (L/h) for the two particles is expected to be similar.

[4] Markus R. Jahn, etal, "*A comparative study of the physicochemical properties of iron isomaltoside 1000 (Monofer_), a new intravenous iron preparation and its clinical implications*"; European Journal of Pharmaceutics and Biopharmaceutics 78 (2011) 480–491

Iron Core Size by Atomic Force Microscopy and total Iron Content

At the nanoparticle level, the size of iron core has an important implication for the core surface area available for dissociation and release of the reduced ferrous iron from the colloidal ferric oxyhydroxide cores.

Imaging of iron-carbohydrate nanoparticles using atomic force microscopy distinguishes the iron-oxyhydroxide core from the carbohydrate shell and permits direct determination of core size.

AFM ANALYSIS	S1 Renibus		S2 VENOFER		S3 INFeD	
Location	1	2	1	2	1	2
Mean Height	**2.38 nm**	**2.43 nm**	**3.88 nm**	**3.49 nm**	**4.20 nm**	**3.23 nm**
Min Height	1.34 nm	1.16 nm	0.99 nm	1.20 nm	1.19 nm	0.91 nm
Max Height	3.62 nm	3.73 nm	8.35 nm	7.76 nm	10.19 nm	7.23 nm
σ	0.61	0.73	1.53	1.33	1.46	1.47
# Particles	21	29	84	52	117	49

The Renibus Iron Sucrose nanoparticle has a core that is significantly smaller than the Venofer Iron Sucrose, but the overall nanoparticle size, Renibus vs Venofer, is the same (ie, the non-absorption clearance rate will be the same Renibus vs Venofer and high renal clearance is not expected for Renibus iron sucrose).

ANALYSIS	S1 Renibus	S2 VENOFER	S3 INFeD
Particle size by DLS (average)	15.30 nm	15.41 nm	16.88 nm
Iron core	2.41 nm	3.69 nm	3.72 nm

This translates into a lower overall iron content in the Renibus Iron Sucrose (per particle), which is supported by the both the titration and ICP-OES data:

	S1 Renibus	S2 VENOFER	S3 INFeD
Osmolality	1540 mOsm/kg	1681 mOsm/kg	529 mOsm/kg
ICP-OES (Total Iron)	1.07 wt%	1.77 wt%	4.51 wt%
Total Fe (titration)	11.87 mg/mL	20.02 mg/mL	51.33 mg/mL
Label Strength	**= 12mg/mL**	**20mg/mL**	**50mg/mL**
Total Organic Carbon	7.69%	12.14%	8.69%

The Renibus and Venofer material have similar osmolarity (ie, # of particles/mg) and the Renibus material has a significantly lower label strength of iron (12mg/mL) vs Venofer (20mg/mL); the titration and ICP-OES data align with the label data for Venofer and INFeD.

The lower iron content is tracked by the similarly lower Total Organic Carbon, indicating that the overall *Iron:Sucrose ratio* is similar (Renibus:Venofer). The NMR and the FTIR data confirm that the TOC is in the form of sucrose and not some other organic species and the TGA and DSC indicate similar degradation profiles, showing that there is an iron-sucrose complex (and the amorphous material does NOT reflect significant quantities of unbound/non-complexed sucrose present), showing that the differences are most likely crystallinity and hydration related.

When the crystallinity is considered, the Renibus Iron sucrose is almost completely amorphous as compared to the Venofer material, which is approximately 40% crystalline.

S1		**S2**		**S3**	
Renibus		**VENOFER**		**INFED**	
XRD (lyophilized material)	Phases Detected (wt%)	XRD (lyophilized material)	Phases Detected (wt%)	XRD (lyophilized material)	Phases Detected (wt%)
$Na_4Fe_2O_5$ – Sodium Iron Oxide		$C_{12}H_{22}O_{11}$ – Sucrose		$Na_4Fe_2O_5$ – Sodium Iron Oxide	
Monoclinic, SG: P21/n (14)		Monoclinic, S.G.: P21 (4)		Monoclinic, SG: P21/n (14)	
PDF# 04-013-8809	5.2	PDF# 02-063-8998	42.9	PDF# 04-013-8809	18.8
Amorphous materials	**94.8**	Amorphous materials	**57.1**	Amorphous materials	**81.2**

Additionally, the sodium content is significantly higher (~2x higher in Renibus vs Venofer), indicating that, in addition to a lower density and crystallinity, the molecular formula for Renibus Iron Sucrose is different than that for Venofer, with significantly more sodium.

ANALYSIS	**S1** **Renibus**	**S2** **VENOFER**	**S3** **INFED**
TOC	7.69%	12.14%	8.69%
ICP-OES (Total Iron)	1.07 wt%	1.77 wt%	4.51 wt%
Total Fe (titration)	11.87 mg/mL	20.02 mg/mL	51.33 mg/mL
Total Na	1.26 wt%	0.50 wt%	0.42 wt%

Venofer = $[Na_pFe_5O_8(OH) \cdot 3(H_2O)]_n \cdot m(C_{12}H_{22}O_{11})$; $p = 2$
Renibus = $[Na_pFe_5O_8(OH) \cdot x(H_2O)]_n \cdot m(C_{12}H_{22}O_{11})$; $p = 4$ *and* $x > 3$*?*

Analysis	**S1** **Renibus**	**S2** **VENOFER**	**S3** **INFED**
Acid Degradation for Labile Iron (III)	1.48%	2.27%	1.34%

Additionally, the quantity of uncomplexed (labile) iron in Renibus material is lower than in Venofer, indicating that the iron in Renibus iron sucrose is NOT the toxic free inorganic iron hydroxide and is primarily the desired iron-sucrose complex and the Renibus material should have lower toxicity than Venofer.

Iron Fe(III) vs Fe(II)

The Renibus Iron Sucrose contains significantly less Fe(II) than Venofer (on a % basis); the biological effect of injected Iron(II) vs Iron(III) is no, but Iron(II) is expected to increase the generation of free radicals and oxidative stress.[5]

ANALYSIS	S1 Renibus	S2 VENOFER	S3 INFED
Total Fe (titration)	11.87 mg/mL	20.02 mg/mL	51.33 mg/mL
Fe(III)	11.43 mg/mL	16.90 mg/mL	50.90 mg/mL
Fe(II)	0.41 mg/mL	3.16 mg/mL	0.44 mg/mL
% Fe(II) (calculated)	3.4%	15.8%	0.8%

The tested Venofer Fe(II) content agrees well with the literature values:

Table 1 Fe(II) and total iron content of intravenous iron formulations

Iron formulation	Lot number Expiry date	Percentage Fe(II) iron	Total iron	
			Measured value (mg/mL)	Labeled value (mg/mL)
Iron dextran (InFed™)	14W21A Oct 2017	0.8	49	50
Ferumoxytol (Feraheme™)	ACO386 Feb 2019	0.26	30	30
Ferric carboxymaltose (Injectafer™)	1501901 Feb 2017	1.4	48	50
Iron gluconate (Ferrlecit™)	A4040 Nov 2017	1.4	12.7	12.5
	A5063 April 2018	1.8	–[a]	–[a]
	Average	1.6		
Iron sucrose (Venofer™)	4351A Nov 2016	10.2	19.4	20
	5163 May 2017	15.5	–[a]	–[a]
	5127A Mar 2017	11.0	–[a]	–[a]
	Average	12.2		

[a] Total iron content was not determined for these lot numbers because analysis of total iron content for the first lot and for the other iron formulations generated label values

[5] Ajay Gupta, Raymond D. Pratt, Alvin L. Crumbliss; "*Ferrous iron content of intravenous iron formulations*"; Biometals (2016) 29:411–415

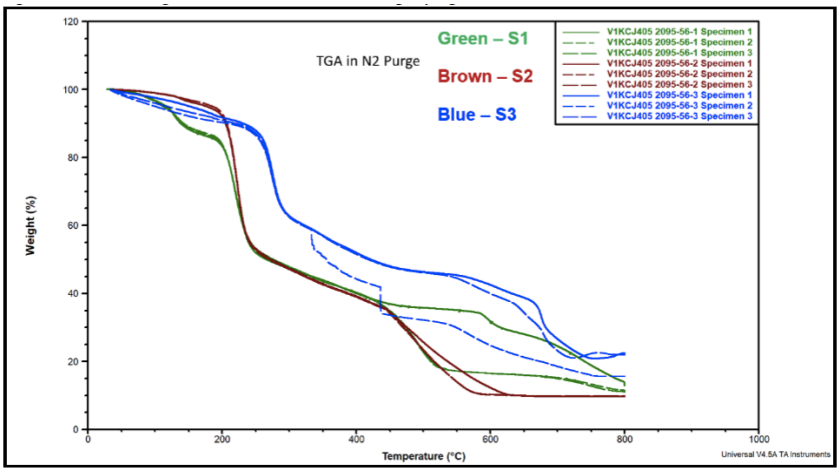
DSC/TGA

The DSC of the Renibus and the Venofer have similar Temperature of initial exotherm, which correlates well with the crystallization of the amorphous material (and the correspondingly larger exotherm (delta H) is proportional to the differences in the quantities of amorphous iron sucrose.

DSC	S1 Renibus	S2 VENOFER	S3 INFED
Texo1 (°C)	33.8	29.2	39.2
ΔHexo1 (J/g)	88.0	47.6	99.9
Texo2 (°C)	154.9	144.6	N/A
Onset Texo2 (°C)	141.0	127.1	N/A

The TGA of the Renibus Iron sucrose shows a larger weight loss < 200C which correlates to a higher % water content of the Renibus iron sucrose. The higher temperature degradation correlates well with the disassociation of the iron-sucrose complexes and sucrose degradation, indicating the two complexes have similar iron-sucrose bonds.

TGA		S1 Renibus	S2 VENOFER	S3 INFED
Temp.		Weight Loss (%)		
RT to 100°C	Nitrogen	3.4	1.1	3.7
	Air	2.5	0.9	4.7
100°C to 245°C	Nitrogen	42.7	45.0	8.2
	Air	43.2	43.0	7.8



PRODUCT SUMMARY

Renibus vs Venofer

Label Strength:	12mg/mL Renibus vs 20mg/mL Venofer (Fe content)
Renal Clearance Rate:	Expected to be the same (based on size of nanoparticle)
RES Absorption:	Faster for Renibus (based on 20% lower MW complex)
Fe Mobilization/RES:	Renibus cellular processing expected to be faster (based on lack of crystallinity)
Labile Iron:	Less % labile (toxic) Iron in Renibus than Venofer
Iron-Sucrose:	Similar Fe-Sucrose chemical bonding (based on NMR/FTIR/TGA)
Iron:Ratio:	Similar Fe:Sucrose ratio
Fe-Sucrose Stability:	Similar (based on TGA)
Water Content:	Renibus Higher (based on TGA)
Fe(II) level:	Venofer much higher level of Fe(II) than Renibus
Na Content:	Renibus much higher level of Na than Venofer (higher Fe(III)-OH solubility)