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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2017

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Mensink, M. (2017). *Solid state stabilization of proteins by sugars: Why size and flexibility matter*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen.

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Influence of miscibility of protein-sugar lyophilizates on their storage stability

AAPS Journal 2016, volume 18, pages 1225-1232

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ABSTRACT

For sugars to act as successful stabilizers of proteins during lyophilization and subsequent storage, they need to have several characteristics. One of them is that they need to be able to form interactions with the protein and for that miscibility is essential. To evaluate the influence of protein-sugar miscibility on protein storage stability, model protein IgG was lyophilized in the presence of various sugars of different molecular weight. By comparing solid state nuclear magnetic resonance spectroscopy relaxation times of both protein and sugar on two different timescales, i.e. $^1\text{H } T_1$ and $^1\text{H } T_{1\rho}$, miscibility of the two components was established on a 2-5 and a 20-50 nm length scale, respectively, and related to protein storage stability. Smaller sugars showed better miscibility with IgG and the tendency of IgG to aggregate during storage was lower for smaller sugars. The largest sugar performed worst and was phase separated on both length scales. Additionally shorter protein $^1\text{H } T_1$ relaxation times correlated with higher aggregation rates during storage. The ELISA assay showed overlapping effects of aggregation and Maillard browning and did not correspond as well with the miscibility. Because of the small scale at which miscibility was determined (2-5 nm) and the size of the protein domains ($\sim 2.5 \times 2.5 \times 5$ nm) the miscibility data give an indirect measure of interaction between protein and sugar. This reduced interaction could be the result of steric hindrance, providing a possible explanation as to why smaller sugars show better miscibility and storage stability with the protein.

INTRODUCTION

Protein pharmaceuticals have become increasingly important in the pharmaceutical industry over the past decade, with 10 out of the 41 drugs approved by the American Food and Drug Administration (FDA) in 2014 being proteins.¹ Most protein formulations are produced as solutions requiring refrigerated storage and handling, the so-called cold chain. This is both costly and impractical. The cold chain can be circumvented by drying the protein formulation in the presence of a stabilizer, using methods such as lyophilization and spray drying.^{2,3} For these situations, sugars are often used as stabilizers.

Much research has been done into the mechanism by which these sugars stabilize proteins both during drying and storage,⁴⁻⁶ producing several widely discussed theories regarding these mechanisms (e.g. vitrification theory, the water replacement theory and refinements focusing on local mobility of the protein).^{5,7} For each of those theories interactions between protein and sugar are needed, and for that miscibility of the protein and sugar is a necessary condition.

To qualify solids as miscible or immiscible, it is important to define the scale of this miscibility and the technique used to quantify miscibility. Two substances can appear miscible using a technique that measures a larger domain size, whilst showing phase separation using a technique that measures a smaller domain size. Therefore, a definition of miscibility should be accompanied by a qualification of the scale at which it was established. In this chapter, miscibility was determined using solid state Nuclear Magnetic Resonance spectroscopy (ssNMR). The method uses the concept of spin diffusion to determine the homogeneity of mixed systems.⁸ In homogeneous systems spin diffusion causes magnetization transfer between neighboring nuclei, resulting in similar relaxation times for both nuclei. For phase separated systems magnetization transfer does not occur and the relaxation times of different components are not averaged.

Spin diffusion is a time dependent phenomenon and the length scale of spin diffusion L is correlated to the relaxation time as follows:

$$(7.1) \quad L = \sqrt{6Dt}$$

D is the spin diffusion coefficient, which is typically assumed to be $10^{-12} \text{ cm}^2/\text{s}$ ^{9,10} and t is the relaxation time. When relaxation times of different timescales are compared, this principle allows differentiation of homogeneity on different length scales. In particular, a spin-lattice relaxation time ($^1\text{H } T_1$) is typically 1-5 s, corresponding to a spin diffusion over a distance of approximately 20-50 nm, while a rotating frame spin-lattice relaxation time ($^1\text{H } T_{1\rho}$) is usually 5-50 ms during which spin diffusion occurs over a distance of around 2-5 nm.

By comparing $^1\text{H } T_1$ and $^1\text{H } T_{1\rho}$ relaxation times of different components in a mixture, in this

case protein and sugar, it is thus possible to establish the intimacy of mixing on two different length scales (figure 7.1). If a system is fully miscible, both the relaxation times will be similar for both components. If a system is phase separated, both the ${}^1\text{H } T_1$ and ${}^1\text{H } T_{1\rho}$ values will be different for the sugar and protein. When the ${}^1\text{H } T_{1\rho}$ values are different, but the ${}^1\text{H } T_1$ values are similar, this indicates phase separation on the 2-5 nm scale, but miscibility on the 20-50 nm scale (partial phase separation). Using these relaxation times, it is thus possible to determine miscibility of these systems on two different length scales.

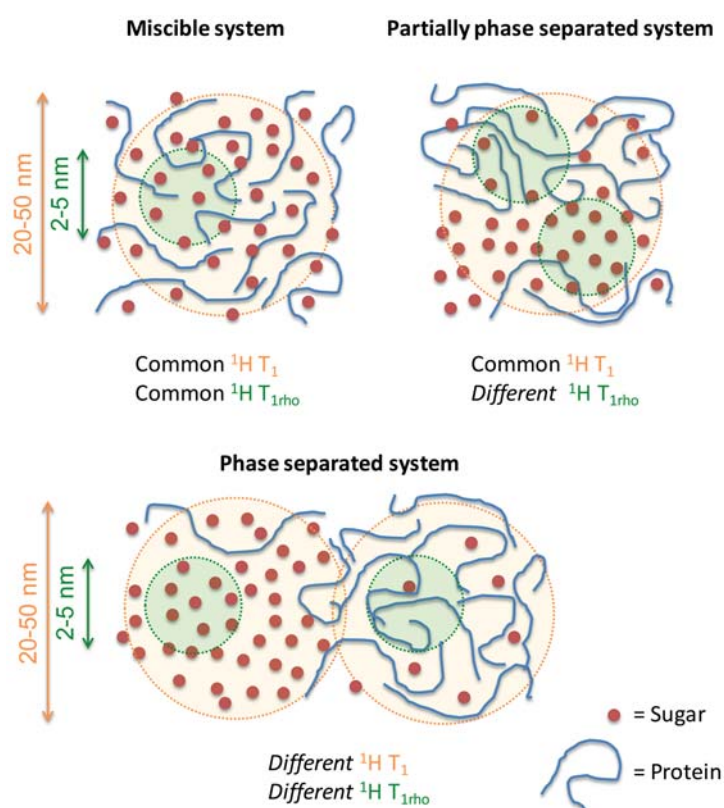


Figure 7.1 Illustration of the effect of spin diffusion on measured area for three different degrees of miscibility. Modified with permission from Yuan *et al.*⁸. Copyright 2015 American Chemical Society.

Recently it was shown that larger and molecularly more rigid sugars (e.g. oligo- and polysaccharides with a backbone through the sugar ring) are less efficient stabilizers of proteins than smaller sugars (e.g. disaccharides).¹¹ It was found that these larger sugars form fewer hydrogen bonds with the protein during the last part of lyophilisation.¹² This

lack of interactions could be responsible for phase separation in these protein-sugar mixtures. Therefore, the influence of protein-sugar miscibility on protein stability will be investigated in this chapter.

MATERIALS & METHODS

MATERIALS

Immunoglobulin G (IgG, from bovine serum) was obtained from Sigma-Aldrich U.S.A. (St. Louis, MO, United States of America). Trehalose was acquired from Ferro Pfanstiehl (Waukegan, IL, United States of America) and dextrans 1.5, 5 and 70 kDa from Pharmacosmos (Holbaek, Denmark). Inulins 1.8 kDa and 4 kDa were generous gifts from Sensus (Roosendaal, The Netherlands). Sodium phosphate buffer components used for the formulations and dialysis (monobasic as monohydrate, dibasic as heptahydrate) were purchased at Fisher Scientific.

FORMULATION PREPARATION

IgG was received frozen and thawed slowly on ice prior to use. The protein solution (IgG) was dialyzed at least 3 times for a minimum of 3 hours against 2 mM sodium phosphate buffer (pH 7.5, SPB) at 4 °C using a Spectra/Por® 6 dialysis tube membrane with a molecular weight cut-off of 2000 Da. After dialysis, the protein solution was filtered using Millex-GV low-protein binding PVDF syringe filters with a 0.22 µm pore size and 33 mm diameter. Subsequently protein concentration was determined spectroscopically at 280nm, using the absorption coefficient $A_{1\text{cm}}^{1\%} = 14$.¹³ The protein solution concentration was adjusted to 10.71 mg/mL by dilution with filtered SPB. Sugar solutions were prepared by dissolving sugars in SPB to a concentration of 100 mg/mL. If needed, solutions were heated to dissolve the sugar. All formulations were also filtered using 0.22 µm PVDF filters. Protein and sugar solutions were mixed to achieve an end concentration of 6 mg/mL protein and 44 mg/mL sugar (12% protein on dry solids basis). When IgG and dextran 70 kDa were mixed, some hazing was observed in the resulting solution. The solutions were transferred to Schott Fiolax® tubular vials of 10 mL with a 13 mm opening. For samples for physico-chemical testing a fill volume of 5 mL was used, for stability testing a fill volume 1 mL was used.

LYOPHILIZATION

Freeze-drying was conducted using a SP Scientific LyoStar 3 (Warminster, PA, USA) equipped with Praxair ControLyo™ controlled ice nucleation technology. Samples were placed on a shelf pre-cooled at 5 °C and kept at that temperature for 30 minutes. The temperature was then lowered to -5 °C at a rate of 1 °C/min and held at that temperature for 30 minutes.

Controlled nucleation was then induced, followed by further cooling to -40 °C at a rate of 1 °C/min. After 60 minutes at that temperature, the pressure was lowered to 87 μBar and the temperature was raised to -25 °C at 1 °C/min. Sample temperature of at least 5 vials was monitored using thermocouples. Shelf temperature was lowered to -28 °C if primary drying was not done after 30 hours or if the product temperature would exceed -33 °C. The end of primary drying was established by comparative pressure measurement (i.e. Pirani vs. MKS Baratron).¹⁴ After primary drying was completed, the shelf temperature was slowly raised to 40 °C at a rate of 0.1 °C/min and secondary drying was continued for 6 hours at this temperature. Lastly, the vials were stoppered in the freeze-drier at 87 μBar using Flurotec™ stoppers and were sealed with aluminum crimpers upon removal from the freeze-drier. All samples were stored at -20 °C up until the moment of analysis. Water content of the lyophilizates was determined using Karl Fisher coulometry and was found to be ≤0.25% for all tested formulations.

SOLID STATE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

All solid state NMR spectra were acquired using a Tecmag Redstone HF3 (Tecmag, Houston, TX) spectrometer operating at 100.6 MHz for ¹³C (9.4 T static magnetic field). The lyophilized powders were packed into 7 mm zirconia rotors and sealed with Kel-F end-caps (Revolution NMR, LLC, Fort Collins, CO) in a glovebox purged with dry nitrogen gas with a relative humidity of <1%. Experiments were performed using a 7 mm double resonance magic angle spinning (MAS) probe (Agilent, Palo Alto, CA). All ¹³C spectra were acquired under magic angle spinning (MAS)¹⁵ at 4 kHz, using ramped-amplitude cross polarization (CP),¹⁶ total sideband suppression (TOSS)¹⁷ and spinal64 decoupling¹⁸ with a ¹H decoupling field of about 66 kHz. A 2 ms contact time was used in all experiments. 3-methylglutaric acid was used to optimize spectrometer settings and the methyl peak was referenced to 18.84 ppm.¹⁹ All spectra were acquired at ambient temperature.

DETERMINATION OF PHASE SEPARATION

To evaluate phase separation of the protein and sugar, the ¹H T₁ and ¹H T_{1ρ} values were determined. The ¹H T₁ relaxation values were measured using a saturation recovery experiment through ¹³C observation. The peak areas were integrated, and plotted in Kaleidagraph (Synergy Software, Reading, PA) and fit to the following equation to determine ¹H T₁ times for the protein and the sugar peaks individually:

$$(7.2) \quad M = M_0 (1 - e^{-\tau / T_1})$$

Where M is the integrated signal intensity, M₀ is an amplitude parameter from the fit, τ is the recovery delay time, ¹H T₁ is the obtained spin-lattice relaxation time. The protein peak

was integrated in the ~164 – 180 ppm region, whereas for the sugar peak the ~90 - 106 ppm region was used. Only the central 1/3rd of the peaks were used. These peaks were chosen because of the lack of overlap between the two species in those areas. For the ¹H T_{1p} experiment the spin locking time was varied from 1 to 30 ms. Similar to the ¹H T₁ experiments, Kaleidograph was used for integration and fitting of the data using equation 7.3.

$$(7.3) \quad M = M_0 e^{-\tau / T_{1p}}$$

The symbols are the same as used in equation 7.2. ¹H T_{1p} is the rotating frame spin lattice relaxation time. Data acquisition parameters for the ¹H T₁ experiments were a 1 s recycle delay, 2048 acquisition points (51.2 ms acquisition time), a 3.8 μs ¹H 90° pulse, (~66 kHz decoupling field), a 2 ms contact time, and a 4 kHz MAS frequency. 12 time points (0.1, 0.3, 1.2, 1.8, 3.0, 4.0, 9.0, and 15 s) were used in the ¹H T₁ determination, and 500 acquisitions were acquired per T₁ point. Data acquisition parameters for the ¹H T_{1rho} experiments were a 4 s recycle delay, 2048 acquisition points (51.2 ms acquisition time), a 3.8 μs ¹H 90° pulse, (~66 kHz decoupling field), a 2 ms contact time, and a 4 kHz MAS frequency. 8 spin locking times (1, 2, 3, 5, 10, 15, 20, and 30 ms) were used, with 500 acquisitions per T₁ point. Data acquisition for the ¹³C spectra were a 4 s recycle delay, a 2 ms cross polarization contact time, 2048 acquisition points (51.2 ms acquisition time) and 1000 acquisitions.

Data processing was done the same way for all spectra, and was done in the TNMR software package (Tecmag, Houston, TX). The first 512 points (13.3 ms acquisition time) was used, and Fourier transformed with 20 Hz of line broadening. The spectra (supporting information) were individually phased and integrated using abovementioned limits and the fit to the equations above. The central 1/3rd of the peaks was used for the integration to obtain the best data, and minimize error of the peak integrations. The error presented for the peaks is the error of the fit from the Kaleidagraph fitting. Protein and sugar relaxation times were considered different if they were more than two fitting errors apart.

STORAGE STABILITY

To evaluate the stabilizing effects of the different sugars, the different formulations were subjected to an accelerated storage stability test. Samples were stored at 60 °C up to 90 days. Samples were taken from the freezer at different time points, so they could all be analyzed together, eliminating assay variations. The storage temperature was below the T_g of all samples tested and they therefore remained physically as original during storage.

HIGH PRESSURE SIZE EXCLUSION CHROMATOGRAPHY (HPSEC)

HPSEC was conducted to provide information on aggregation of the IgG. The samples which were reconstituted with 1 mL ultrapure water were diluted further to a concentration of 1

mg/mL IgG with 20 mM NaH_2PO_4 with 150 mM NaCl (pH 7.5). The samples were filtered using a 0.45 μm filter before being injected on a Superose 6 GL 10/30 column in 100 μL aliquots. The mobile phase was the same as the dilution buffer and the flow used was 0.5 mL/min. Detection was done spectrophotometrically at 214 nm.

Baseline corrections based on the signals between 6 and 7 minutes and 26-27 minutes were carried out for the obtained chromatograms. The area under the curve of the monomer peak (~16 minutes) was determined from half height to half height of the peak. Degradation kinetics were established by correlating monomer peak area and the square root of storage time and carrying out a linear regression analysis, the slope of that curve was used as a measure for the aggregation rate. To limit aggregation after reconstitution of the samples, each sample was measured only once.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Since HPSEC only provides information about the aggregation of IgG but does not give information about functionality, an ELISA was conducted on the same samples to provide additional information. The integrity of the epitope of IgG was used as a measure for its stability. This was established using a bovine IgG ELISA kit supplied by Bethyl (Montgomery, TX, United States of America). Lyophilized samples were reconstituted using 1 mL of ultrapure water and diluted to concentrations between 500 and 7.8 ng/mL using diluent buffer, which consisted of 50 mM Tris with 0.14 M NaCl and 0.05% Tween 20 (pH 8.0). Analysis was carried out on 96-wells plates, which were pre-incubated with affinity purified bovine IgG coating antibody for an hour at room temperature and subsequently blocked with blocking solution, using the diluent buffer, at the same temperature for 30 minutes. The diluted samples were incubated for an hour at room temperature, followed by incubation of horseradish peroxidase conjugated bovine IgG detection antibody for an hour. Lastly a substrate solution containing 3,3',5,5'-tetramethylbenzidine (TMB) was added and incubated in the dark during 15 minutes at room temperature. The reaction was stopped by addition of 0.18 M H_2SO_4 , after which the conversion of TMB by peroxidase was measured spectrophotometrically at 450 nm. The plates were washed 5 times with diluent buffer between all steps up to the addition of TMB. The absorbance was related to an unprocessed reference sample, giving an indication of the amount of epitopes still intact. Results were not related to $t=0$ as some formulations already showed loss of epitopes during lyophilization. Each sample was measured in 4 dilutions, results from the two optimal dilutions were averaged. A duplicate analysis of a large part of the formulations provided nearly identical results.

RESULTS

SOLID STATE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

For the formulations of trehalose, dextran 1.5 kDa, and both inulins the $^1\text{H } T_1$ relaxation times of protein and sugar are indistinguishable (figure 7.2 (top)). For dextran 5 kDa and 70 kDa the values of the protein and sugar are clearly different, with a bigger difference in $^1\text{H } T_1$ relaxation times for the formulation of dextran 70 kDa. This is indicative of phase-separation at the 20-50 nm scale for formulations with dextran 5 kDa and 70 kDa and IgG. For the formulations with IgG and trehalose, both inulins, and dextran 1.5 kDa there is no indication of phase separation on this scale.

In addition to phase separation, there is a trend in the $^1\text{H } T_1$ relaxation time, where the lower molecular weight sugar systems had the longest relaxation time, and the protein relaxation time decreases as the sugar molecular weight increases. Since $^1\text{H } T_1$ relaxation times are indicators of molecular mobility, the longer relaxation times would correlate with a lower molecular mobility.^{20,21} The absolute $^1\text{H } T_1$ relaxation time of IgG is highest for the smallest sugar trehalose and decreases when the molecular weight of the combined sugar increases, suggesting less molecular mobility of the protein when incorporated in the low molecular weight sugar.

The $^1\text{H } T_{1\rho}$ relaxation times of IgG do not show an increase with lower molecular weight (figure 7.2 (bottom)). $^1\text{H } T_{1\rho}$ relaxation times are indistinguishable for trehalose and IgG, somewhat different for the inulin formulations, and, even more different for the formulations containing dextran. This indicates miscibility down to the 2-5 nm scale for trehalose. The other formulations show immiscibility on this scale. Therefore, combinations of IgG with both inulins and dextran 1.5 kDa are classified as partially phase separated, whereas dextran 5 kDa and 70 kDa are phase separated and trehalose and IgG are fully miscible.

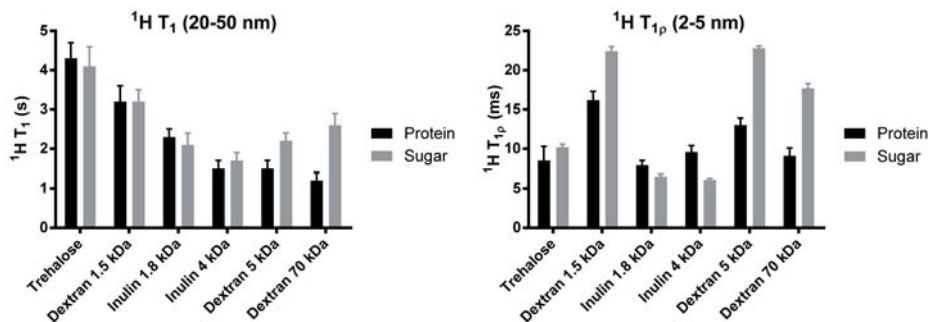


Figure 7.2 Spin-lattice relaxation times ($^1\text{H } T_1$, top) and rotating-frame spin-lattice relaxation times ($^1\text{H } T_{1\rho}$, bottom) of both protein and sugar from lyophilizates of IgG with various sugars. The formulations are ordered by molecular weight of the sugar, increasing from left to right.

STORAGE STABILITY - HPSEC

During storage at 60 °C the formulation containing trehalose shows nearly no aggregation of IgG (figure 7.3). For the other sugars, there is an increasing degree of IgG aggregation with increasing molecular weight of the sugar, with the exception of dextran 5 kDa. The formulation with dextran 1.5 kDa shows ~20% of aggregation of IgG after 90 days of storage, for inulin 1.8 kDa and dextran 5kDa this is ~40%. Inulin 4 kDa shows ~50% loss of monomer and lastly there was no clear difference in aggregation between the formulation without sugar and that with dextran 70 kDa. The aggregation results correlate with the $^1\text{H T}_1$ relaxation times of the protein, and are consistent with the more stable samples being fully or partially miscible, and the phase separated samples having the poorest stability.

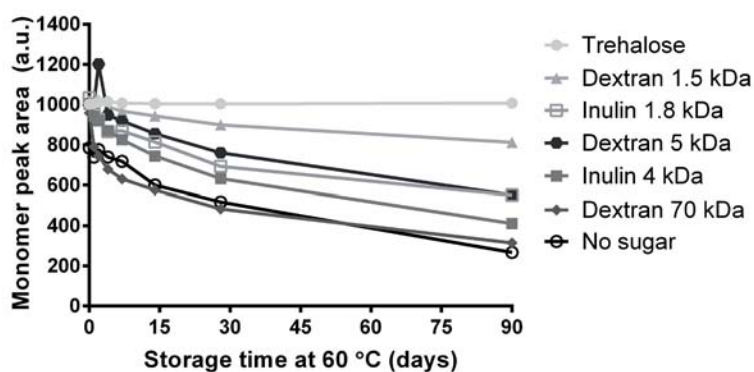


Figure 7.3 Amount of unaggregated IgG (soluble monomer) of IgG lyophilized with various sugars during storage at 60 °C during 90 days.

STORAGE STABILITY - ELISA

As with aggregation, the formulation of IgG and trehalose shows little or no change in amount of intact epitopes during storage (figure 7.4). Inulin 4 kDa shows a loss of around 30% of epitopes, the formulations with dextran 70 kDa and the formulation without sugar lost ~40% of epitopes. Remarkably, the smaller inulin 1.8 kDa and the two smaller dextrans 1.5 and 5 kDa show a bigger loss of intact epitopes than the formulation without sugar, a total loss of ~50%, ~70% and ~60% respectively. This indicates a deteriorative effect of the sugar on the protein, which could indicate that the reducing groups of the sugar reacted with the amine groups of the protein, the so-called Maillard reaction. Initial Maillard browning could affect the functional epitopes of IgG and therewith the ELISA data, but because of the molecular weight of these sugars compared to that of IgG, it would likely not impact HPSEC results significantly. Some discoloration was observed in the samples with

dextran 1.5 kDa, dextran 5 kDa and inulin 1.5 kDa after 90 days. Most discoloration was found in the formulation containing dextran 1.5 kDa. There was no observable discoloration for formulations with trehalose, inulin 4 kDa and dextran 70 kDa.

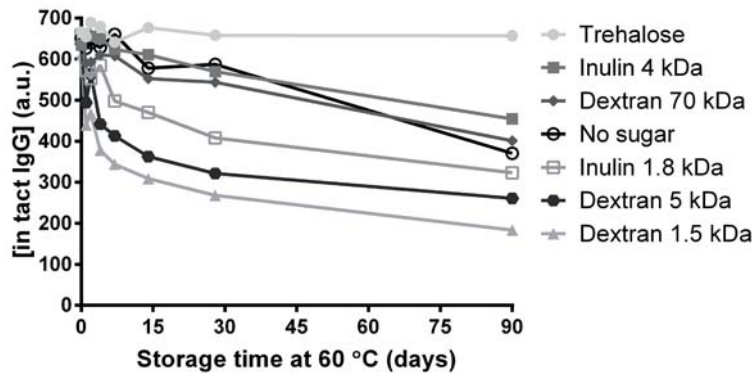


Figure 7.4 Amount of intact epitopes of IgG lyophilized with various sugars during storage at 60 °C during 90 days.

DISCUSSION

In this chapter, the influence of miscibility of protein-sugar lyophilizates on the storage stability was investigated using model protein IgG. Miscibility was evaluated by comparing ssNMR relaxation times at two timescales, providing information on miscibility on 2-5 nm and 20-50 nm length scales. When $^1\text{H } T_1$ relaxation times of protein and sugar were different for a single sample, this indicated immiscibility of the system already on the larger 20-50 nm scale and these combinations were classified as immiscible. When $^1\text{H } T_1$ relaxation times were similar, but $^1\text{H } T_{1p}$ relaxation times, with a diffusion length scale of 2-5 nm, were different, the combination was considered partially phase separated. If both $^1\text{H } T_1$ and $^1\text{H } T_{1p}$ relaxation times were similar, there was no indication of phase separation and the sample was deemed miscible. Table 7.1 shows the classifications of all the tested combinations, based on this rationale.

Previously we reported that smaller and molecularly more flexible sugars were better capable of stabilizing proteins during lyophilization,¹¹ as they were less inhibited by steric hindrance and configurational inflexibility and therewith better able to form hydrogen bonds with the protein during lyophilization.¹² Here, it was found that miscibility and absolute $^1\text{H } T_1$ relaxation times decreased with increasing molecular weight of the sugar used. $^1\text{H } T_1$ relaxation times have been shown to be predictive for physical stability of amorphous

systems²² and protein storage stability,²⁰ presumably because the molecular mobility involved in generating the $^1\text{H } T_1$ relaxation process is similar to the molecular mobility that causes degradation. Here too, protein $^1\text{H } T_1$ relaxation times correlate with protein aggregation rates (figure 7.5).

Table 7.1 Classification of miscibility based on ssNMR relaxation times of protein-sugar lyophilizates.

Sugar	MW (kDa)	20-50 nm	2-5 nm	Classification
Trehalose	0.3	•	•	Miscible
Dextran	1.5	•	•	Partially phase separated
Inulin	1.8	•	•	Partially phase separated
Inulin	4	•	•	Partially phase separated
Dextran	5	•	•	Phase separated
Dextran	70	•	•	Phase separated

MW = molecular weight; • = similar relaxation times; • = different relaxation times

The overall size of IgG is significantly larger than 2-5 nm, with a total maximum length of ~16 nm.²³ However, the protein is not a sphere and its subdomains (chains) are smaller, with dimensions of approximately 2.5 by 2.5 by 5 nm.²⁴ Therefore, these miscibility data likely describe the ability of sugars to enter empty spaces in the protein's three-dimensional structure and form intimate contact. Thus, the miscibility we describe here could possibly indirectly indicate steric hindrance of the protein and sugar. This is in line with our previous findings, as a close proximity of the sugar to the protein is prerequisite for hydrogen bonding and therewith stabilization. It is therefore not surprising that the smallest sugar tested, disaccharide trehalose, is the best stabilizer of IgG as represented by both the HPSEC and

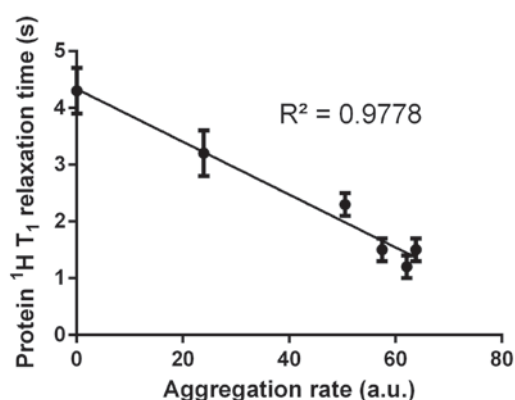


Figure 7.5
Correlation between protein aggregation rate (loss of monomer as determined with HPSEC per the square root of time during storage) versus protein $^1\text{H } T_1$ relaxation times for the different formulations.

ELISA storage stability data, and that stabilizing capacity diminishes with increasing molecular weight of the stabilizer.

The ELISA data do not correlate as well with the miscibility data as the HPSEC results do. Most likely, this is because of an additional mechanism of degradation, being the Maillard reaction. Inulin without the glucose end group and dextran both have a reducing end group, which is relatively more abundant in lower molecular weight sugars.²⁵ This explains why the smaller dextrans (1.5 and 5 kDa) and smaller inulin (1.8 kDa) maintain less functional epitopes than the protein without sugar. As mentioned previously, chemical bonding of a sugar of 1.5-5 kDa, as happens in the initial steps of the Maillard cascade, does not have a large impact on the total mass and three-dimensional size of IgG, which has a molecular weight of approximately 150 kDa. Therefore, HPSEC is insensitive to Maillard reactions with relatively small oligosaccharides, unlike the ELISA assay which will detect these changes if they impact the functional epitope directly or indirectly do so by causing conformational changes.

Considering the above, smaller non-reducing sugars are preferred for stabilizing proteins as they are less limited by steric hindrance and are thus more miscible with the protein. However, these sugars are generally characterized by a lower glass transition temperature (T_g) and with that care should be taken that vitrification of the formulation is maintained during storage.²⁶ That is, one should not exceed the glass transition temperature. This is particularly a problem in case of higher levels of residual moisture or moisture sorption, as water has a strong plasticizing effect on sugar glasses.²⁷ In cases where the T_g would not be adequate for the planned storage conditions (i.e. approximately 10-20 °C higher than the storage temperature),²⁸ combining a large polysaccharide with a non-reducing disaccharide is a suitable alternative.^{29,30} This limits steric hindrance as much as possible, whilst achieving a high enough T_g.¹¹

CONCLUSION

In summary, ssNMR is able to detect miscibility on a level close to the size of the protein molecules and the obtained miscibility classifications and ¹T H₁ relaxation times correlate well with aggregation rates of IgG during storage. The ELISA storage stability data show degradation by both aggregation and Maillard browning, which cannot be deconvoluted accurately. Smaller sugars show better protein-sugar miscibility as they are less influenced by steric hindrance and this makes them more suitable as stabilizers of proteins.

ACKNOWLEDGEMENTS

This research was jointly financed by Royal FrieslandCampina, the European Union, European Regional Development Fund and The Ministry of Economic Affairs, Agriculture and Innovation, Peaks in the Delta, the Municipality of Groningen, the Provinces of Groningen, Fryslân and Drenthe as well as the Dutch Carbohydrate Competence Center.

We acknowledge ms. de Ruiter and mr. Scheffer of Royal FrieslandCampina for their analytical support.

MJN and EJM acknowledge support from the NSF I/UCRC Center for Pharmaceutical Development (IIP-1063879 and industrial contributions) for financial support. The solid-state NMR results presented here are from academic work performed at the University of Kentucky, and no data from Kansas Analytical Services are presented. EJM is a partial owner of Kansas Analytical Services, a company that provides solid-state NMR services to the pharmaceutical industry.

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CHAPTER 7

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SUPPORTING INFORMATION

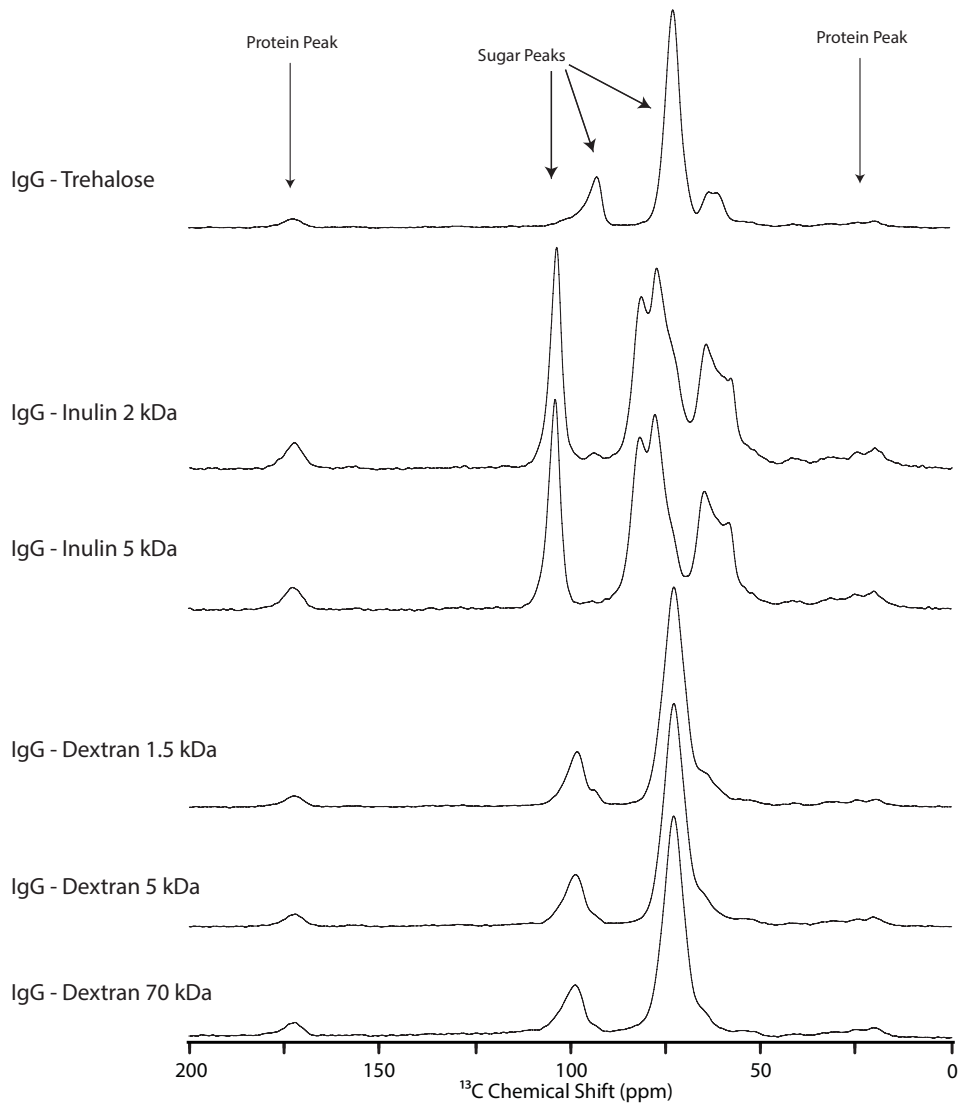


Figure 7.S1 Solid state Nuclear Magnetic Resonance spectra of the various formulations.

