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## Comparison of NMR and Dynamic Light Scattering for Measuring Diffusion Coefficients of Formulated Insulin: Implications for Particle Size Distribution Measurements in Drug Products

Sharadrao M. Patil<sup>1</sup>, David A. Keire<sup>2</sup>, and Kang Chen<sup>1,3</sup>

<sup>1</sup>Division of Pharmaceutical Analysis, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland 20993, USA.

<sup>2</sup>Division of Pharmaceutical Analysis, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, St. Louis, Missouri, USA.

### Abstract

Particle size distribution, a measurable physicochemical quantity, is a critical quality attribute of drug products that needs to be controlled in drug manufacturing. The non-invasive methods of dynamic light scattering (DLS) and Diffusion Ordered Spectroscopy (DOSY) NMR can be used to measure diffusion coefficient and derive the corresponding hydrodynamic radius. However, little is known about their use and sensitivity as analytical tools for particle size measurement of formulated protein therapeutics. Here, DLS and DOSY-NMR methods are shown to be orthogonal and yield identical diffusion coefficient results for a homogenous monomeric protein standard, ribonuclease A. However, different diffusion coefficients were observed for five insulin drug products measured using the two methods. DOSY-NMR yielded an averaged diffusion coefficient among fast exchanging insulin oligomers, ranging between dimer and hexamer in size. By contrast, DLS showed several distinct species, including dimer, hexamer, dodecamer and other aggregates. The heterogeneity or polydisperse nature of insulin oligomers in formulation caused DOSY-NMR and DLS results to differ from each other. DLS measurements provided more quality attributes and higher sensitivity to larger aggregates than DOSY-NMR. Nevertheless, each method was sensitive to a different range of particle sizes and complemented each other. The application of both methods increases the assurance of complex drug quality in this similarity comparison.

### Keywords

diffusion; particle size distribution; physicochemical equivalence; protein drug product; similarity

<sup>3</sup>To whom correspondence should be addressed. kang.chen@fda.hhs.gov.

COMPLIANCE WITH ETHICAL STANDARDS

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## INTRODUCTION

Protein-based drug products present a challenge for quality review because of complex formulations and inherent heterogeneity in protein structures (1). Higher-resolution analytical methods are needed to ensure structural and compositional similarities between protein therapeutics containing the same drug substance. In addition to protein higher-order structure probed by NMR, circular dichroism (CD) or other approaches (2), sub-visible protein aggregation presents an immunogenicity risk to drug safety (3–5). For most solution systems, particle size measurements can be inferred from the measured diffusion coefficient of a molecule and its proportionality to hydrodynamic radius. Therefore, diffusion coefficients provide information about molecular size of protein oligomers and higher-order complexes (6). Two modern analytical methods viz. dynamic light scattering (DLS) and Diffusion Ordered Spectroscopy (DOSY) NMR are capable of non-invasively measuring molecular translational diffusion coefficients and particle size distributions (PSD) directly in drug products (7–12). In DLS, the translational diffusion of molecules is measured by virtue of decay in scattered light intensity as a function of time (13). In DOSY-NMR, the decrease in protein magnetization intensity as a function of gradient strength is measured. These intensity functions are correlation functions of molecular translational diffusion. Of note, DLS and NMR measure the ensemble averaged diffusion coefficients of all equilibrated particles. In theory, DLS weighs higher molecular weight species more heavily than smaller components because the scattered light intensity is proportional to the sixth power of molecular radii. By contrast, DOSY-NMR weighs lighter molecular species more because smaller molecules yield sharper NMR lines than do larger molecules (7,14–17). DLS and DOSY-NMR methods offer a convenient and non-invasive way to measure a particle size with reduction of convoluted data to get simpler results. Here, these methods are compared with a focus on applicability to complex protein formulations to obtain information on the protein oligomerization/ aggregation and poly-dispersity found in a given drug product solution.

Insulin forms dimer, hexamer, and higher-order structures in solution depending on the insulin concentration and the presence of  $Zn^{2+}$  and/or phenol (18–21). Dynamic insulin exchange among dimer, tetramer, and hexamer has been proposed previously to explain observed spectroscopic data (22,23). Formulations used for this study contain protein at sub-mM concentration and excipients such as  $Zn^{2+}$ , *m*-cresol and/or phenol. These formulation conditions stabilize insulin dimer, hexamer and also higher-order structure formation (19,24,25). Hexameric or higher-order structures have also been observed for insulin analogues under these conditions (18,26–28). Therefore, insulin could be present in polydisperse form in the five formulations investigated and were designated as a model “complex protein system” for purposes of this study.

To examine if DOSY-NMR and DLS give the same result when used to measure protein diffusion coefficients, first, a test was performed on a known protein standard. Then, the same protocol was applied to five insulin drug products from different manufacturers. The results demonstrated that these two methods are sensitive to different molecular size ranges, structural forms, and, for DOSY-NMR, to exchange kinetics among oligomers. To our

knowledge, these data are the first such assessment of the orthogonality of these two non-invasive particle size distribution assay methods for analyzing complex protein therapeutics.

## MATERIALS AND METHODS

### Protein Sample Preparation

Lyophilized powder of ribonuclease A (RNase A) (Sigma-Aldrich, St. Louis, MO) was dissolved in 75 mM PBS buffer (Quality Biological Inc., Gaithersburg, MD) to get a final protein concentration of 1 mM RNase A. The sample was filtered through 0.02- $\mu$ m Antop 10 plus filter (GE Healthcare UK Limited, Buckinghamshire, UK) using a 1-mL syringe (Becton Dickinson and Company, NJ). Insulin formulations were purchased as 10-mL vials at 100 U/mL, equivalent to a concentration of 0.6 mM in monomeric form. Three different lots for each of the insulin drug products Apidra<sup>®</sup>, Humalog<sup>®</sup>, Humulin R<sup>®</sup>, Novolin R<sup>®</sup> and Novolog<sup>®</sup> were purchased and used directly for NMR and DLS measurements without filtering. For each NMR sample, 30  $\mu$ L D<sub>2</sub>O (Cambridge Isotope Laboratories, Tewksbury, MA) with 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid (TMSP-*d*<sub>4</sub>) (Sigma-Aldrich, St. Louis, MO) was mixed with 500  $\mu$ L each of insulin drug products before loading to a 5-mm NMR tube (Wilmad lab-glass, NJ). The same NMR samples were applied on polystyrene flat bottom 96-well plate (Greiner Bio-One GmbH, Frickenhausen, Germany) for DLS data acquisition.

### 2D DOSY-NMR

The Bruker <sup>1</sup>H 2D DOSY pulse sequence modified with 3919 water suppression (see supplementary information for the pulse program code) was used to measure diffusion coefficients. NMR data were acquired on a Bruker 850-MHz NMR instrument equipped with a triple-gradient TXI room temperature probe capable of generating gradient field strength of 54 G/cm. The DOSY time interval and gradient pulse were set at 600 ms ( $\tau$ ) and 1.2 ms ( $\delta$ ), respectively. A total of 64 gradient increments, linearly varied from 2 to 98%, were collected with 128 scans for each increment. The total experiment time for each sample was approximately 9 h.

NMR data processing was performed using MestReNova 11.0.3 software (Mestrelab Research S.L.). Each free induction decay (FID) series in 2D DOSY dataset was apodized using Gaussian 1-Hz broadening function, zero-filled to 128-k data points, and baseline corrected with a third-order Bernstein polynomial fit. Phase correction and baseline correction were performed on the first FID and applied to all 64 FIDs in the diffusion dimension. Bayesian DOSY processing (BDT) functionality in MestReNova was used to process the second-dimension data. A molecular species with diffusion coefficient higher than  $1 \times 10^{-7}$  cm<sup>2</sup>/s was not observed in the initial analysis; therefore, diffusion range limit between  $1 \times 10^{-4}$  and  $1 \times 10^{-7}$  cm<sup>2</sup>/s and a resolution factor of 98 were used for BDT processing with two repetitions. A total of 256 trace points were processed for improved resolution with a reduction factor of 1 for vertical and horizontal scales. Peak suppression functionality of MestReNova was used only for samples of drug product Apidra<sup>®</sup> to suppress strong polysorbate-20 excipient peak. Five peaks corresponding to insulin between 0 and 1.3 ppm were selected from each 2D DOSY-NMR spectrum, and the average diffusion

coefficients were calculated for a drug sample. Standard deviation from five repetitions on the same lot of Novolin R® was adopted as DOSY method variation data. Reference diffusion coefficients for RNase A and insulin (pdb 3aiy) monomer, dimer and hexamer were calculated from reported crystal structures using simulation software HYDROPRO (29) and HYDRONMR (30).

### Dynamic Light Scattering

DLS experiments were performed using a 96-well plate, and 110  $\mu\text{L}$  of NMR sample was loaded per well. Samples were equilibrated at 25°C for 30 min. Data were acquired with acquisition time of 4 s and a total of 40 scans per well. The curve fittings were performed using regularization functionality in DYNAMICS 7.5.17 (Wyatt Technologies) with baseline limit of 0.02 and maximum sum of squares (SOS) for the correlation function fit set to 300. A default Dynals™ analysis was applied to the autocorrelation function for the calculation of diffusion coefficients ( $D$ ), percent poly-dispersity (%Pd), relative scattered light intensity (%Intensity) and mass weight (%Mass) for a maximum of four species per curve. %Pd is the ratio between the width of diffusion coefficient distribution to its mean value. Normalized diffusion coefficients for DLS were calculated using equation  $D_N = \sum_{i=1}^n W_i D_i$ , where  $n$  is 3 or 4,  $D_N$  is the normalized diffusion,  $D_i$  is the diffusion coefficient of a discrete species, and  $W_i$  is the %Mass of the respective species. DLS data were obtained in duplicate for all samples, and the averaged values were reported as the results. Standard deviation from five repetitions on the same lot of Novolin R® was adopted as DLS method variation data.

## RESULTS AND DISCUSSION

### Method Orthogonality

To cross-check DLS and 2D DOSY-NMR for measuring protein diffusion coefficients, a standard protein RNase A was adopted as a test model. DLS and DOSY-NMR investigations were performed on identical samples. The representative 1D  $^1\text{H}$  NMR spectrum, 2D DOSY-NMR spectrum and DLS data for RNase A are shown in Fig. 1a–c, respectively. The average diffusion coefficients for RNase A obtained from DOSY-NMR and DLS fitting were  $1.17 \pm 0.02$  and  $1.19 \pm 0.02 \times 10^{-6} \text{ cm}^2/\text{s}$ , respectively. The diffusion coefficients obtained from the two methods were nearly identical within experimental error. The average %Pd obtained from DLS was  $14.0 \pm 0.4\%$ , which represent a realistic metric for a mono-disperse protein solution. The poly-dispersity value of 14% was designated as the minimum requirement to assign a discrete species to be “mono-disperse” for further analysis. The diffusion coefficient of RNase A was also calculated using its reported crystal structure. The calculated translational diffusion coefficient of  $1.15 \times 10^{-6} \text{ cm}^2/\text{s}$  was obtained for RNase A monomer, consistent with experimental data. The diffusion coefficient values obtained using both methods compared very well with the literature diffusion coefficient values for RNase A (31) and demonstrate the suitability of regularization curve fitting in analyzing DLS autocorrelation curves and BDT algorithm in processing 2D DOSY-NMR spectra.

### Insulin DOSY-NMR Analysis

The representative NMR spectra of 1D  $^1\text{H}$  and 2D DOSY for drug product Novolin R<sup>®</sup> are shown in Fig. 2a, b, respectively. The diffusion coefficients of insulin regular, drug substance in Novolin R<sup>®</sup>, were read directly from insulin methyl peaks. For all DOSY spectra, only one insulin species was identified. The diffusion coefficients of all the measured insulin lots ranged from  $9.91 \times 10^{-7}$  to  $1.21 \times 10^{-6}$   $\text{cm}^2/\text{s}$  (Table I), all of which were between calculated dimer and hexamer diffusion coefficient values (Fig. 3). The insulin analogue drug products (Apidra<sup>®</sup>, Novolog<sup>®</sup>, and Humalog<sup>®</sup>) showed larger diffusion coefficients, close to dimer, than that of regular insulin drug products (Novolin R<sup>®</sup> and Humulin R<sup>®</sup>). The regular insulin might be experiencing fast exchange between dimer and hexamer, which caused them to diffuse slower. NMR line broadening makes higher molecular weight species (higher than hexamer) more difficult to identify unambiguously in DOSY-NMR spectra. Thus, higher-order oligomer species could have been present but are not directly detectable by NMR due to broadened lines and low concentration.

A repetition of five DOSY measurements on one lot of Novolin R<sup>®</sup> yielded the averaged diffusion value of  $1.08 \times 10^{-6}$   $\text{cm}^2/\text{s}$  with relative standard deviation (RSD) of 1% (Table S1), which represented DOSY precision of diffusion coefficient measurement for insulin drug product. For each brand of insulin, three lots of drug products were subject to DOSY measurement (Table I). Among them, only Novolin R<sup>®</sup> showed the lowest inter-lot variation of 0.1%, and other inter-lot variations observed in Apidra<sup>®</sup>, Novolog<sup>®</sup>, Humulin R<sup>®</sup>, and Humalog<sup>®</sup> are above the 1% of the DOSY method precision, attributed to measurable lot-to-lot differences in these insulin products (Table S2).

### Insulin DLS Analysis

A DLS signal decay curve for Novolin R<sup>®</sup> is shown in Fig. 2c. Typical DLS regularization curve fitting algorithm was applied. The calculation assumes that the correlation function is composed of several (up to four) distinct particles differing by at least one order of magnitude in diffusion coefficient. The deconvolution of a DLS curve results in weighting parameters percent intensity (%Intensity) for each species. The %Intensity is a much bigger number than %Mass, which is derived from %Intensity according to Mie's scattering law. The %Mass normalized diffusion coefficient values were calculated as the DLS diffusion coefficient. For all lots of insulin DLS data (Tables S3 and S4), the %Intensity from insulin aggregates can be appreciable; however, aggregates of insulin were down to less than 1% in mass. Therefore, normalized diffusion coefficient values were obtained for each lot of insulin and were nearly the same as the value of the smallest species (Tables II and S4). The DLS diffusion values between  $1.26$  and  $1.40 \times 10^{-6}$   $\text{cm}^2/\text{s}$  were observed for Novolin R<sup>®</sup> and Humulin R<sup>®</sup>, both of which are regular human insulin. These values range between monomer and dimer diffusion coefficients (Fig. 3 and Table II). The DLS diffusion coefficients of the insulin analogue drug products Apidra<sup>®</sup>, Humalog<sup>®</sup>, and Novolog<sup>®</sup> were  $0.91 \pm 0.02$ ,  $0.96 \pm 0.05$ , and  $1.04 \pm 0.04 \times 10^{-6}$   $\text{cm}^2/\text{s}$ , respectively (Table III), which suggested the dominance of hexamer species for this measurement (Fig. 3) and differed from their DOSY-NMR results where the dimer species dominated. In addition, a distribution of species around the mean diffusion coefficient of each species is assumed. The %Pd values indicate the broadness and heterogeneity of the distribution. Most %Pd values for the

primary species are close to the 14% value observed for RNase A, suggesting homogeneity. Some lots of Humalog® and Humulin R® showed high %Pd of 20%, indicating higher heterogeneity in these products.

A repeat of five DLS measurements on one lot of Novolin R® yielded a normalized diffusion coefficient value of  $1.31 \times 10^{-6} \text{ cm}^2/\text{s}$  with 2% RSD, representing DLS accuracy to be 2% (Table S3). For all five brands of insulin, the inter-lot variations in diffusion coefficient are above the 2% of the DLS method precision, attributed to measurable lot-to-lot differences in these insulin products (Tables III and S4).

### Comparison of the Two Methods

Different from model protein RNase A, DOSY and DLS never reported the same diffusion coefficient value on any insulin drug product. For insulin analogue products, Apidra®, Humalog® and Novolog®, the DOSY-NMR reported lower molecular weight species than DLS. For the regular insulin products, Humulin R® and Novolin R®, DOSY experiments showed smaller diffusion coefficients, between dimer and hexamer, while DLS reported dimer and higher-order oligomer species (Fig. 3). Regular insulin might experience fast exchange among monomer, dimer, hexamer and dodecamer, which could lower the averaged diffusion coefficient values measured in DOSY, but does not affect the DLS signals for all particles regardless of slow or fast exchange among them.

In addition to comparing the absolute diffusion coefficient values to assess orthogonality of DLS and DOSY-NMR measurements, the standard deviations across different lots were also compared. For each of the five brands, the inter-lot RSD from DOSY-NMR ranged between 0.1 and 6%, with Novolin R® being the most consistent solution, whereas Humalog® and Humulin R® had the largest inter-lot deviations (Table III). The inter-lot RSD values for DLS ranged between 2 and 5% with the highest variation obtained for Humalog® as well. Humalog® also has the largest %Pd of  $20 \pm 10\%$  (Table III), indicating heterogeneity in its lot-to-lot comparison. The RSD for Humulin R® was slightly smaller in DLS analysis (4%) than DOSY-NMR (5%); however, the %Pd of Humulin R® was the seconded largest  $15 \pm 5\%$ , meaning more heterogeneity in the primary species. Products of Apidra®, Novolog® and Novolin R® yielded less inter-lot variations and their %Pd values are also lower, less than 13% (Table III), indicating higher homogeneity in the primary species. Generally, the inter-lot variations were consistent between DLS and DOSY methods.

While the inter-lot variation is of interest for product consistency within each brand, the inter-brand variation is indicative of method sensitivity and differentiability. The range of variation in DLS diffusion coefficient values for all 15 tested lots of insulin range from 0.88 to  $1.4 \times 10^{-6} \text{ cm}^2/\text{s}$ , which was 2.5 times wider than the diffusion coefficient range from DOSY-NMR, 0.99 to  $1.2 \times 10^{-6} \text{ cm}^2/\text{s}$  (Tables I and II). In the DOSY method, the RSD of the DOSY diffusion coefficients obtained for all the tested 15 insulin lots from the five brands was only 6%. In the DLS method, the RSD from all the tested 15 lots was 17%; nearly three times more than the variation of the DOSY-NMR method. Thus, DLS differentiated insulin brands more effectively than DOSY-NMR.

## CONCLUSIONS

In the present study, DOSY-NMR and DLS were demonstrated to be robust and orthogonal methods in measuring the diffusion coefficient for drug products directly and could be used to infer particle size distributions. Clinically, in general, protein aggregate size matters more than aggregate proportion in eliciting immunogenicity (32,33). Therefore, from a drug safety standpoint, a technique more sensitive to larger size aggregates (i.e., DLS) is more fit for this purpose than DOSY-NMR. For insulin drug products, trace amount of aggregates (<0.1% in mass) were detected using DLS. Within each insulin drug product, the oligomerization equilibrium and exchange kinetics are modulated by excipients and protein sequence. DLS analysis rapidly differentiated individual brands of insulin better than DOSY-NMR because of the greater sensitivity to higher molecular weight species. These higher-order oligomers were not directly observable by DOSY-NMR due to substantial line broadening. DOSY-NMR was able to evaluate lower molecular weight insulin complexes, exchange kinetics among oligomers and the diffusion properties of the individual excipients present. Importantly, the averaged diffusion coefficients observed for insulin in DOSY-NMR imply the presence of lower molecular weight complexes that are concealed from DLS.

Taken together, for protein drug quality attributes such as particle size, each result should be specified by the analytical method and the processing parameters employed. More non-invasive and orthogonal analytical methods certainly cover broader attributes of drug products, leading to tighter control of the product either within the same brand before and after manufacture change, or across the brand between the originator and a biosimilar or generic drug version. The combined results from DOSY-NMR and DLS allowed better understanding of protein oligomerization, aggregation, equilibrium and kinetics, which are affected by excipients of drug product-specific formulation. The application of orthogonal analytical methods is crucial in demonstrating part of the physicochemical equivalence between any two products even though the requirement for the sameness in drug substance identity and concentration has been met.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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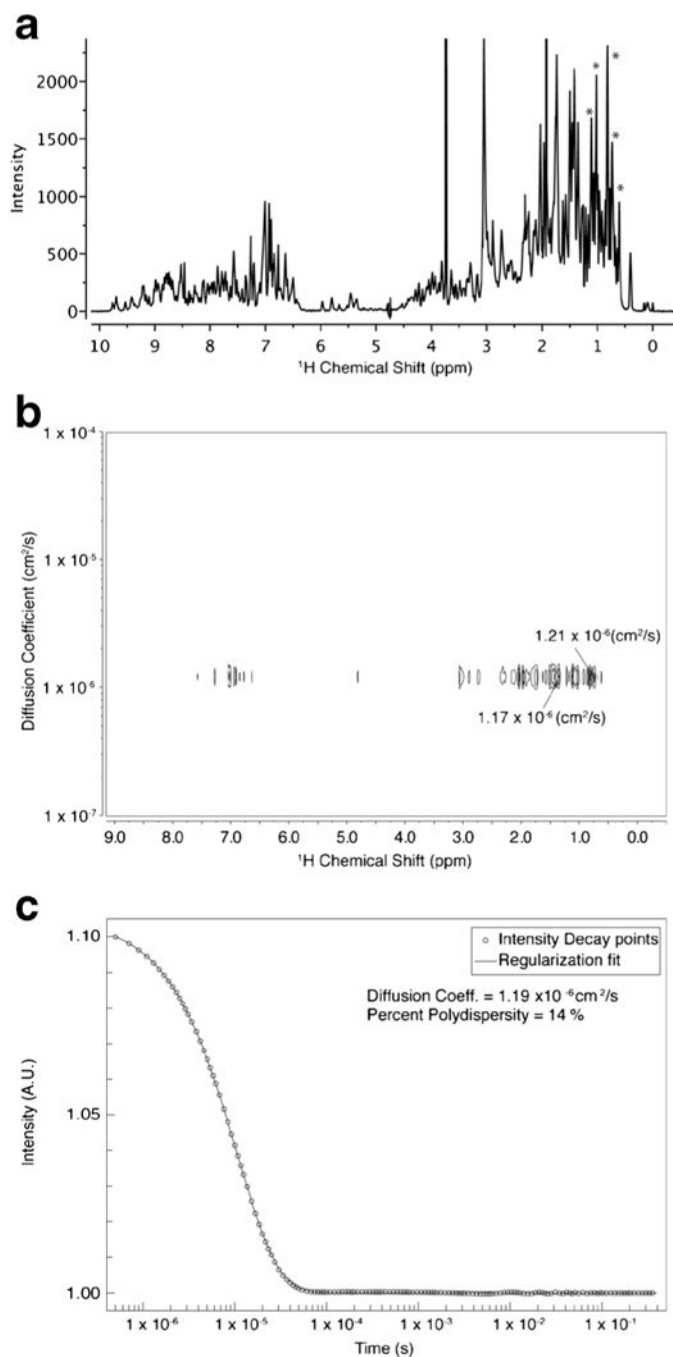
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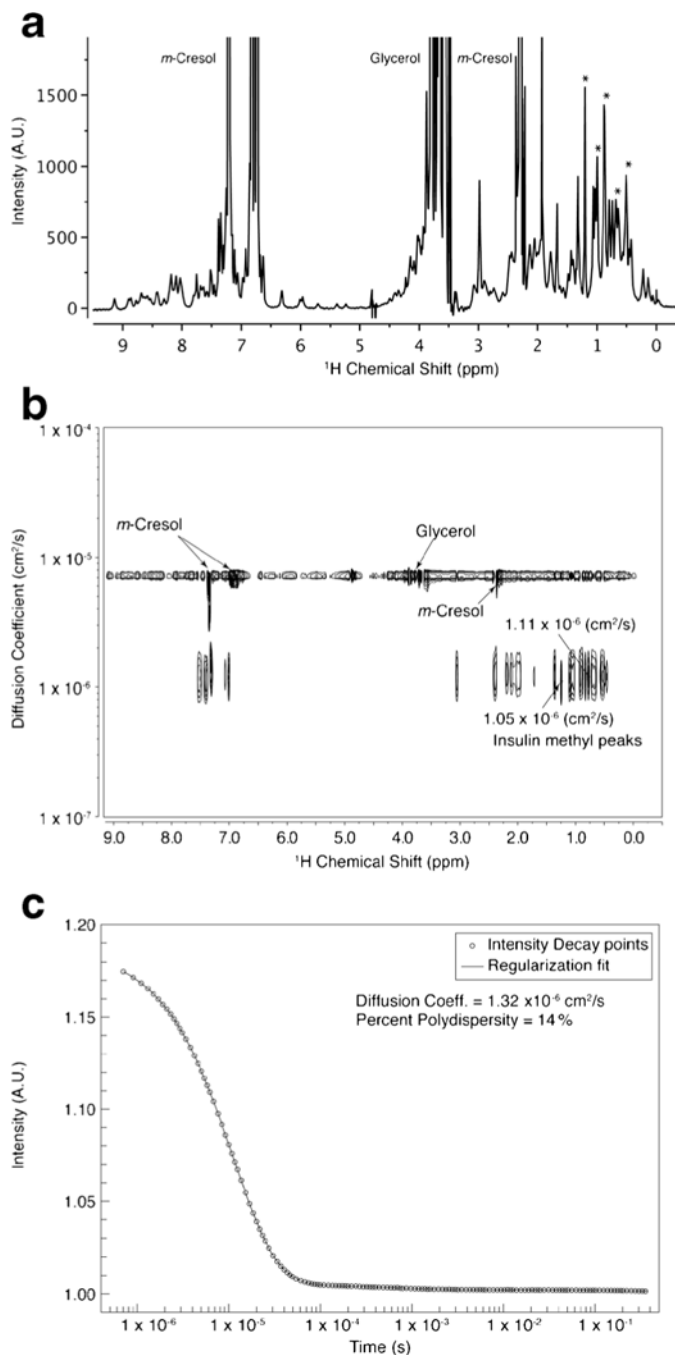
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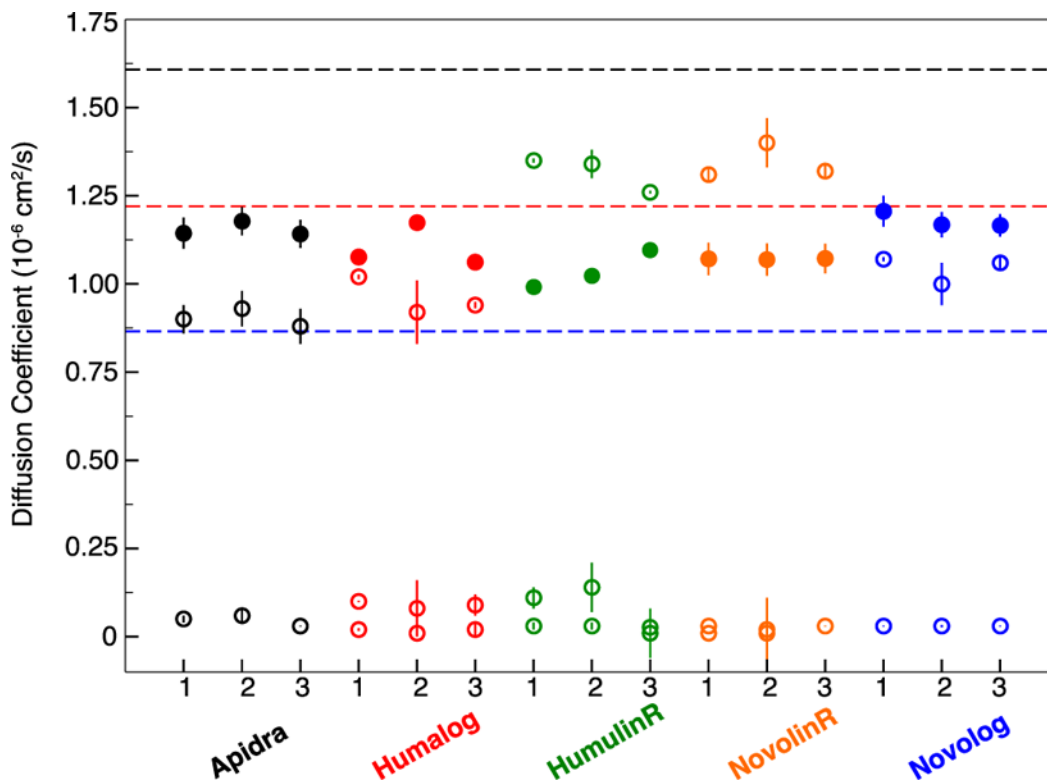
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**Fig. 1.** Representative data plots for two diffusion coefficient analysis methods for RNase A sample. **a** 1D  $^1\text{H}$  NMR spectrum. **b** 2D DOSY-NMR spectrum. **c** DLS autocorrelation curve. Strong protein methyl peaks (*asterisk* in **a**) were used to read out diffusion coefficient in a DOSY spectrum (**b**)



**Fig. 2.** Representative data plots for diffusion coefficient analysis of insulin in drug product Novolin R®. **a** 1D  $^1\text{H}$  NMR spectrum of Novolin R®. **b** 2D DOSY-NMR spectrum. **c** DLS autocorrelation curve. Strong insulin methyl peaks (*asterisk* in **a**) were used to read out diffusion coefficient in a DOSY spectrum (**b**)



**Fig. 3.** Experimental diffusion coefficients for three lots of five insulin drug products. 2D DOSY-NMR (*solid circles*) and DLS (*open circles*) results are plotted with the calculated diffusion coefficients of insulin (pdb 3aiy) monomer ( $1.61 \times 10^{-6}$  cm<sup>2</sup>/s), dimer ( $1.22 \times 10^{-6}$  cm<sup>2</sup>/s), and hexamer ( $0.87 \times 10^{-6}$  cm<sup>2</sup>/s) shown as *black, red, and blue dashed lines*, respectively

**Table I.**

Diffusion Coefficients for Insulin Drug Products Obtained Using DOSY-NMR

Drug product	Drug substance	Lot no.	DOSY diffusion coefficient <sup>a</sup> (10 <sup>-6</sup> cm <sup>2</sup> /s)
Apidra®	Analogue Glulisine	1	1.14
		2	1.18
		3	1.14
Humalog®	Analogue Lispro	1	1.08
		2	1.17
		3	1.06
Humulin R®	Insulin regular	1	0.99
		2	1.02
		3	1.10
Novolin R®	Insulin regular	1	1.07
		2	1.07
		3	1.07
Novolog®	Analogue Aspart	1	1.21
		2	1.17
		3	1.17

<sup>a</sup>The averaged DOSY diffusion coefficient from all 15 lots of insulin was  $1.11 \times 10^{-6}$  cm<sup>2</sup>/s and the RSD was 6%

**Table II.**

Diffusion Coefficients for Insulin Drug Products Obtained Using DLS

Drug product	Lot no.	Normalized diffusion coefficient <sup>a,b</sup> ( $10^{-6}$ cm <sup>2</sup> /s)	% Poly-dispersity <sup>c</sup> (%Pd)	Hydrodynamic radius <sup>d</sup> (nm)
Apidra®	1	0.90 ± 0.04	9.9 ± 0.3	2.7 ± 0.1
	2	0.93 ± 0.06	9 ± 4	2.6 ± 0.1
	3	0.88 ± 0.05	11 ± 1	2.7 ± 0.1
Humalog®	1	1.02 ± 0.01	10.6 ± 0.3	2.4 ± 0.0
	2	0.92 ± 0.09	30 ± 10	2.7 ± 0.2
	3	0.93 ± 0.01	14 ± 7	2.6 ± 0.0
Humulin R®	1	1.35 ± 0.01	15 ± 1	1.8 ± 0.0
	2	1.34 ± 0.04	11 ± 2	1.8 ± 0.0
	3	1.26 ± 0.01	20 ± 1	1.9 ± 0.0
Novolin R®	1	1.31 ± 0.02	11 ± 1	1.9 ± 0.1
	2	1.40 ± 0.07	13.2 ± 0.4	1.8 ± 0.1
	3	1.32 ± 0.02	16 ± 6	1.8 ± 0.0
Novolog®	1	1.07 ± 0.01	12 ± 0.1	2.3 ± 0.0
	2	1.00 ± 0.01	15 ± 5	2.4 ± 0.1
	3	1.06 ± 0.03	13 ± 3	2.3 ± 0.1

<sup>a</sup>The experimental variation was from two technical repeats

<sup>b</sup>The averaged DOSY diffusion coefficient from all 15 lots of insulin was  $1.11 \times 10^{-6}$  cm<sup>2</sup>/s and the RSD was 17%

<sup>c</sup>Percent poly-dispersity (%Pd) of the major species is shown. The %Pd value for RNase A solution was  $14.0 \pm 0.4\%$

<sup>d</sup>Hydrodynamic radius of the major specie is shown

**Table III.**

Comparison of Inter-Lot Diffusion Coefficient Results from DOSY-NMR and DLS

Method	DOSY-NMR		DLS			
	Drug product	Inter-lot averaged diffusion coefficient ( $10^{-6}$ cm <sup>2</sup> /s)	Relative standard deviation (RSD) <sup>a</sup>	Inter-lot averaged diffusion coefficient ( $10^{-6}$ cm <sup>2</sup> /s)	Relative standard deviation (RSD) <sup>a</sup>	Inter-lot averaged %Pd <sup>b</sup>
Apidra®		1.15 ± 0.02	2%	0.91 ± 0.02	2%	10 ± 1
Humalog®		1.10 ± 0.06	6%	0.96 ± 0.05	5%	20 ± 10
Humulin R®		1.04 ± 0.05	5%	1.32 ± 0.05	4%	15 ± 5
Novolin R®		1.07 ± 0.001	0.1%	1.34 ± 0.05	4%	13 ± 3
Novolog®		1.18 ± 0.02	2%	1.04 ± 0.04	4%	13 ± 2

<sup>a</sup>The RSD is the relative standard deviation from results of three lots in Tables I and II

<sup>b</sup>Percent poly-dispersity (%Pd) of the major species is shown