Hydrophilic Interaction Liquid Chromatography – A Potential Alternative for the Analysis of Dextran-1

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A method for the analysis of dextran-1 has been developed using hydrophilic interaction liquid chromatography (HILIC) with charged corona aerosol detection (CCAD) as a potential alternative to the size exclusion chromatography method described in the European Pharmacopoeia (EP). The EP method is in excess of 500 minutes and is considered long for routine analysis of multiple samples. The developed method retains resolution between monomers, whilst performing separation within 13 minutes.

Introduction

Dextran is a complex mixture of polysaccharides, principally of the α-1,6-glucan type, which is produced by the fermentation of sucrose by certain lactic acid bacteria, e.g. *Leuconostoc mesenteroides and Streptococcus mutans*. Clinical grade dextran is produced using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof.[1] The polymeric structure of dextran is shown in Figure 1.

Being a product of a fermentation process, dextran consists of different polymer lengths. Pharmacopoeial grade dextran is split into a number of different fractions, as defined in Table 1. The larger dextran fractions have been use clinically to reduce thrombosis.[2] In some patients the larger dextran fraction have the potential to induce anaphylactic reactions; this risk is reduced significantly when dextran-1 is pre-administered.[3]

The pharmacopoeial test for the average relative molecular mass is used as a quality property to define and control the overall distribution of glucose units present in the polymer length. The pharmacopoeial method uses size-exclusion chromatography (SEC)[4] and has a run time in excess of 500 minutes. Based on the known capabilities of HILIC to retain highly polar analytes[5], a potential alternative method, of 13 minutes, is described.

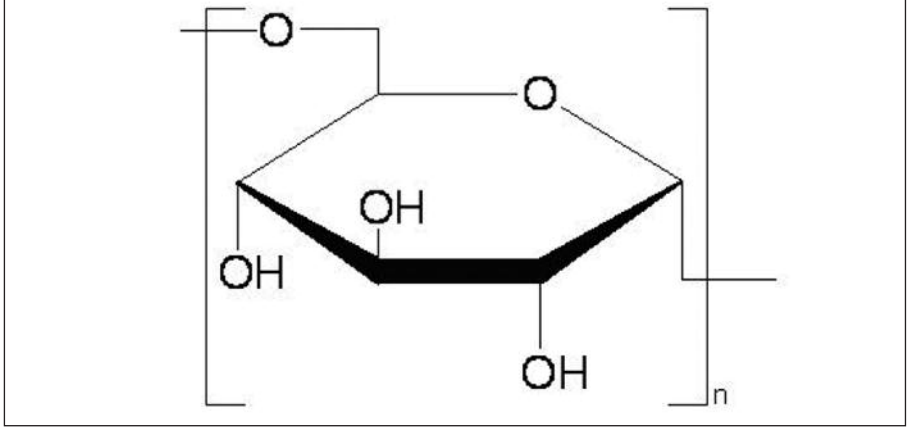

Figure 1: Structure of dextran

Component	Average relative molecular mass	Average relative molecular mass Range
Dextran-1	~1000	850 to 1150
Dextran 40	~40 000	35 000 to 45 000
Dextran 60	~ 60 000	54 000 to 66 000
Dextran 70	~70 000	64 000 to 76 000

Table 1: Dextran fractions

Experimental

Samples and Reagents

Purified water was produced from a Millipore Milli-Q system and was of 18.2 MΩcm and < 3ppb TOC. Acetonitrile (Chromasolv grade) was obtained from Sigma Aldrich, as was sodium chloride (ACS grade). Ammonium acetate (Analytical puriss HPLC grade) was purchased from Fluka. Pharmacopoeial grade Dextran-1 was purchased from Pharmacosmos A/S.

Solutions were prepared by dissolving the appropriate amount of Dextran-1 in the appropriate method diluent (see Tables 2 and 3).

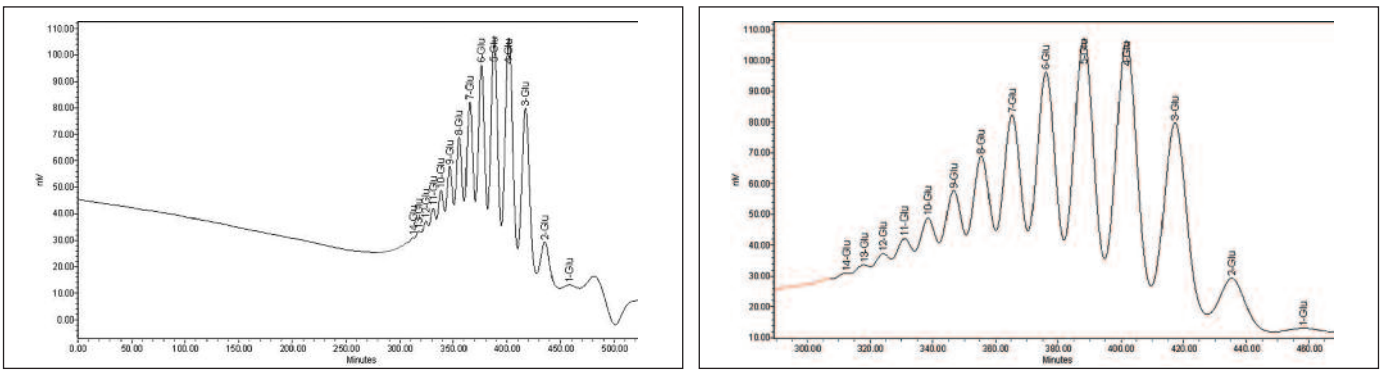

Figure 2: Typical SEC chromatogram for Dextran-1 150 mg/mL Solution for Injection (full and expanded scale)

Column:	2 × Superdex Peptide 10/300 GL, 10 mm × 30 cm column
Flow:	0.08 mL/min
Temperature:	20°C to 25°C
Injection Volume:	100 µL
Detection:	Refractive index at a temperature of 30°C
Mobile Phase	2.92 g/L sodium chloride
Sample Concentration	~ 6mg/mL Dextran-1 in 2.92 g/L sodium chloride (aq)

Table 2: Chromatographic conditions for pharmacopoeial method for Dextran-1 by Size-exclusion Chromatography

Column:	Phenomenex Luna HILIC 3µm 150 mm × 4.6 mm column				
Flow:	1.0 mL/min				
Temperature:	25°C				
Injection Volume:	20 µL				
Detection:	CAD with 100 pA range				
Mobile Phase A:	Water				
Mobile Phase B:	Acetonitrile				
Mobile Phase C:	100 mM Ammonium acetate (aq)				
Gradient:	Time (Minutes)	0	7	7.1	13
	% A	30	40	30	30
	% B	65	55	65	65
	% C	5	5	5	5
Sample Concentration	~ 4.5 mg/mL Dextran-1 in water/acetonitrile 35/65 v/v				

Table 3: Chromatographic conditions for HILIC method for Dextran-1

Instruments and Methods

European Pharmacopeia Methodology

The size exclusion method following the European Pharmacopeia method for determination of average molecular weight by SEC was run on an Agilent 1100 system equipped with a Waters 2414 Refractive Index Detector, using the conditions presented in Table 2.

HILIC Methodology

The HILIC method was developed using basic starting method development conditions for the Phenomenex Luna HILIC columns[6]; and run on an Agilent 1100 system equipped with ESA Corona Plus Charged Aerosol Detection. Modifications to the gradient were used to improve the separation between the glucose units. The final chromatographic conditions are presented in Table 3.

Results and Discussion

Typical chromatograms obtained for dextran-1 using the pharmacopoeial size exclusion methodology and the alternative HILIC methology are present in Figure 2 and Figure 3 respectively.

With the pharmacopoeial size exclusion method for dextran-1, the last peak to elute (glucose) was found to have a retention time of approximately 459 minutes, with each successive polymer unit eluting prior to the previous. This is as expected with SEC, as the smaller the molecule, more retention is experienced as it can penetrate more through the solid phase pores, whilst the larger the molecule becomes the less interaction it has with the solid phase pores and will elute earlier.

Using the HILIC column, the first peak to elute is glucose, and whilst full baseline separation is not achieved, the chromatography is acceptable. With a pore size of 200 Å, there is potential for some size exclusion effect, however, the change in elution order suggests that the hydrophilic interaction is the dominant effect.

As a short comparison, the molecular weight distribution for the dextran-1 was determined for both methodologies using the pharmacopoeial equation (Equation 1). The results for the molecular weight fractions and the average molecular mass are presented in Table 4.

Equation 1 $$M_w = \sum w_i \times m_i$$

M_w= average molecular mass of Dextran-1

w_i= molecular mass of oligosaccharide i

m_i= weight proportion of oligosaccharide i

The data shows minor differences in the molecular weight distribution for the dextran-1. By both methods the sample meets the pharmacopoeial criteria in the monograph of:

- Average molecular mass (Mw): between 850 and 1150
- Fraction with less than 3 glucose units: less than 15%
- Fraction with more than 9 glucose units: less than 20%

Conclusion

The 13 minute HILIC method demonstrates a similar molecular mass distribution pattern for dextran-1. There is potential that this method may be a suitable alternative, to the pharmacopoeial method for the determination of the molecular mass distribution of dextran 1, though further work may be required to understand the differences between the two methods.

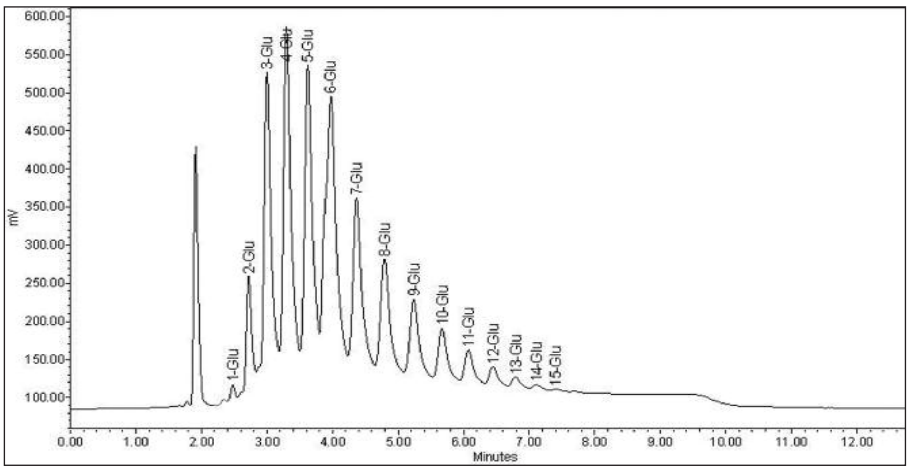

Figure 3: Typical HILIC chromatogram for Dextran-1 150 mg/mL Solution for Injection

		Size Exclusion Determination		HILIC Determination	
Glucose Unit (i)	Mi	%Area	Wi * Mi	%Area	Wi * Mi
1	180	0.25	0.5	0.35	0.6
2	342	3.59	12.3	3.78	12.9
3	504	13.15	66.3	12.57	63.4
4	666	17.03	113.4	15.12	100.7
5	828	16.14	133.7	14.28	118.2
6	990	13.52	133.8	18.67	184.8
7	1152	10.56	121.6	10.81	124.5
8	1314	7.96	104.6	8.22	108.0
9	1476	5.91	87.2	5.84	86.2
10	1638	4.23	69.2	4.08	66.9
11	1800	3.03	54.5	2.67	48.0
12	1962	2.11	41.5	1.71	33.5
13	2124	1.54	32.7	1.04	22.1
14	2286	0.99	22.7	0.58	13.3
15	2448			0.28	6.8
Mw=∑(Wi * Mi)			993.8		990.0
<3 glucose units,%			3.8		4.1
>9 glucose units, %			11.9		10.1

Table 4: Comparison of molecular weight fractions for Dextran-1 by SEC and HILIC

References

1. *European Pharmacopoeia 7.1 – Dextran-1 for Injection Monograph*
2. *Moncrieff J. A., Darin J. C., Canizaro P. C., Sawer R. B. Annals of Surgery (1963), 158(4), 553*
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5. *Grumach E. S., Wagrowski-Diehl D. M., Mazzeo J. R., Alden B., Iraneta P. C., LCGC North America (2004), 22(10), 1010*
6. *Phenomenex Luna HILIC Method Development Guidelines*