Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 353 (2008) 21–27
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Molecular weight determination for colloidal iron by Taguchi optimized validated gel permeation chromatography ☆

R.B. Shah, Y. Yang, M.A. Khan, P.J. Faustino *

Division of Product Quality Research, Office of Testing and Research, Office of Pharmaceutical Science, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave. Silver Spring, MD 20993, USA

Received 10 August 2007; received in revised form 1 November 2007; accepted 5 November 2007
Available online 22 November 2007

Abstract

Method development of gel permeation chromatography (GPC) is a time-consuming task, since finding appropriate operating conditions has traditionally been a trial-and-error process. A novel approach in the field of GPC using experimental design called Taguchi is presented. This experimental design was used to compare the net effects of various conditions which were both qualitative and quantitative in nature. Quantitative factors included mobile phase pH, flow rate, temperature of column and detector, and injection volume. The qualitative factors were treated as noise which included enclosure of GPC system and position of waste container with respect to refractive index detector. The method was efficient as opposed to a one-factor-at-a-time approach. Taguchi optimized conditions included pH of 7.2, flow rate of 0.4 mL/min, temperature of 35 °C for column and detector, as well as injection volume of 10 μL. The optimized factors yielded acceptable results in terms of weight average molecular weight (m.w.), standard deviation and signal-to-noise ratio. Standard curves were constructed using dextran m.w. standards (12,000–270,000 Da) over the analytical range. The method was validated according to ICH guidelines. Log-linear function was used for m.w. standard curve and weight average m.w. was calculated utilizing trapezoidal approach. A correlation coefficient of >0.99 was obtained for both intra-day and inter-day standard calibration curves. Inter-day accuracy ranged from 91 to 108% and precision was <2.0%.
Published by Elsevier B.V.

Keywords: Gel permeation chromatography; Taguchi design; Design of experiments; Iron sucrose; Validation; Weight average molecular weight

1. Introduction

Parenteral iron formulations containing iron complexed with carbohydrates are currently used for the treatment of anemia associated with chronic kidney disease (Kudasheva et al., 2004). Iron dextran (DexFerrum®), iron sucrose (Venofer®), and ferric gluconate (Ferrlecit®) have been used for this purpose (PPI Venofer, 2005; PPI DexFerrum, 1998; PPI Ferrlecit, 2001). Preservative free iron sucrose has been widely used due to its superior safety profile as compared to iron dextran and wider application in terms of patient population with non-dialysis-dependent, hemodialysis-dependent or peritoneal-dialysis-dependent chronic kidney disease patients (Venofer monograph). Iron sucrose injection is a sterile, colloidal solution of ferric hydroxide in complex with sucrose in water for injection (Iron sucrose monograph). The molecular weight (m.w.) of iron sucrose ranges from 34,000 to 60,000 Da and pH ranges from 10.5 to 11.1 (Venofer monograph; Iron sucrose monograph).

There are numerous literature reports on use of gel permeation chromatography (GPC) for the assessment of molecular weights (Iron sucrose monograph; Karmarkar et al., 2006; Mulloy et al., 1997; Van and Daenens, 1993; Meredith, 1984; Richter et al., 1983; Nilsson and Nilsson, 1974; Komatsu et al., 1993). However, method development is time consuming as the chromatographic separation can be influenced by mobile phase pH, column type, temperature, detector temperature, to name few. For refractive index detection, room temperature might also play a role which is difficult to control. Unlike normal or reverse phase chromatography GPC is considerably more sensitive to these environmental factors and operating parameters thus impacting efficient method development. Currently there is no systematic study approach to optimize a GPC method by

☆ *Disclaimer*: The opinions expressed in this work are those only of the authors, and do not necessarily reflect the policy and statements of the FDA.

* Corresponding author at: 10903 New Hampshire Ave. Silver Spring, MD 20993 FDA, USA. Tel.: +1 301 796 0021; fax: +1 301 796 9816.
E-mail address: Patrick.Faustino@fda.hhs.gov (P.J. Faustino).

0378-5173/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2007.11.025

utilizing the Design of Experiments (DoE) principle (Fisher, 1971) but most approaches use a one-factor-at-a-time approach (Mulloy et al., 1997; Van and Daenens, 1993; Meredith, 1984; Richter et al., 1983; Nilsson and Nilsson, 1974).

Molecular weight affects two biologic characteristics of iron sucrose that are directly relevant to therapeutic use in patients: rate of release of iron from the ferric oxyhydroxide core and rate of clearance of agent from the plasma after intravenous administration (Danielson, 2004). Thus m.w. is an important marker for product performance *in vivo*. The present work was aimed at studying the influence and interaction of operating factors on m.w. determination of iron sucrose preparations.

Taguchi's method of optimization (Taguchi and Wu, 1980; Taguchi, 1987; Roy, 2001) has been traditionally used to develop products that work well in spite of natural variation in materials, operators, suppliers, and environmental change. Thus it is alternatively referred to as robust engineering. The Taguchi arrays include two-level, three-level, and mixed-level fractional factorial designs. The unique aspects of this approach are the use of inner and outer arrays, signal-to-noise (S/N) factors, and S/N ratios. Here signal factors are control inputs whereas noise factors are variables that are typically difficult or expensive to control. Thus dividing the factors as signal or noise becomes a key input in such approaches. An inner design constructed over the control factors finds optimum settings. An outer design over the noise factors looks at how the response behaves for a wide range of noise conditions. The experiment is performed on all combinations of the inner and outer design runs. Thus in case of Taguchi's design, the response variable is not the raw response or quality characteristic but it is the S/N ratio (JMP). In the present work, the experimental design was applied to the optimization of an analytical method for the first time.

2. Materials and methods

2.1. Chemicals

Dextran molecular weight standards (12,000, 25,000, 50,000, 80,000, 150,000, and 270,000 Da), sodium azide and glacial acetic acid were purchased from Sigma–Aldrich (St. Louis, MO). Venofer® (iron sucrose injection, USP, 100 mg elemental iron/5 mL ampules), lot number Lot # 5394, was purchased from Washington Wholesale Drug Exchange (Savage, MD). Sodium phosphate, dibasic and sodium phosphate, monobasic and monohydrate were purchased from Mallinckrodt Baker (Phillipsburg, NJ). All other chemicals were of reagent grade and were used as received. Filtered 18 MOhm water was supplied in house by a Millipore Milli-Q System (Bedford, MA).

2.2. Preparation of calibration, quality control and system suitability samples

The first set of six dextran m.w. standards of 12,000, 25,000, 50,000, 80,000, 150,000 and 270,000 Da were prepared to a final concentration of 5 mg/mL from stock solution I with mobile phase for preparation of calibration curve. Quality control standards were prepared daily in mobile phase from the molecular weight quality control (QC) stock solution II (5 mg/mL) to produce nominal molecular weights of low QC (12,000 Da), QC intermediate standard (80,000 Da) and QC high standard (270,000 Da). Five solutions of each m.w. level were prepared. This set served as quality control standard samples. For system suitability sample, a dextran standard of 50,000 Da from stock solution III was used in similar concentration of 5 mg/mL in mobile phase. All m.w. standard solutions were equilibrated in mobile phase for 24 h prior to injecting onto the GPC.

2.3. Calibration curve

To construct the calibration curve, the logarithm of the molecular weight of dextran standards ranging from 12,000 to 270,000 Da was plotted as a function of the retention time for each standard ($n = 3$).

2.4. Taguchi's experimental design

Table 1 shows the chromatographic factors, noise factors and response studied. A four-factor three-level including two noise two-level selected for L-9 orthogonal array Taguchi experimental design was generated using JMP software (JMP 5.1 version). Table 2 shows the values of the experimental factors.

2.5. Chromatographic system

All standards and samples were analyzed by high performance-GPC. Two replicate analysis of each standard were conducted per analytical run. An Agilent Series 1100 (Wilmington, DE) high performance liquid chromatography system was equipped, a 1362A refractive index detector, a 1311A series quaternary pump, a 1316A thermostated column compartment, a 1329A series thermostated automated injector and 1322A series solvent degasser modules. Chromatographic separation was achieved on two 7.8-mm × 30-cm Tosoh G4000PW$_{XL}$ polymer-based columns with pore sizes of 500 Å (Supelco, Bellefonte, PA) set up in series. The column and detector compartment were maintained either at 35, 40 or 45 °C as per the experimental scheme shown in Table 2. The flow rate of 0.4–0.6 mL/min

Table 1
Chromatographic and noise factors and response variable for Taguchi's experimental design (L-9 orthogonal array)

Chromatographic factors	Levels used		
	Low	Medium	High
X_1: pH of mobile phase	6.4	6.8	7.2
X_2: flow rate (mL/min)	0.4	0.5	0.6
X_3: temperature of column/detector (°C)	35/35	40/40	45/45
X_4: injection volume (μL)	10	20	30

Noise factors	Levels used	
	Low	High
Position of waste container	Below detector	Above detector
Chromatography system enclosure	Not enclosed	Enclosed

Dependent variable, Y: molecular weight of iron sucrose.

Table 2
Taguchi experimental design randomized runs and the response

Run	X_1	X_2	X_3	X_4	Y_{--} [a]	Y_{-+} [b]	Y_{+-} [c]	Y_{++} [d]	Y_{avg} [e]	Y_{std} [f]	S/N [g]
1	6.4	0.4	35	10	82,587	87,130	60,407	91,997	80,530	13,955	98
2	6.4	0.5	40	20	112,711	109,312	115,516	100,784	109,581	6,390	101
3	6.4	0.6	45	30	125,314	131,736	128,120	141,001	131,543	6,832	102
4	6.8	0.4	40	30	64,000	64,548	62,511	64,635	63,924	983	96
5	6.8	0.5	45	10	57,228	53,438	58,361	56,502	56,382	2,107	95
6	6.8	0.6	35	20	69,385	67,796	69,257	66,876	68,328	1,207	97
7	7.2	0.4	45	20	51,421	49,306	52,296	54,228	51,813	2,042	94
8	7.2	0.5	35	30	53,332	55,191	56,156	54,915	54,899	1,172	95
9	7.2	0.6	40	10	52,304	52,584	51,861	52,609	52,340	348	94

[a] Waste container below the detector and GPC system not enclosed.
[b] Waste container below the detector and GPC system enclosed.
[c] Waste container above the detector and GPC system not enclosed.
[d] Waste container above the detector and GPC system enclosed.
[e] Y_{avg}: average of the response.
[f] Y_{std}: standard deviation of the response.
[g] S/N: signal-to-noise ratio.

was used for the Taguchi experiments. Sodium phosphate buffer of 40 mM with 0.02% sodium azide (NaN_3), pH adjusted to 6.4, 6.8, or 7.2 with sodium hydroxide, was used as the mobile phase and sample diluent in respective experiments as shown in Table 2. The solvent reservoir was an external 4 L reservoir. The injection volume for samples and standards ranged from 10 to 30 μL.

2.6. Analytical method and validation

The HP-GPC is a chromatographic technique in which analytes are separated based on their molecular size in solution. Basically, the polymer-based GPC columns containing pore sizes up to 500 Å act as sieves through which the smaller molecules will permeate, while the larger molecules or particles are excluded by being swept around the pores (Fig. 1). Due to this action, HP-GPC is also referred to as size-exclusion chromatography (SEC), where substances of different sizes are eluted from the columns and are separated from largest to smallest. As each separated substance is eluted, a refractive index detector measures the substance's peak response. The peak retention time of chromatogram represents the relative molecular weight. The longer the retention time is, the smaller the molecular weight is, and vice versa. Several validation characteristics including linearity, accuracy, precision, stability and reproducibility were validated according to USP, FDA guidelines. Analytical range was established according to the ICH (Validation USP <1225>; ICHQ2A, 1995; ICHQ2B, 1997; Reviewer Guidance FDA, 1994; Heyden et al., 2002). The Taguchi optimized validation parameters are shown in Table 3.

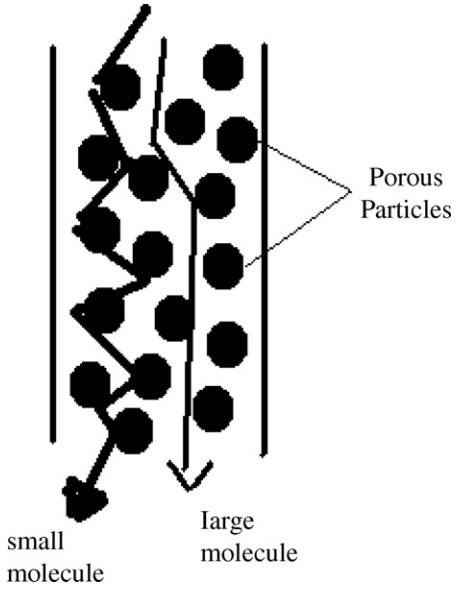

Fig. 1. Separation principle in gel permeation chromatography.

2.7. Calculation of weight average molecular weight

To construct the calibration curve, the logarithm of the molecular weight of dextran standards ranging from 12,000 to 270,000 Da was plotted as a function of the retention time ($n = 3$). From the chromatogram the iron sucrose peak was man-

Table 3
Taguchi optimized GPC method for m.w. assessment of iron sucrose

Parameter	Experiment conditions
Chromatographic separation	Gel permeation chromatography (GPC)
System	Agilent 1100
Detection	Refractive index (35 °C)
Column 1	TSK-GEL 4000 PW-XL 300 mm × 7.8 mm (35 °C)
Column 2	TSK-GEL 4000 PW-XL 300 mm × 7.8 mm (35 °C) (polyhydroxy-cross-linked methyl acrylate)
Mobile phase	0.02% sodium azide with acetic acid
Elution	Isocratic
pH	7.2 with sodium hydroxide
Flow rate	0.4 mL/min
Injection volume	10 μL
HPLC system and columns	Shrouded
Position of waste container	Above the detector

ually selected. The negative peak value was converted to positive value, if necessary. A straight line was drawn between the initial and final point. The area under the curve (AUC) was calculated by trapezoidal summation from the initial point to the final point. Then the average retention time was calculated, and from this average retention time the weight average molecular weight was obtained based on the calibration curve. A linear as well as polynomial fit was obtained. The log-linear fit equation was found to be acceptable and used for the calculation of m.w.

2.8. Statistical analysis

Statistical analysis was performed using regression analysis.

2.9. Molecular weight determination of a colloidal iron drug product

Three individual sample vials of the colloidal iron (iron sucrose) drug product Venofer® were analyzed in triplicate by GPC using the weight average molecular weight approach.

3. Results and discussion

3.1. Taguchi optimization

Many times the one-factor-at-a-time series does not progress efficiently as negative results may discourage or will not allow a selection of parameters which need to be changed in the next experiment. The data might be insufficient to draw any significant conclusions and the main problem of understanding the basis of parameter or their interactions will still remain unsolved. A well-planned set of experiments, in which all parameters of interest are varied over a specified range, is a much better approach to obtain systematic data. A method based on orthogonal array experiments which gives much reduced variance for the experiment with optimum settings of control parameters was chosen for the purpose. The primary aim of the Taguchi experiments is to minimize variations in output even though noise is present in the process. Thus it helps to increase robustness of the process. The aim is to make a product or process less variable (more robust) in the face of variation over which one has little or no control. Conventionally, it can be viewed as an experiment in four factors. Taguchi has pointed out the usefulness of viewing it as a set-up of four inner array factors (pH, flow rate, temperature, and injection volume) over which we have design control, plus an outer array of factors over which we have control only in the laboratory (shrouding of equipment, position of waste container). Refractive index detector which is a bulk property detector is one of the least sensitive LC detectors. Conversely, refractive index detectors are highly affected by changes in ambient temperatures, pressure changes, flow rate changes, or changes in solution density. The later factor does not necessarily depend on sample concentration, but can change due to environmental factors such as temperature and pressure. Even small changes in ambient temperature can cause baseline drift. Therefore, to stabilize the refractive index detector from the effects of operating environmental temperatures, it is recommended that the refractive index detector system should be kept away from air conditioning vents, chance breeze or direct sunlight. Therefore we selected enclosure of the entire GPC with the detector system as one of the noise factors, by keeping the system shrouded (enclosed) and non-shrouded (open to the environment). It is generally recommended by refractive index detector manufacturers to keep the waste container above the detector in order to avoid back pressure pulses from a dripping waste tube. The reason being, that this back pressure change can cause short-term baseline drifts. RI detectors operate with significantly lower back pressures of 20–40 bar and are therefore more sensitive to small changes in backpressure caused by waste container position. This effect was observed during the preliminary experiments, and therefore was included as another noise factor to observe the effect of position of the waste container and in other terms, the effect of back pressure on the reproducibility and overall quality of chromatography. However, the effect was not significant with respect to the shrouding of the system to prevent it from environmental changes or keeping the waste container to prevent back pressure. The baseline drift did not remain an issue with the samples eluted as the refractive index unit (y-axis on chromatogram) for the iron sucrose eluted was significantly higher than the noise due to baseline drifts.

Taguchi orthogonal experimental designs [L-9] offer the possibility of investigating four independent variables at three levels in presence of noise. The selection of factors and levels in the design would be based on the results of a preliminary investigation. GPC methods are very sensitive to small fluctuations in experimental factors such as operating parameters and environmental influences. Obtaining an analytical method to analyze accurate m.w. and small changes in m.w. for colloidal iron preparations requires information on the effect that chromatographic and sample variables have on the chromatographic performance.

Taguchi experimental design and the values of the response variable are shown in Table 2. Note that there are four outputs measured on each row. These correspond to the four 'outer array' design points. Each row yields a mean and standard deviation percent of weight average m.w. Ideally, there would be one row that had the optimized m.w., lowest standard deviation, and the highest S/N ratio. Row 9 has the lowest standard deviation and row 3 has the highest S/N ratio. There is no row with all desired characteristics so simply 'picking a winner' approach was not feasible. Also it should be noted that rows 1–3, 4, and 6 yielded an m.w. for iron sucrose which was outside the acceptable range. Additionally, the pH of 6.4 yielded highest standard deviation and thus the process was found to be highly variable due to experimental factors, although there was minimum effect of noise factors.

A model was generated and S/N ratio was calculated. Fig. 2 shows the actual S/N ratio versus the predicted ratio with R^2 of 0.998 confirming the validity of the model. Pareto plot showed the effect of main factors on the outcome (Fig. 3). It is clear from the figure that pH of the mobile phase had maximum effect on Y-variable. It is also shown in Fig. 4. The prediction profiler is a quick way to find settings that give the highest signal-to-noise ratio for the experiment (Fig. 4). The profile traces indicate that different settings on the first factor (pH) have maximum impact

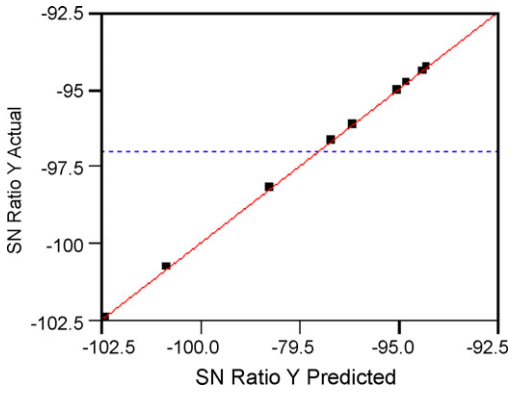

Fig. 2. Actual by predicted plot.

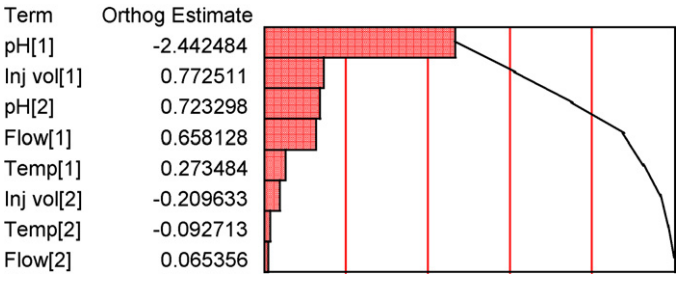

Fig. 3. Pareto plot.

on *Y*-variable as well as S/N ratio. The pH of the mobile phase affects largely the properties of the colloidal iron molecules, and therefore was shown to be the most significant parameter in terms of its elution on GPC. Although to a lesser extent pH, according to classical GPC theory, does effect the interactions of the particles with the GPC stationary phase. This is based on the charges present on the particles which are not end-capped as in case of reversed phase columns. Therefore these charges, although small, can interact with the analyte of interest depending upon the pH. The phenomenon was observed in case of iron sucrose elution with respect to pH. The flow rate of the mobile phase, temperature of column and detector as well as injection volume also effect the *Y*-variable, however, the effect was lower as compared to that of mobile phase pH. Iron sucrose monograph has m.w. range from 34,000 to 60,000 Da and thus

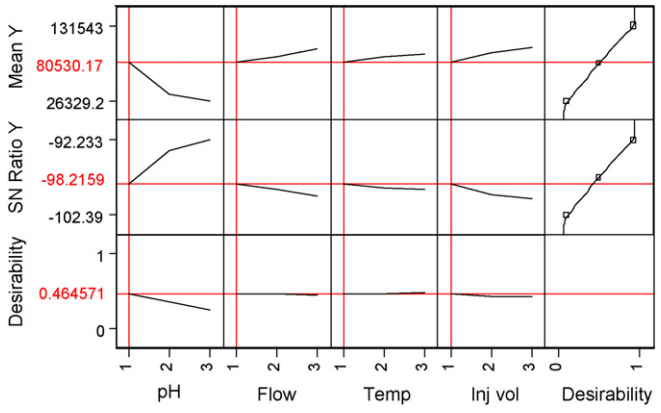

Fig. 4. Prediction profiler.

Table 4
Parameters of calibration curve ($n = 3$)

Parameter	Day 1	Day 2	Day 3
Linear range (Da)	12,000–270,000	12,000–270,000	12,000–270,000
Calibrators	6	6	6
R^2 value	0.9965	0.9966	0.9962
Slope	−0.3511	−0.3499	−0.3514
Intercept	22.296	22.256	22.322
Retention time R.S.D. (%) of system suitability	0.042	0.071	0.077

Table 5
Intra-day precision and accuracy ($n = 5$)

Standards (Da)	Precision (%R.S.D.)			Accuracy (%)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
12,000	0.27	0.30	0.48	93	93	93
80,000	0.25	1.10	0.68	108	108	109
270,000	1.04	1.18	0.54	91	91	92

to obtain such result, experimental setting which gives a lower *Y*-variable value needed to be chosen. Also the value of factors which yield maximum S/N ratio was considered. In order to obtain minimum desirability, L3 of pH was chosen as optimum condition whereas other three factors did not change the desirability significantly. However they should be at L1 considering slightly better S/N ratio. Therefore pH 7.4, flow rate of 0.4 mL/min, temperature of 35 °C, and injection volume of 10 μL were chosen as optimized factors. The experiment performed with these settings gave accurate and reliable results and therefore the method was validated at these operational parameters.

3.2. Analytical method validation

In this study, several validation characteristics including linearity, accuracy, precision, stability and reproducibility were addressed. Six commercially available high molecular weight standards were used to construct the standard calibration curve. The calibration curve was linear over the validated ranging from 12,000 to 270,000 Da when the logarithm of the molecular weight was plotted against the retention time. The correlation coefficient was greater than 0.99 on three different days (Table 4).

Intra-assay and inter-assay precision and accuracy results are listed in Tables 5 and 6. The mean intra-day precisions were 0.27–1.18%. The mean intra-day accuracies were 91–109%. The mean inter-day precisions were 0.35–0.92%. The mean inter-day

Table 6
Inter-day precision and accuracy ($n = 15$)

Standards (Da)	Precision (%R.S.D.)	Accuracy (%)
12,000	0.35	93
80,000	0.68	108
270,000	0.92	91

Table 7
Molecular weight determination of the colloidal iron drug product Venofer® ($n = 3$)

Venofer®	Molecular weight (m.w.)	S.D.	%R.S.D.
Sample 1	47,400	750	1.58
Sample 2	48,050	650	1.35
Sample 3	49,650	550	1.10

accuracies were 91–108%. The acceptance ranges of accuracies are 85–115% for the low, intermediate and high QCs. The allowable limits of %R.S.D. are 15% for the low, intermediate and high QCs (Guidance for Industry, 2001). Therefore, both accuracy and precision were found to be acceptable. When taken together, acceptable accuracy, precision and linearity were used to establish the analytical range.

Two additional determinations of molecular weight for iron sucrose were made on different days with two different analysts. These experiments were conducted to establish the reproducibility and ruggedness of the molecular weight procedure. The results are shown in Table 7. The acceptance criterion for the %R.S.D. is less than 3%.

The response factor (retention time) of dextran standards solutions was found to be unchanged for up to 30 days under temperature of 4 °C. Less than 0.3% retention time difference was found between the solutions freshly prepared and those aged 30 days. The solutions can therefore be used within this period without the results being affected.

To obtain the selectivity for iron sucrose and its components such as sucrose was essential. The reason is not only that iron sucrose and its components have to be separated but also some possible degraded fraction with similar retention times. The current method effectively resolved the peak from formulation excipients as shown in Fig. 5.

3.3. Calculation of weight average molecular weight

One important step for GPC in the data treatment to derive m.w. is the modeling of the calibration curves. The weight average m.w. was calculated for each experiment by constructing a standard curve with m.w. dextran standards ranging from 12,000 to 270,000 Da for each of the runs. However the calibration curves were not linear for m.w. of standard versus retention time. The standard calibration curve obtained for GPC was linear for log m.w. versus retention time. The polynomial fit also yielded good R^2 value (∼0.99). However it is reported in the literature that making the polynomial more complex, i.e. using a higher order polynomial, the model will fit the data better (Heyden et al., 2002). Therefore the use of such models should be avoided as much as possible since mathematical changes such as the application of higher order polynomials can affect the analytical accuracy and therefore the validity of the results overall. In the current study, log linear calibration curves were utilized for the calculation of the weight average m.w.

The retention time cannot be just selected as peak retention time as it will not yield the weight average m.w. For this purpose, calculations were realized on excel after exporting the data from ChemStation chromatographic software (Agilent Technologies, version 8.03). The iron sucrose peak was selected and negative values of peak heights were first converted to positive values. A straight line was drawn from peak onset up to the end of the peak and slope and intercept values were used to calculate area under curve for each of the trapezoid by trapezoidal rule. After that the sum of each trapezoidal AUC multiplied by average line value divided by total AUC and considering the equation of straight line gave rise to weight average retention time. Once this value was obtained, a standard calibration curve was used to calculate the weight average m.w.

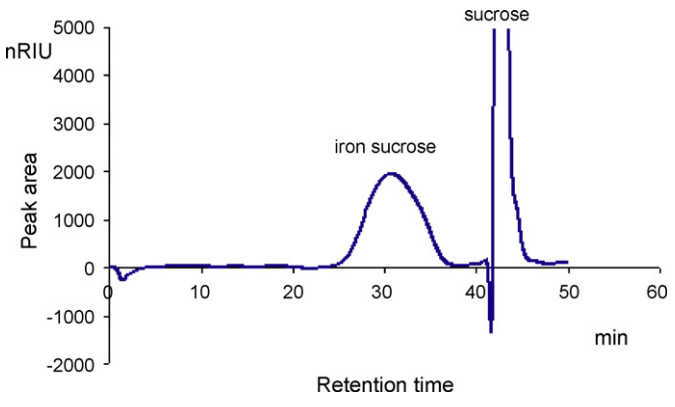

Fig. 5. Chromatogram of iron sucrose containing free sucrose.

3.4. Determination of the weight average molecular weight of an iron sucrose colloidal iron drug product

Three individual samples of the iron sucrose colloidal iron drug product Venofer® were analyzed in triplicate by GPC (Fig. 5) using the weight average molecular weight approach. The average molecular weight of Venofer® was determined to be approximately 48,000 Da (Table 7). These data are consistent with the manufacturing product insert that specifies the molecular weight range to be between 34,000 and 60,000 Da.

4. Conclusion

Gel permeation chromatographic factors were optimized using a Taguchi orthogonal experimental design. The pH of the mobile phase had a significant influence on the m.w. of iron sucrose. The other less influential factors included the flow rate of the mobile phase, temperature of the column and detector as well as the injection volume. The factors were optimized considering an optimum m.w. as obtained from the m.w. range given for iron sucrose products, low standard deviation as well a high signal-to-noise ratio. Validation results indicated that the method shows acceptable linearity, precision, and accuracy, over the analytical range. The method was also shown to be rugged. The method was used successfully to determine the molecular weight of the colloidal iron drug product Venofer®.

Acknowledgements

Authors thank Dr. Andre S. Raw and Dr. Lawrence X. Yu from Office of Generic Drugs (OGD) for their invaluable help. OGD is also acknowledged for providing partial funding for the project.

References

Danielson, B.G., 2004. Structure, chemistry, and pharmacokinetics of intravenous iron agents. J. Am. Soc. Nephrol. 15, S93–S98.

Fisher, R., 1971. Design of Experiments, ninth ed. Macmillan and Company, New York.

Guidance for Industry: Bioanalytical Method Validation, 2001. FDA CDER guidance page, http://www.fda.gov/cder/guidance/index.htm.

Heyden, Y.V., Popovici, S.T., Schoenmaker, P.J., 2002. Evaluation of size-exclusion chromatography and size-exclusion electrochromatography calibration curves. J. Chromatogr. A 957, 127–137.

ICHQ2A, 1995. Guidance for industry, Text on Validation of Analytical Procedures. International Conference on Harmonization-Quality.

ICHQ2B, 1997. Guidance for industry, Validation of Analytical Procedures: Methodology. International Conference on Harmonization-Quality.

Iron Sucrose, USP Monograph USP29-NF24, 2006.

JMP Help Menu, JMP 5.1.

Karmarkar, S., Garber, R., Kluza, J., Koberda, M., 2006. Gel permeation chromatography of dextrans in parenteral solutions: calibration procedure development and method validation. J. Pharm. Biomed. Anal. 41, 1260–1267.

Komatsu, H., Takahata, T., Tanaka, M., Ishimitsu, S., Okada, S., 1993. Determination of the molecular-weight distribution of low-molecular-weight heparins using high-performance gel permeation chromatography. Biol. Pharm. Bull. 16, 1189–1193.

Kudasheva, D.S., Lai, J., Ulman, A., Cowman, M.K., 2004. Structure of carbohydrate-bound polynuclear iron oxyhydroxide nanoparticles in parenteral formulations. J. Inorg. Biochem. 98, 1757–1769.

Meredith, S.C., 1984. The determination of molecular weight of proteins by gel permeation chromatography in organic solvents. J. Biol. Chem. 259, 11682–11685.

Mulloy, B., Gee, C., Wheeler, S.F., Wait, R., Gray, E., Barrowcliffe, T.W., 1997. Molecular weight measurements of low molecular weight heparins by gel permeation chromatography. Thromb. Haemost. 77, 668–674.

Nilsson, G., Nilsson, K., 1974. Molecular-weight distribution determination of clinical dextran by gel permeation chromatography. J. Chromatogr. 101, 137–153.

PPI DexFerrum® (Iron Dextran Injection, USP) Patient Package Insert, 1998.

PPI Ferrlecit® Product Package Insert, 2001.

PPI Venofer® (Iron Sucrose Injection, USP) Product Package Insert, 2005.

Reviewer Guidance: Validation of Chromatographic Methods, 1994. FDA CDER guidance page, http://www.fda.gov/cder/guidance/index.htm.

Richter, W.O., Jacob, B., Schwandt, P., 1983. Molecular weight determination of peptides by high-performance gel permeation chromatography. Anal. Biochem. 133, 288–291.

Roy, R., 2001. Design of Experiments using the Taguchi Approach: 16 Steps to Product and Process Improvement. Wiley-Interscience, New York.

Taguchi, G., Wu, Y., 1980. Introduction to Off-line Quality Control, Japan: Central Japan Quality Control Association (available from American Supplier Institute, 32100 Detroit Industrial Expressway, Romulas, MI 48174).

Taguchi, G., 1987. In: Clausing, D. (Ed.), System of Experimental Design: Engineering Methods to Optimize Quality and Minimize Costs, vols. 1 & 2. UNIPUB, Krass International Publications, New York.

Van, D.J., Daenens, P., 1993. Molecular weight identification of polyethylene glycols in pharmaceutical preparations by gel permeation chromatography. J. Pharm. Sci. 82, 938–941.

Venofer Formulary Monograph. Available at http://www.venofer.com/VenoferHCP/new_home.asp.

Validation of Compendial Methods General Chapter <1225>. United States Pharmacopoeia 29/National Formulary 24, 2006.