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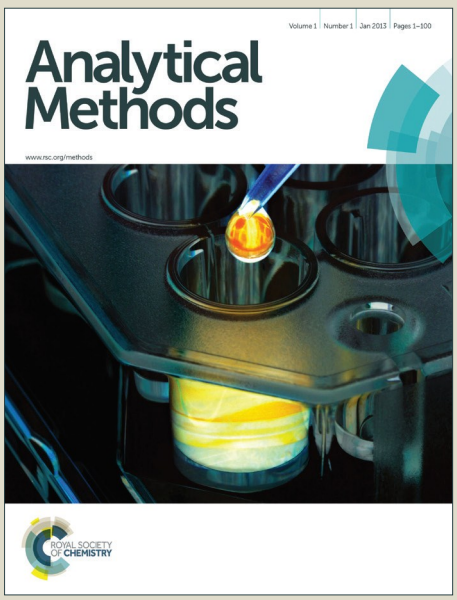

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ARTICLE

Fast and efficient method for molecular weight analysis of cellulose pulp, in-process and finished product

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Abstract:The purpose of this study is to establish method for molecular weight analysis of cellulose pulp using lesser number of GPC columns, with shorter analysis time and lesser cost of analysis using HPLC which is applicable in processing or manufacturing unit. Pullulan polysaccharides are used as standard and 0.5% LiCl in N,N-dimethylacetamide as eluent. Typically four columns were used for molcular weight determination, which is replaced with two columns as an alternative method. The comparative data of four columns versus two columns is represented in the results and discussion section.The method is validated to cover system precision, linearity, accuracy, method precision, robustness and solution stability to support the study.

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A Introduction

In the production of pulp & cellulose fibre, there is occurrence of vigorous polymerization that can precede either uniformly or non-uniformly. Accurate information about changes in the molecular weight and molecular weight distribution of the wood pulp, viscose and lyocell is necessary[1].

The parameters which are highly relevance for analysis of molecular weight and its distribution are as follows:

Degree of polymerization (DP): It is usually defined as the number of monomeric units in a macromolecule or polymer or oligomer molecule. [2,3,4] Degree of polymerisation and Viscosity (cP values) are industrial known parameters which are related to mechanical property of material. Some authors, however, define DP as the number of repeat units, where for copolymers the repeat unit may not be identical to the monomeric unit.[5,6]

Number average molecular weight (Mn): The number average molecular weight is the statistical average molecular weight of all the polymer chains in the sample

Weight average molecular weight (Mw): The weight average molecular weight takes into account the molecular weight of a chain in determining contributions to the molecular weight average. The more massive the chain, the more the chain contributes to Mw.

Higher average molecular weights (Mz, Mz+1): The higher averages are increasingly more sensitive to high molecular weight polymers and accordingly are increasingly more difficult to measure with precision. They tend to be associated with methods that measure the motion of polymer molecules, such as diffusion or sedimentation techniques. Although the z-averages are not commonly quoted for polymers, several important methods for measuring the dimensions of chains that yield z-average molecular weights. [7]

The polydispersity index (PDi) is used as a measure of the broadness of a molecular weight distribution of a polymer, and is defined by: Polydispersity index = Mw/Mn

The larger the polydispersity index, broader the molecular weight. SEC is the only technique that measures Mn, Mw, Mz and Mz+1 at the same time by measuring the entire distribution of the polymer.[7]

Peak molecular weight (Mp): The peak molecular weight Mp, defined as: Mp = molecular weight of the highest peak. Therefore, Mp is the mode of the molecular weight distribution.[7]

Size Exclusion Chromatography (SEC) or Gel Permeation Chromatography (GPC) is the preferred method of determining molecular weight and molecular weight dis-tribution. The fundamental of SEC / GPC is the separation of solvated molecules on column packed with material having a broad distribution of pore sizes. Differences in effective molecular size in solution separates molecule in sample.[8]

Calibration is necessary so that the elution volume of chromatograms can be related to polymer molecular weight. There are several methods but a calibration based on well characterized polymers with very narrow molecular distribution is mostly in practice. Ideally with a set of standards with Mw/Mn is 1, which is highly monodispers. A calibration curve can be constructed if a number of samples of different molecular weights are available. It has been shown experimentally that, over the useful separation range of a GPC column, the functional relationship between the molecular weight M of a molecular species, and its elution volume v is of the approximate form [9,10]. Using narrow and low molecular weight fractions, a plot of log M vs. retention time (or volume) is fitted with 3rd or 5th polynomial to get a calibration curve.[11,12]

Previous studies have characterized values of Mw of pulp celluloses by methods such as fractionation or GPC or SEC of cellulose derivatives. Fractionation methods are tedious and impractical for routine analysis. GPC is more practical, but it requires converting cellulose into a derivative that dissolves in organic solvents. This derivatization exposes the cellulose chains to degradation[13].

A discovered solvent system for cellulose is a mixture of N-N,-dimethylacetamide (DMAc) and lithium chloride[14,15]. Evidence suggests that this solvent system effects dissolution by complex formation [14].In the dissolution mechanism by McCormick, suggests that the hydroxyl protons of the anhydroglucose units and the chloride anions from the dissociated salt form hydrogen bonding[16]. The chloride anion also associates with a Li+(DMAc) macrocation. Each hydroxyl group in a cellulose molecules complexes with only one LiCl molecule[17]. There are also methods available where refractive index (RI) detector and multi angle light scattering (MALS) are connected in-line for molecular weight determination.[18]

Typically four SEC columns were used, which usually takes 50 minutes to complete the analysis.[19] This leads to more consumption of solvent, time and testing cost. In view of raw material characterisation, in-process monitoring and finished goods testing; similarly taking consideration of reducing the factors related to cost and time for analysis, the proposed method is developed. This new method deals with two PLgel columns instead of four columns and RI detector. The results obtained by comparative study of four columns versus two columns are mentioned in results and discussion section. The developed method was validated to support the study by considering parameters like system precision, method precision, accuracy, linearity, robustness and solution stability.

B Experimental

Reagents: N,N-Dimethylacetamide (DMAc): GC Grade, 99.0%, manufactured by Sigma Aldrich.

Lithium Chloride: Reagent Plus, 99.0%, manufactured by Spectrochem.

Pullulan Polysaccharide standards (Molecular Weight (Mw) range from 180, 667, 6200, 10000, 21700, 48800, 113000, 200000, 366000 and 805000 dalton). Manufactured by Agilent Technologies.

Filter: Ultipor N Nylon 6,6 membrane (Ultipor N) or equivalent with pore size of 0.2 µm and diameter of 47 mm. Syringe filters Nylon (Syringe filters) or equivalent with pore size of filter 0.2 µm and diameter of 25 mm

Instrument: Waters HPLC equipped with Refractive Index detector 2414, Auto sampler 2707, HPLC pump 515, Temperature Control module II and Empower2 GPC Software.

Chromatographic condition:

Column: PLgel 20µ Mixed-A LS 300 x 7.5mm and a guard column of Agilent make.

Refractive Index detector: Temperature: 35°C, Sensitivity: 8

Column temperature: 70°C

Mobile phase flow rate: 1.0 ml/min

Sample: Pulp sample, In-process sample and Fibers supplied by pulp manufacturing company from Canada.

Procedure

Mobile phase preparation (0.5% LiCl in DMAc): Taken 5 gm of LiCl in 1000ml of DMAc. Stirred for one hour and filtered using Ultipor N.

Standard solution preparation (Standard solution concentration 0.1%): Weighed 10mg of Pullulan Polysaccharide standard of each molecular weight in different culture vial. Added 10 ml of mobile phase to each vial. Stirred vigorously for 24 hours on magnetic stirrer without heating. Filtered the solution using Syringe filters.

Sample preparation: Weighed 100 mg of sample in beaker. Added 100 ml of deionized water and boiled for 2 hours. Stirred occasionally with glass rod. Filtered the solution using Ultipor N. Washed with 25ml hot deionized water three times and collected the residue. Taken residue in beaker and added 40ml of methanol. Stirred for 30 min on magnetic stirrer without heating. Filtered the solution using Ultipor N. Washed with15 ml methanol three times and collected the residue. Soaked the residue with DMAc for 30 min with stirring and filtered using Ultipor N. Washed with15 ml DMAc three times and collected the residue. Taken freshly prepared 10ml of 8% LiCl in DMAc (0.8gm LiCl in 10ml DMAc) in culture vial. Added 20 mg of cellulose with respect to initial amount (one-fifth of the initial 100mg sample) in culture vial. Stirred continuously with heating till 40°C for two hours. The dissolved cellulose sample is diluted with DMAc to make the concentration of 0.5% of LiCl in the sample solution by taking 1ml of dissolved sample in 15ml DMAc and stirred for one hour. Filtered the sample using Syringe filters. Filled the filtered sample in sample vial and run the sample for analysis.

Four column versus two column:

Connected in series four columns of PLgel in the column oven and analyse the pullulan polysaccharide standards. Similarly, in series two columns of PLgel in the column oven and ran the pullulan polysaccharide standards. Calibration linearity graph was plotted log Mw versus elution volume and is further discussed in result and discussion section.

Method validation protocol:

Using the chromatographic condition as mentioned, two columns were connected and carried out the validation. Following are the parameter used for method validation:

System precision: System precision of the system was performed by six replicates injection of standard solution of Mw 180, 113000 and 805000 dalton. The criteria set for system precision is %RSD of molecular weight Not More Than (NMT) 15.0%.

Calibration Linearity: Linearity parameter was demonstrated by preparing ten standard solutions ranged from Mw = 180 to 805000 dalton and performed triplicate injections for each solution. The criteria set for linearity is coefficient regression = R^2 ≥ 0.980.

Accuracy: Accuracy of the method was performed by triplicate injection of a standard solution of Mw = 180, 113000 and 805000 dalton. Similarly, triplicate injections of sample solution of fibre were injected for accuracy study. The criteria set for accuracy of standard solution is % Error = ±10%. % error is measured as deviation from the standard value of molecular weight. For example, If observed value of Mw is 188 dalton for theoretical value of Mw 180 dalton, then % error will be 4% (Calculated as ((188/180)x100)-100 = 4%).

Method precision: Method precision is carried out for repeatability and intermediate precision. Two chemist performed six replicates of standard solutions of Mw = 113000 dalton for polydispersity (PDi) determination. The criteria's set for method precision were %RSD of PDi NMT 15.0%.

Robustness: Robustness is the parameter in which deliberate changes are made to the system or equipment parameters. In this experiment following parameters are deliberately changed:
1. Change in the column temperature by ± 5°C,

2. Change in the flow rate of eluent by± 0.2ml/min and

3. Change in the concentration of LiCl in eluent by ± 20% from the value specified in the method. Injected standard solution of Mw = 180, 113000 and 805000 dalton. The criteria's set for robustness is % error in determination of Mw is NMT 10 %.

Solution solubility: Solution stability parameter is the study of stability of working standard and fibre sample. It was carried out by performing injection of pullulan polysaccharide of Mw 113000 and fibre sample after every six hours of interval for 60 hours and data was collected.

The criterias set for solution stability is the PDi observed to measure with ±5% of Average PDi.

C Result & Discussion

Comparative study - Four columns versus two columns

The comparative study between four columns versus two columns was carried out to support the method development. The R^2 for four columns and two columns are 0.9988 and 0.9995 respectively. This shows very less significant variation in data. Also run time required for four columns is 50 minutes while for two columns 25 minutes. Hence, the method also enhanced the productivity of analysis by two folds. This will help in production plant where turnaround time is priority for batch process. (Refer table no.1 and figure no. 1 & 2)

Table 1. Comparative study

Molecular Weight Mw	Retention time (RT) of four column	Retention time (RT) of two column
180	-	17.82
667	32.37	17.20
6200	29.85	15.90
10000	29.38	15.62
21700	28.36	15.16
48800	27.22	14.65
113000	26.20	14.09
200000	25.20	13.60
366000	24.25	13.21
805000	23.63	12.86
R^2	**0.9988**	**0.9995**

Figure 1. Four columns

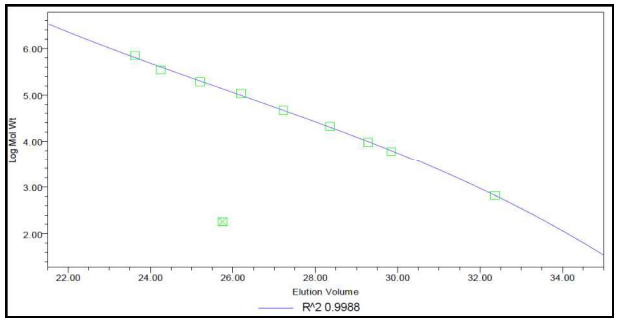
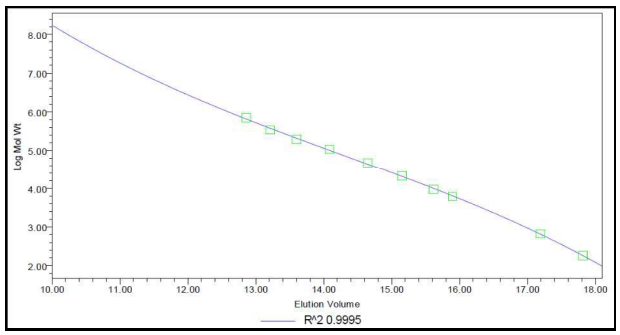


Figure 2. Two columns



System precision study

Results obtained are average of six replicates of each molecular weight for system precision. The set criteria is achieved thus concludes that the system is precise. (Refer table no. 2, figure 3, 4 and 5).

Table 2. System Precision for HPLC

Theoretical Mw	Average Observed Mw	%RSD
180	192	0.6
113000	114364	2.0
805000	747803	0.3

Figure 3. Typical chromatograph of Blank.

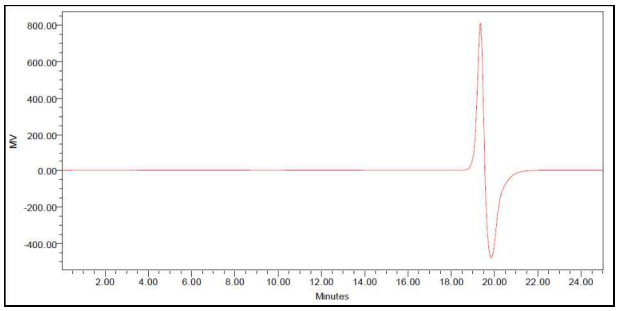


Figure 4. Typical chromatograph of pullulan polysaccharide standard

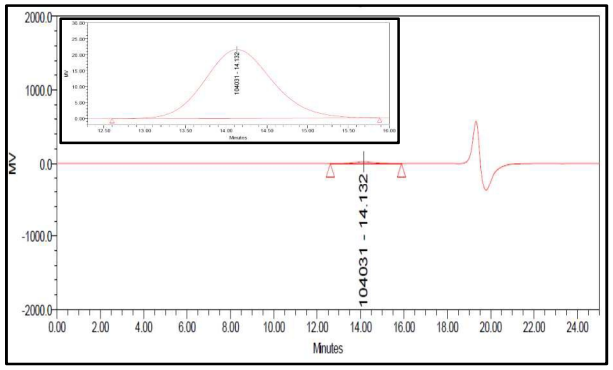
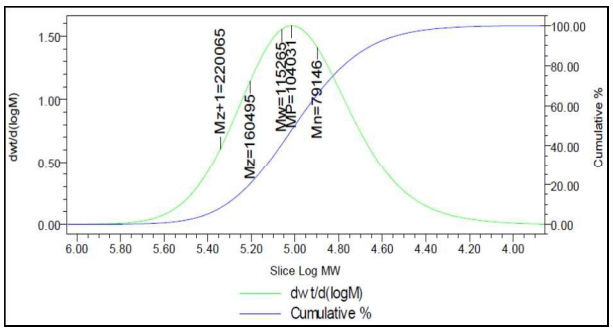


Figure 5. Typical chromatograph of pullulan polysaccharide standard with Mwd plots



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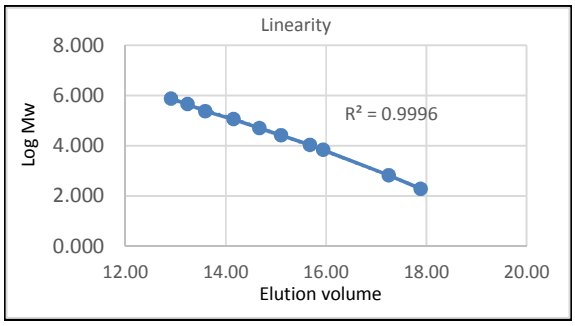
Calibration - Linearity study

Results obtained for linearity is shown by linearity graph of log Mw versus elution volume is shown in figure 6. The study derives R^2 = 0.999 with 3rd polynomial fitted calibration curve which meets the set criteria and concludes that the system is linear.

Figure 6. Linearity study on HPLC



Accuracy study

cP and DP values are industrial known parameters which are related to mechanical property of material. The given value of cP and Dp of sample is mentioned in table no. 4. Raw material (pulp) is having more number of repeating unit, which result into higher molecular weight as compare to its in-process and finished product (fibre). From the results it is seen that Observed Mw is decreasing from raw material (pulp) to finished product (fibre) which matches with the available Dp value. The result obtained from the study for standard and sample concludes that the method is accurate and are shown in table no. 3 and 4. (Refer figure 7 & 8)

Table 3. Summary table of accuracy for standard

Theoretical Mw	Observed Mw	% Error
180	191	6
113000	112469	0.5
805000	749356	7

Table 4. Accuracy study for samples

Sample	cP	Dp	Observed Mw	Observed Mn	PDi
Pulp	17.6	845	495341	74401	6.65
In-process	12.0	570	322930	117361	2.75
Fiber	5.7	266	126810	40930	3.10

Figure 7. Typical chromatograph of sample - Pulp

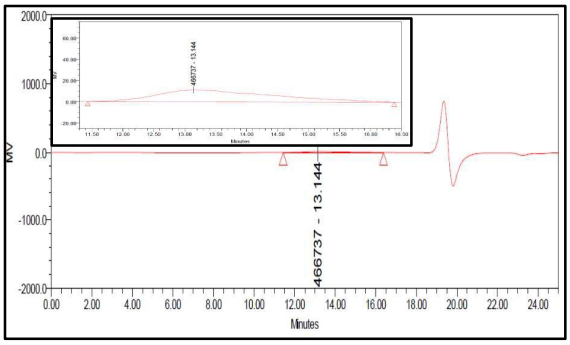
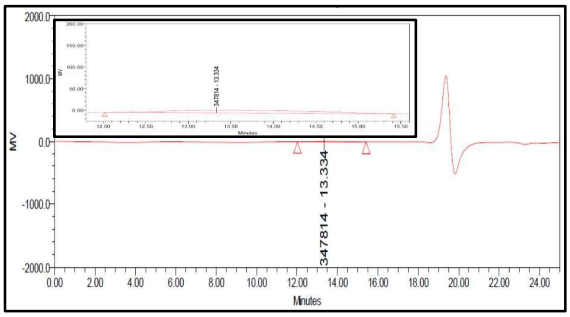


Figure 8. Typical chromatograph of sample – In-process



Method precision study

Results obtained are average of polydispersity from six replicates are shown in table no. 5 for first and second chemist. The study concludes that the %RSD is less than 15.0%. This shows that method is precise and reproducible

Table 5. Method precision study

Chemist	Observed Mw	Observed Mn	Average of PDi	Standard Deviation	%RSD
One	114364	78435	1.46	0.005	0.3
Two	112257	73885	1.52	0.069	4.6

Robustness study

Results obtained are summarized by taking average of six replicates for the robustness study and are shown in table no. 6. From the detail study of robustness, it is observed that for change in column temperature the method is robust for all the molecular weight, for change in flow rate of eluent, there is adverse effect at higher Mw and for change in concentration of LiCl in case of +20% affects at

higher Mw. Thus, method is sensitive to flow rate of mobile phase and concentration of LiCl. Hence, it is essential to take care of flow rate of eluent and concentration of LiCl during analysis.

Table 6. Robustness study – Summary table

Theoretical Mw	Average of observed Mw of six replicate each	%Error
Change in column temperature by +5°C		
180	188	4.5
113000	112809	-0.2
805000	729015	-9.4
Change in column temperature by -5°C		
180	188	4.5
113000	111966	-0.9
805000	743954	-7.6
Change in flow rate by +0.2ml		
180	187	4.0
113000	113046	0.04
805000	658281	-18.2
Change in flow rate by -0.2ml		
180	188	4.6
113000	115411	2.1
805000	707924	-12.1
Change in concentration of LiCl in DMAC by +20%		
180	186	3.4
113000	112648	-0.3
805000	693441	-13.9
Change in concentration of LiCl in DMAC by -20%		
180	190	5.7
113000	112360	-0.6
805000	733671	-8.9

Solution stability study

Results obtained by solution stability study are shown in run chart form in figure no. 9 and 10 for standard and sample respectively. The study concludes that the solution of standards are stable for 60 hours, showing the result within ±5% of average PDi. But sample is stable only for 24 hours. Till 24 hours PDi is within the set criteria. Using four columns one can analyse only 24 samples in 24 hours. Hence samples may degrade after 24 hours. In case of analysis performing using two columns, requires 25 minutes per sample. Thus, one can analyze 48 samples in the same duration without sample degradation. Thus analysis throughput will increase in production plant to monitor the process sample.

Figure 9. Solution stability study for standard with Mw = 113000

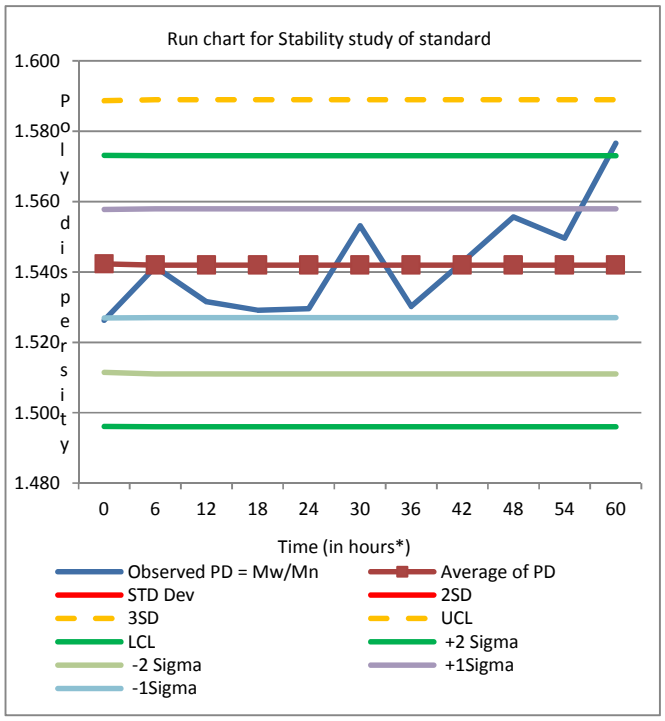
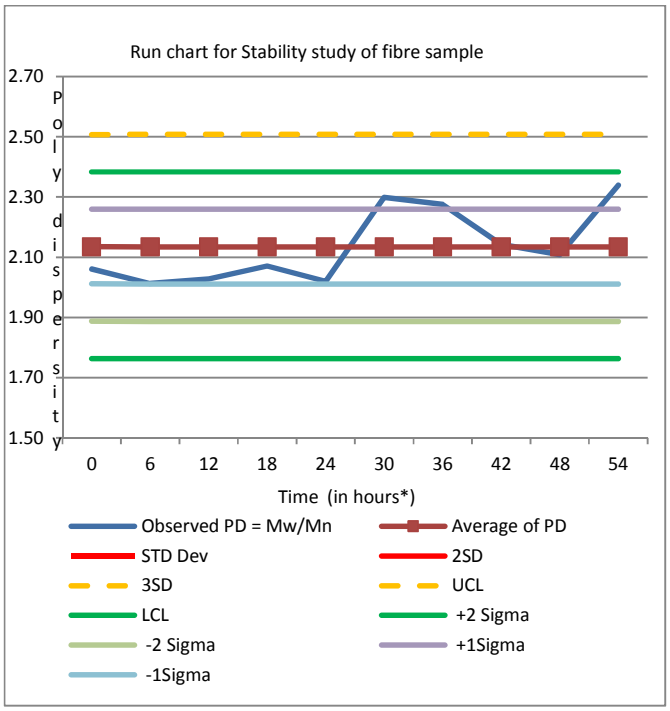


Figure 10. Solution stability study for fibre sample



Conclusions

The method validated for the determination of molecular weight of raw material, in-process sample and finished goods (fibre) using pullulan polysaccharide as a standard from the molecular weight range from 180 to 805000 dalton using two PLgel column in HPLC exhibits precise, linear and accurate. Also the method was shown to be robust with the change in the temperature. But flow rate of eluent and concentration of LiCl, affects the analysis. Hence, there should be control on flow rate of eluent and concentration of LiCl during analysis. The stability study for standard as well as sample is carried out for 60 hours which concludes stable for 60 hours in case of standard solution and 24 hours for sample solution. Thus, the developed method is economic with less turnaround time and easily usable in manufacturing unit where turnaround time is critical point.

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Notes and references

Names of the companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the Aditya Birla Science and Technology Company Private Limited or Aditya Birla Group over others not mentioned.

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