Study No.: [ ]

Test Report

Molecular weights and stability test of polymer under acidic and basic conditions (supplied as product name [ ])

Client

[ ]

Date: May 15, 2020

tested by

Korea Polymer Testing & Research Institute (KOPTRI), Ltd.

(ISO/IEC 17025 Certified Laboratory)

Contents

Pictures, tables, and figures captions

1. Test name

Molecular weights and stability test under the acidic and basic conditions of sample name [ ] (Study No.: [ ]

2. Client

[ ]

3. Testing institute, analyzer and author

Address: Korea Polymer Testing & Research Institute (KOPTRI)
21, Hwarang-ro 18ga-gil, Seongbuk-gu, Seoul, 02791, Korea

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Signature: Jiyoung Park Date: 2020. 5. 15
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Signature: Jungmi Kim Date: 2020. 5. 15
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Signature: Jinny Sung Date: 2020. 5. 15
Tel: +82-2-3499-8511, Fax: +82-2-963-2587, e-mail: jinny.sung@polymer.co.kr

4. Test period: 2020. 3. 24 ~ 2020. 5. 15

5. Testing sample (supplied by the manufacturer)

(1) Sample name : supplied as a name of [ ]

(2) Manufacturer/distributor : [ ] [ ] Company LLC

(3) Appearance : Dark brown viscous liquid

(4) Chemical name: [ ]

(5) Cas No. : Not assigned

6. Test contents

(1) Molecular weights, polydispersity

Content of molecular weight below 1 000 Da (%)

Content of molecular weight below 500 Da (%)

(2) Stability at acidic and basic condition

test buffer：pH 1.2, pH 4.0, pH 7.0, pH 9.0

test concentration：4 000 mg/L

test temperature：40 ℃

test period：2 weeks（1day for pH 1.2）

7. Sample preparation

The [ ] was supplied by the manufacturer. The sample was used directly for the test without any further treatment.

8. Testing methods

The molecular weight results (number-average and weight-average molecular weight and distribution) were measured by GPC (Gel permeation chromatography) according to OECD Guideline 118. The stability test in the acidic and basic conditions were performed by GPC and FT-IR (Fourier Transform Infra-red Spectrometer), which followed the method based on OECD Guideline 120 and Guideline of National Institute of Environmental Research (in Korea).

9. Test results summary

9-1. Molecular weight

Table 1. Molecular weights, polydispersity, content of molecular weight below 1 000 Da (%) and content of molecular weight below 500 Da (%) of [ ]

Sample name	Run	Mn	Mw	Mw/Mn	content of molecular weight below 500 Da (%)	content of molecular weight below 1 000 Da (%)	data
[ ]	Run1	[				]	Figure 2-1 Figure 2-3 Table 1-1 Table 1-2
	Run2						
	SD						
	CV						
	Average						**-**

a) Eluent: THF + 2

Column: 2 x TSKgel SupermultiporeHZ-M + TSKgel SuperHZ-2500

Detector: RI

b) Mn: number-average molecular weight

c) Mw: weight-average molecular weight

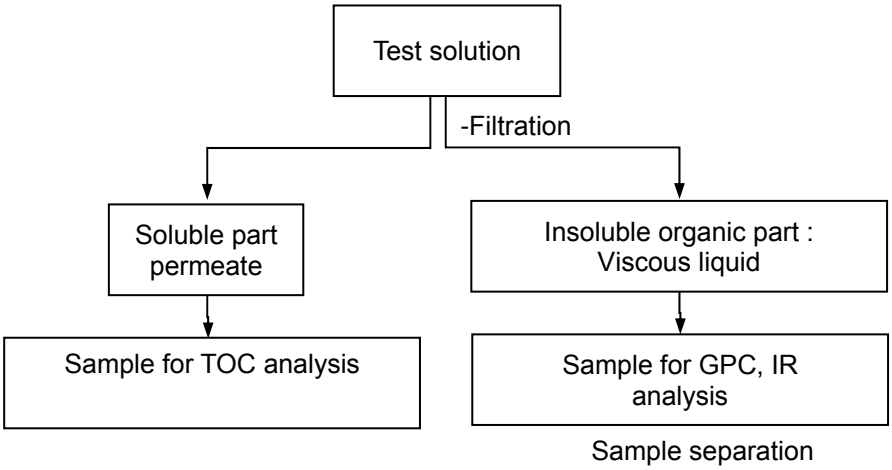
d) Mw/Mn: polydispersity

e) Polymer standards: Polystyrene

f) SD: standard deviation

g) CV: coefficient of variation

9-2. Stability test in the acidic and basic condition and basic condition



pH 1.2: drying remnant liquid sample after buffer decantation
pH 4: drying remnant liquid sample after buffer decantation
pH 7: drying remnant liquid sample after buffer decantation
pH 9: drying remnant liquid sample after buffer decantation

Scheme 1. Stability testing flow scheme

Table 2. Molecular weight change of [ ] after the test

pH	Mn	Mw	ΔMn	[ΔMn/ Mn] x 100 (%)	ΔMw	[ΔMw/ Mw] x 100 (%)	GPC chromatogram
Original1	[	]					Figure 2-1
Original2							Figure 2-3
Average							
1.2			-37	-1.10	-415	-2.78	Figure 3-1
4			-45	-1.34	-997	-6.68	Figure 3-2
7			-27	-0.80	79	0.53	Figure 3-3
9			26	0.77	267	1.79	Figure 3-4

Table 3. IR spectral change of [ ] after the test

pH	IR spectrum	IR spectra (overlapped, aligned)	Spectral change
Original	Figure 4-1	Figure 4-6 Figure 4-7	-
1.2	Figure 4-2		no
4	Figure 4-3		no
7	Figure 4-4		no
9	Figure 4-5		no

Table 4. Elemental analysis

Sample name	C (wt%)	H (wt%)	N (wt%)
[ ]	83.02	13.23	1.61

Table 5. Blank test at each pH

pH	1.2	7	9
TOC(mg/L)	0.71	0.49	0.33

Table 6. TOC content measurement after the stability test

pH	condition	Sample conc. (mg/L)	TOC (mg/L)	ΔDOC (mg/L)	Average ΔDOC (mg/L)	Theoretical maximum TOC (mg/L)	S_1(mg/L) S_2(mg/g) S_3(%)
1.2	40℃ 1day	4 000	26.17 26.22	25.46 25.51	25.49	3 320.6	S_1: 30.70 S_2: 7.68 S_3: 0.77
4	40℃ 2weeks	4 000	-	-	-	3 320.6	-
7	40℃ 2weeks	4 000	19.03 18.94	18.54 18.45	18.50	3 320.6	S_1: 22.28 S_2: 5.57 S_3: 0.56
9	40℃ 2weeks	4 000	8.97 8.73	8.64 8.40	8.52	3 320.6	S_1: 10.26 S_2: 2.57 S_3: 0.26

a) TOC at pH 4 was not measured because the pH buffer solution had high TC value itself.

b) Theoretical maximum TOC (mg/L) = sample concentration x carbon percentage = {sample weight (mg) / buffer volume (L)} x {carbon percentage in elemental analysis (%) x 0.01}

c) Solubility, S_1 (mg/L)

d) Solubility, S_2 (mg/g)

e) Solubility, S_3 (%, Dissolved organic carbon concentration) =
(Measured ΔDOC / Theoretical maximum TOC) x 100

Table 7. Summary of the stability test of [ ]

pH	Temp (℃)	Time (h)	S_1(mg/L) S_2(mg/g) S_3(%)	Solubility Change in THF after test	MW change by GPC	IR spectral change	Stability
1.2	40	24	S_1: 30.70 S_2: 7.68 S_3: 0.77	soluble to soluble	no	no	stable
4	40	336	-	soluble to soluble	no	no	stable
7	40	336	S_1: 22.28 S_2: 5.57 S_3: 0.56	soluble to soluble	no	no	stable
9	40	336	S_1: 10.26 S_2: 2.57 S_3: 0.26	soluble to soluble	no	no	stable

9-3. Conclusions

1. MW measurement: The number-average molecular weight (Mn) of [ ] polymer was [ ]. Content of molecular weight below 1 000 Da [ ] and content of molecular weight below 500 Da was [ ], respectively.

2. Stability test: After the stability test at pH 1.2, pH 4, pH 7 and pH 9, the water solubility (mg/g), S_2 was 2.57 ~ 7.68 (mg/g). The molecular weight changes were negligible in all pH conditions. There were no notable FT-IR spectral changes in all pH conditions.

Picture 1. [ ] sample.

pH 1.2 pH 4 pH 7 pH 9

Picture 2. [ ] samples in flasks after the stability test at pH 1.2, pH 4, pH 7 and pH 9.

10. Detail testing methods and results

10-1. Molecular weight determination

10-1-1. Objective

The purpose of using this method is to determine the molecular weight and molecular weight distribution of [ ] by GPC (gel permeation chromatography).

10-1-2. GPC instruments

(1) Solvent: THF + 20 % DEA
(2) Column (maker, model no.): 2 x TSKgel SupermultiporeHZ-M + TSKgel SuperHZ-2500
(3) Temperature: 40 ℃
(4) Detector: Differential Refractometer
(5) Flowing speed: 0.35 mL/min
(6) Data system: EcoSEC
(7) Injection amount: 30 μL, Concentration: 3 mg /mL

10-1-3. GPC calibration

(1) Standard sample: Polystyrene, Agilent Technologies
(2) Injection amount: 30 μL, Concentration: 1 mg/mL
(3) Calibration plot (Figure 1-2)

10-1-4. GPC results

The number-average molecular weight (Mn) and weight-average molecular weight (Mw) of [ ] were measured by GPC using THF as a solvent. The results are summarized in Table 1.

10-2. Stability tests at acidic and basic conditions

10-2-1. Objective and principle

The following method is used to investigate the stability of polymer in water at pH 1.2, pH 4, pH 7, and pH 9 in 40℃. A sample of the test substance was agitated in the water at pH 1.2 in 40℃ for 1 day and at pH 4, 7, and 9 in 40 ℃ for 2 weeks (14 days). Afterwards the insoluble part was characterized by analytical instruments (GPC, FT-IR) in order to investigate Mw decrease or structural changes in the acidic and basic conditions. However if the sample was completely soluble in water, the polymer samples for the analysis of GPC and FT-IR were obtained by freeze-drying method which is used for polymers in aqueous solutions. If necessary, the freeze-dried samples were treated with the solid phase extraction (SPE)method using the adequate eluents (eg, MeOH/THF) to yield the salt-free polymer samples. The amount of total carbon content dissolved in water at different pH is determined by TOC analyzer. The stability test in the acidic and basic conditions were based on OECD Guideline 120 and Guideline of National Institute of Environmental Research (in Korea). The buffer solution was prepared by OECD Guideline 111.

10-2-2. Reagents and Materials

(1) pH 1.2: 32.25 mL HCl (0.2 N) + 25 mL KCl (0.2 N) dilute to 100 mL
(2) pH 4.0: 0.40 mL NaOH (0.1 N) + 50 mL potassium biphthalate (0.1 M) dilute to 100 mL
(3) pH 7.0: 29.63 mL NaOH (0.1 N) + 50 mL monopotassium phosphate (0.1 M) dilute to 100 mL
(4) pH 9.0: 21.30 mL NaOH (0.1 N) + 50 mL boric acid(0.1 M) dilute to 100 mL

10-2-3. Apparatus and instrument

(1) Tapered neck Erlenmeyer (flat bottom flask) of 250 mL with their glass stoppers
(2) Beakers and test tube 50 mL for TOC measurement
(3) Analytical balance (OHAUS AS220 R2)
(4) pH meter (EUTECH EcoScan pH 60zk)
(5) Isothermal shaking bath with a temperature programming controller
(6) Total carbon analyzer (Shimadzu TOC-Vcsn)
(7) Gel Permeation Chromatography: Tosoh EcoSEC HLC-8320 GPC
(8) FT-IR (Fourier Transform Infra-red Spectrometer): JASCO FT-IR 4600 system

Condition

Method: ATR (attenuated total reflection)

Mode: Transmission

Resolution: 4 cm^{-1}

Scan: 32

10-2-4. Sampling

The recommended concentration of test sample is in the range of 100 ~ 4 000 mg/L. In this test, the highest concentration (4 000 mg/L) was adopted. The sample (0.8 g) was placed into a 250 mL Erlenmeyer. 200mL of buffer solution of each pH was added into the Erlenmeyer with a stopper. Then, the Erlenmeyer was placed in the shaking machine in 40 ℃. The sample at pH 1.2 was agitated for 1 day and the other samples at pH 4, pH 7, and pH 9 were agitated for 14 days respectively.

10-2-5. Procedure

(1) Soluble part

After stopping agitation, transparent aqueous solution was taken by a glass pipette, purified with a syringe-filter (0.45 μm) and measured by the total organic carbon(TOC) analyzer.

(2) Insoluble part

The insoluble [ ] was characterized by GPC and FT-IR (ATR method) in order to investigate molecular weight changes and structural changes of [ ] under the acidic and basic conditions.

10-2-6. Results

(1) GPC

After 14 days of the test, the molecular weight change of [ ] was determined by GPC. The GPC chromatograms are displayed in Figure 2-1 ~ Figure 3-4. The GPC results are summarized in Table 2.

(2) FT-IR

The sample was characterized by FT-IR spectroscopy. The IR spectra are displayed in Figure 4-1 ~ Figure 4-5. Figure 4-6 and Figure 4-7 show the overlapped and aligned FT-IR spectra of [ ] before the test and after the test. The IR results are summarized in Table 3.

(3) TOC

The dissolved organic carbon (DOC) concentration in the filtrate was measured by TOC analyzer. Blank was also measured for each buffer solution. The DOC at pH 4 was not measured because the buffer solution contained considerable amount of organic carbon itself. The TOC results are summarized in Table 6.

DOC was obtained from the next equation.

DOC = [DOC in the filtrate] − [DOC in the buffer solution]

DOC concentration, S_3 was calculated by the next equation.

S_3 (%, ΔDOC concentration) = (Measured ΔDOC/Theoretical maximum TOC) x 100

The stability test results were summarized in Table 7.

11. Archives

Test substance will be stored in the lab for 3 months. Final test report, raw data in the final report and information documents supplied by the sponsor (manufacturer) are stored in our computer as an electronic file with security. Client can ask for the electronic file anytime. Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in KOPTRI for five years.

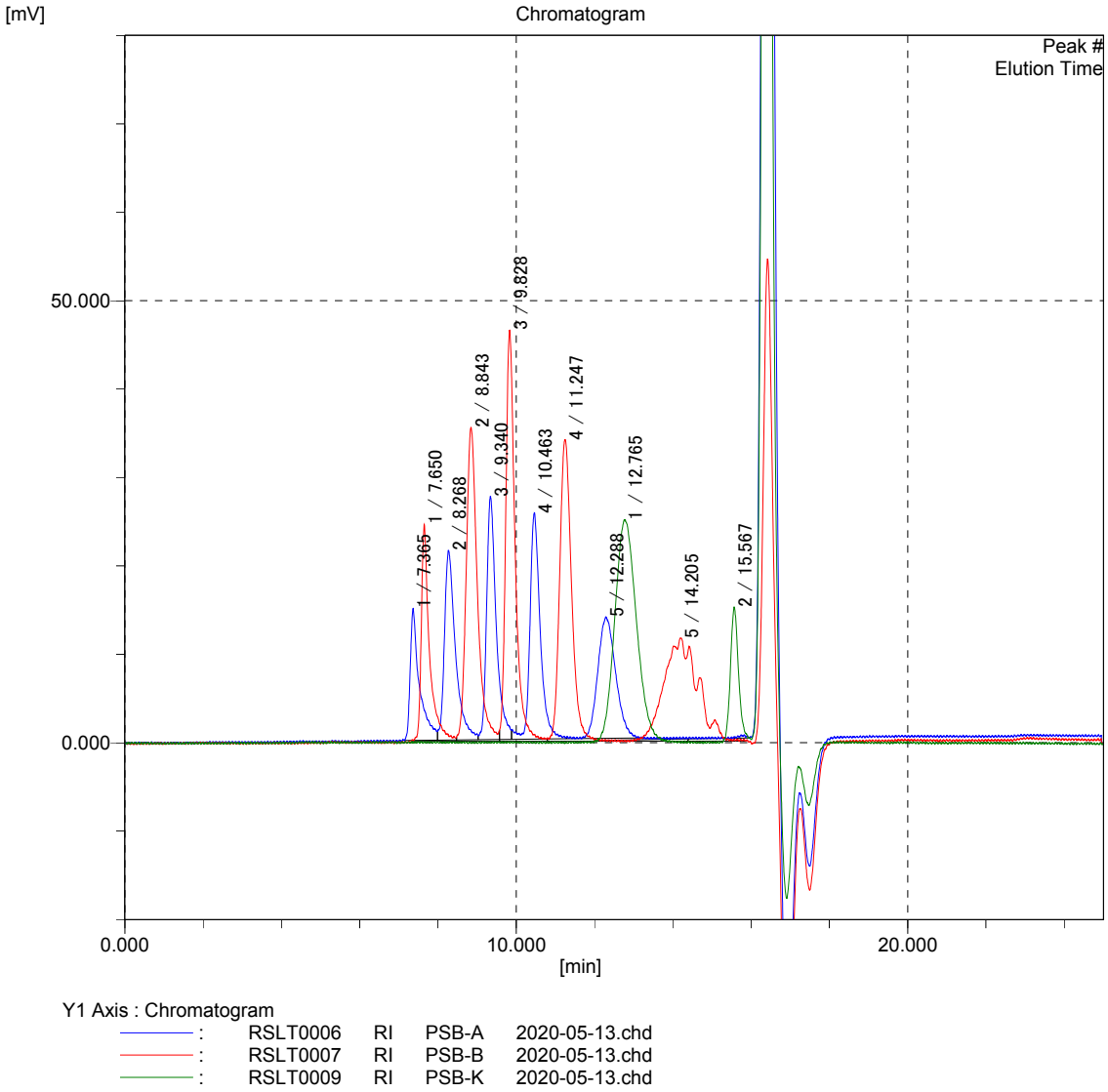
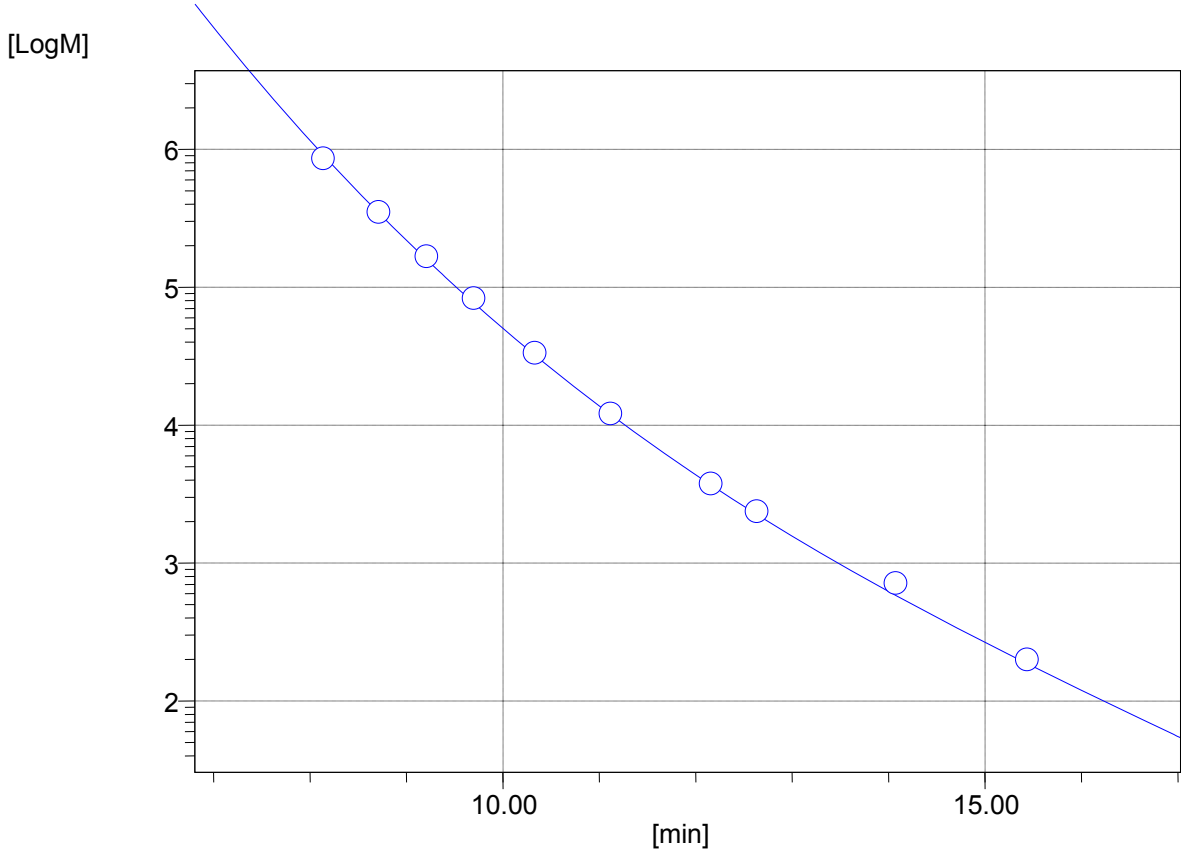


Figure 1-1. GPC chromatograms of Polystyrene standard samples.

Calibration curve

Database name	SystemDataBase.syd
Method name	200513_THF+DEA



Calibration data (RI)

Time [min]	Molecular weight	Error [%]	Weight	Mark	Data name		Coefficient	
8.268	696,000	-4.48235	1	STD	RSLT0006		A =	-1.765057e-003
8.843	283,800	1.02438	1	STD	RSLT0007		B =	9.046629e-002
9.340	135,700	4.01280	1	STD	RSLT0006		C =	-1.878689e+000
9.828	67,600	5.18811	1	STD	RSLT0007		D =	1.620800e+001
10.463	27,060	-0.21680	1	STD	RSLT0006			
11.247	9,820	-4.39523	1	STD	RSLT0007		Correlation	-0.993
12.288	3,050	-5.53352	1	STD	RSLT0006			
12.765	1,920	-3.03225	1	STD	RSLT0009			
14.205	580	10.24486	1	STD	RSLT0007			
15.567	162	-4.06593	1	STD	RSLT0009			

Figure 1-2. The calibration curve obtained from Polystyrene standard samples.

Chromatogram report

Header

Title			Data acquisition date and time	2020-05-13 21:08:11
Sample name	[ ]		Calculation date and time	2020-05-14 10:08:02
Database name	[ ]		Acquisition time [min]	0.000 - 25.000
Data name	RSLT0016		Sampling interval [msec]	100
Method name	200513_THF+DEA		Cup number	16
Channel	RI		Calculation type	Molecular Weight

[ ]

Result of molecular weight calculation (RI)

[ ]

Figure 2-1. GPC chromatogram of [ ] (Run 1).

Table 1-1. GPC Slice table (Run 1)

slice table

Molecular distribution list (RI)

Differential distribution factor	1
Formula of distribution	TOSOH
Sampling interval	100

Peak no. 1 Time(Range) [min] 9.37 - 10.97 - 15.74

[ ]

[ ]

[ ]

[ ]

[ ]

[ ] egral]

Figure 2-2. Cumulative Mw distribution and Mw distribution curve of [ ] (Run1).

Chromatogram report

Header

Title			Data acquisition date and time	2020-05-13 21:33:12
Sample name	[ ]ORI		Calculation date and time	2020-05-14 10:03:50
Database name	2020-05-13.chd		Acquisition time [min]	0.000 - 25.000
Data name	RSLT0017		Sampling interval [msec]	100
Method name	200513_THF+DEA		Cup number	16
Channel	RI		Calculation type	Molecular Weight

[ ]

Result of molecular weight calculation (RI)

[ ]

Figure 2-3. GPC chromatogram of [ ] (Run 2).

Table 1-2. GPC Slice table (Run 2)

slice table

Molecular distribution list (RI)

Differential distribution factor	1
Formula of distribution	TOSOH
Sampling interval	100

Peak no. 1 Time(Range) [min] 9.44 - 10.96 - 15.75

[ ]

[ ]

[ ]

[ ]

[ ]

[ ]

Figure 2-4. Cumulative Mw distribution and Mw distribution curve of [ ] (Run 2).

Chromatogram report

Header

Title			Data acquisition date and time	2020-05-13 21:58:14
Sample name	[ ] pH 1.2		Calculation date and time	2020-05-14 10:07:36
Database name	2020-05-13.chd		Acquisition time [min]	0.000 - 25.000
Data name	RSLT0018		Sampling interval [msec]	100
Method name	200513_THF+DEA		Cup number	17
Channel	RI		Calculation type	Molecular Weight

[ ]

Result of molecular weight calculation (RI)

[ ]

Figure 3-1(a). GPC chromatogram of [ ] after test at pH 1.2.

[ ]l]

[LogM]

Figure 3-1(b). Mw distribution of [ ] after test at pH 1.2.

Chromatogram report

Header

Title			Data acquisition date and time	2020-05-13 22:23:14
Sample name	[ ]pH 4		Calculation date and time	2020-05-14 10:07:04
Database name	2020-05-13.chd		Acquisition time [min]	0.000 - 25.000
Data name	RSLT0019		Sampling interval [msec]	100
Method name	200513_THF+DEA		Cup number	18
Channel	RI		Calculation type	Molecular Weight

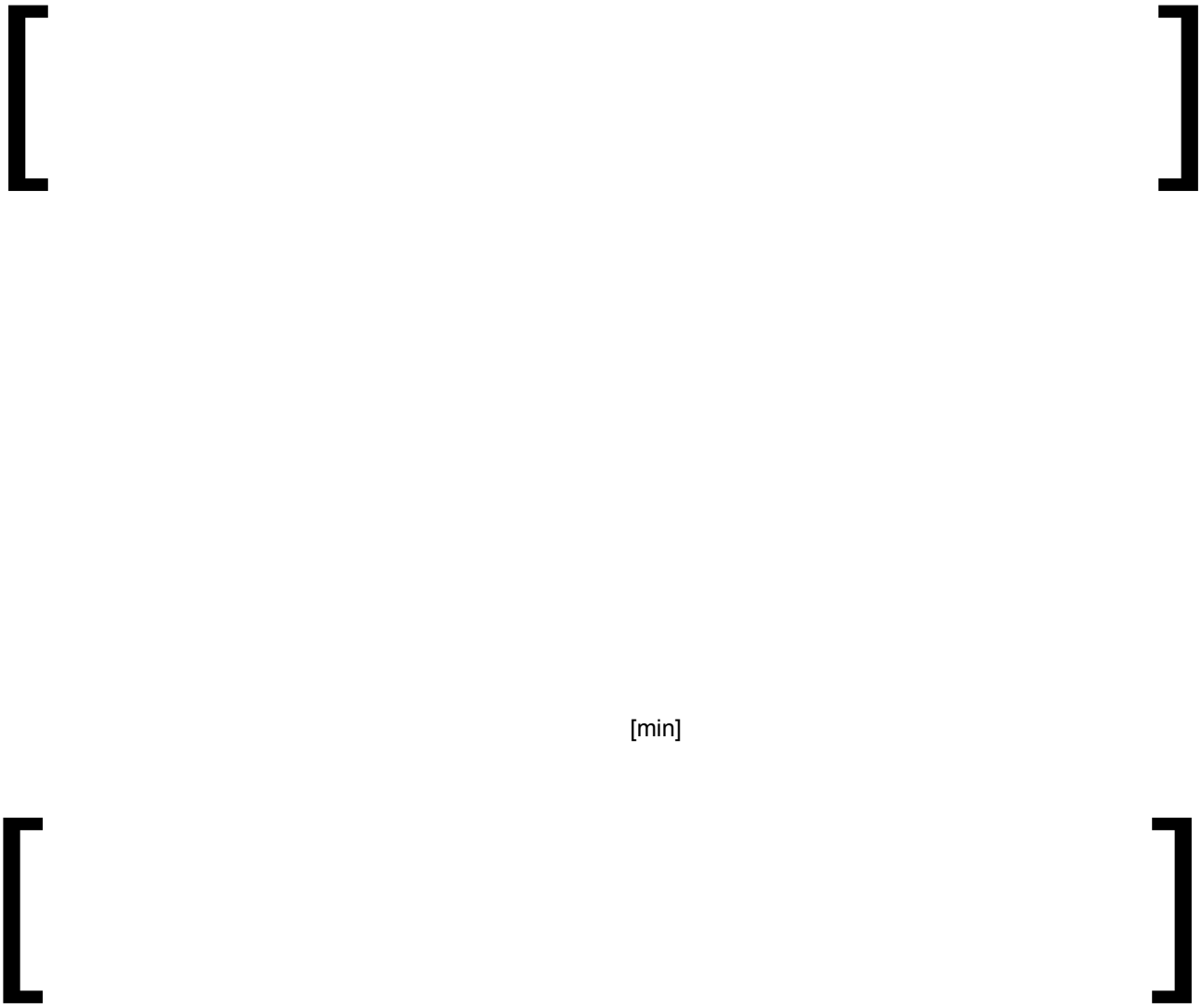


Figure 3-2(a). GPC chromatogram of [ ] after test at pH 4.

[ ]

Figure 3-2(b). Mw distribution of [ ] after test at pH 4.

Chromatogram report

[ ]

[ ]

Result of molecular weight calculation (RI)

[ ]

Figure 3-3(a). GPC chromatogram of [ ] after test at pH 7.

[ ]

Figure 3-3(b). Mw distribution of [ ] after test at pH 7.

Chromatogram report

[ ]

[ ]

Result of molecular weight calculation (RI)

[ ]

Figure 3-4(a). GPC chromatogram of [ ] after test at pH 9.

[ ] al]

Figure 3-4(b). Mw distribution of [ ] after test at pH 9.

[ ]

[ ]

Figure 3-5. Comparison of GPC chromatograms before and after test

[ ]

Figure 4-1. IR spectrum of [ ] before test.

[ ]

Figure 4-2. IR spectrum of [ ] after test at pH 1.2.

[ ]

Figure 4-3. IR spectrum of [ ] after test at pH 4.

[ ]

Figure 4-4. IR spectrum of [ ] after test at pH 7.

[ ]

Figure 4-5. IR spectrum of [ ] after test at pH 9.

[ ]

Figure 4-6. IR spectra of [ ] before and after test (overlapped).

[ ]

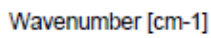


Figure 4-7. IR spectra of [ ] before and after test (aligned).

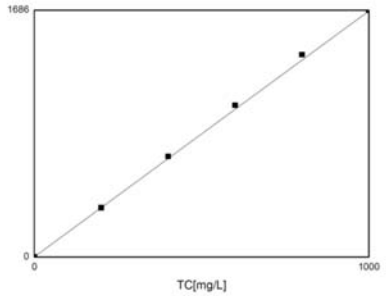
[ ]

Operator ID:	JY
Company name:	Koptri
Method filename:	C:\Eager 300 for EA1112\2020\CHNS\0427\N C H S 20200427 system.mth
Method name:	NCHS_200427
Analysed:	2020-04-28 16:35
Printed:	2020-04-29 16:52
Elemental Analyser method:	
Sampler method:	[ ]
Sample ID:	
Analysis type:	UnkNown
Chromatogram filename:	[ ]
Calibration method:	Least Squares to Linear fit
Sample weight:	1.702
Protein factor:	6.25

Element Name	
Nitrogen	[ ]
Carbon	
Hydrogen	
Totals	

Figure 5. Elemental analysis of [ ]

TC (1 000 ppm calibration curve)

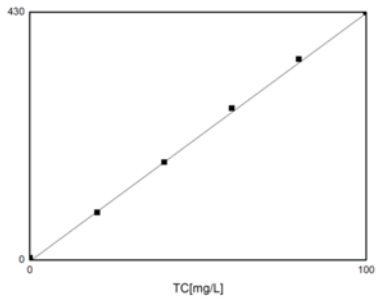


CALIBRATION CURVE : TC4 6-POINTS
[CONDITION]
INJECTION VOLUME : 20 uL
ACID RATIO : 0.0 %
SPARGE TIME : 0.0 sec
NO OF WASHES : 2
[DATA]

STD	AREA	SD	CV %
1000 mg/L	1686	20.5	1.22
800.0 mg/L	1382	3.54	0.26
600.0 mg/L	1037	5.66	0.55
400.0 mg/L	687.3	3.46	0.50
200.0 mg/L	336.4	2.26	0.67
0.000 mg/L	1.426	0.06	4.12

PROTECT : ON
CAL CURVE FORM : LINEAR REGRESSION
SHIFT OPTION : SHIFT TO ORIGIN
R^2 : 0.9996
DATE : 2020-01-21

TC (100 ppm calibration curve)

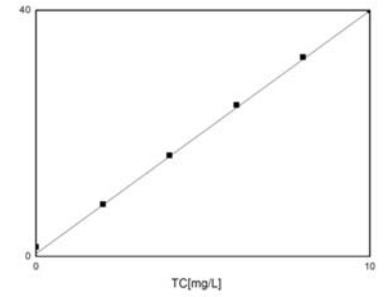


CALIBRATION CURVE : TC2 6-POINTS
[CONDITION]
INJECTION VOLUME : 50 uL
ACID RATIO : 0.0 %
SPARGE TIME : 0.0 sec
NO OF WASHES : 2
[DATA]

STD	AREA	SD	CV %
100.0 mg/L	429.9	5.16	1.20
80.00 mg/L	348.8	1.63	0.47
60.00 mg/L	263.3	1.41	0.54
40.00 mg/L	169.8	0.49	0.29
20.00 mg/L	82.50	1.06	1.29
0.000 mg/L	3.442	0.02	0.66

PROTECT : ON
CAL CURVE FORM : LINEAR REGRESSION
SHIFT OPTION : SHIFT TO ORIGIN
R^2 : 0.9995
DATE : 2020-01-17

TC (10 ppm calibration curve)

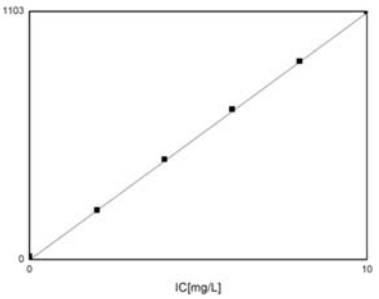


CALIBRATION CURVE : TC5 6-POINTS
[CONDITION]
INJECTION VOLUME : 50 uL
ACID RATIO : 0.0 %
SPARGE TIME : 0.0 sec
NO OF WASHES : 2
[DATA]

STD	AREA	SD	CV %
10.00 mg/L	39.85	0.11	0.27
8.000 mg/L	32.29	0.20	0.64
6.000 mg/L	24.52	0.04	0.17
4.000 mg/L	16.37	0.23	1.43
2.000 mg/L	8.465	0.09	1.02
0.000 mg/L	1.540	0.04	2.66

PROTECT : ON
CAL CURVE FORM : LINEAR REGRESSION
SHIFT OPTION : SHIFT TO ORIGIN
R^2 : 0.9996
DATE : 2020-01-22

IC (10 ppm calibration curve)



CALIBRATION CURVE : IC0 6-POINTS
[CONDITION]
INJECTION VOLUME : 800 uL
ACID RATIO : 1.5 %
NO OF WASHES : 1
[DATA]

STD	AREA	SD	CV %
10.00 mg/L	1103	0.71	0.06
8.000 mg/L	882.2	0.28	0.03
6.000 mg/L	668.8	0.00	0.00
4.000 mg/L	446.1	0.07	0.02
2.000 mg/L	219.9	0.35	0.16
0.000 mg/L	13.89	0.11	0.76

PROTECT : ON
CAL CURVE FORM : LINEAR REGRESSION
SHIFT OPTION : SHIFT TO ORIGIN
R^2 : 0.9999
DATE : 2020-01-16

Figure 6. The TC and IC calibration curves for TOC measurement

The end.