Chemical stability of Q Sepharose BB

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1 Introduction

This analytical report describes the results of the tests for chemical stability of Q Sepharose BB. Three batches of Q Sepharose BB were stored for 160-170 hours in basic and acidic solutions at different storage temperatures (see Analytical report 1). After storage the incubation solutions were sampled from the Q Sepharose BB beads and analysed. The content of Total Organic Carbon (TOC), Total Nitrogen (TN) and bromine/bromide in the storage solutions were analysed (see Analytical report 2). The chemical stability for Q Sepharose BB is determined by calculation of the weight of each element in the storage solutions related to the total weight of each element in wet sedimented gel and dried gel.

Bromine is released when storing Q Sepharose BB in alkaline solutions. Investigations of what form the released bromine is in and if the released bromine can be eliminated by a simple buffer wash has been performed.

This report also describes the dry weight variation of 8 Q Sepharose BB batches that have been stored in shipping solution (20% ethanol) for at least 8 years. If Q Sepharose BB decomposes the dry weight of the particles would be expected to decrease.

2 Materials and methods

Analytical report 1, “Sample preparation for investigating the impurities, extractables and chemical stability of Q Sepharose BB” describes the procedure for sampling the chemical stability samples.

3 Results

The TOC/TN and bromine data for all samples is given in Analytical report 2,” Elemental analysis for investigating the impurities, extractables and chemical stability of Q Sepharose BB”.

3.1 Evaluation of the results from the storage stability

3.1.1 Original amounts of carbon, nitrogen and bromine in Q Sepharose BB

To calculate the loss of carbon, nitrogen and bromine in Q Sepharose BB, the original amount of these elements was investigated. Dried Q Sepharose BB samples, prepared as described in Analytical report 1 section 2.2, were sent to the accredited laboratory Mikrokemi AB (Seminariegatan 19, Uppsala, Sweden) for carbon, hydrogen, sulphur, nitrogen and oxygen analysis. The averaged percentages of carbon and nitrogen in Q Sepharose BB were calculated, according to Table 1. It was not practical possible to quantify bromine and to estimate the bromine amount in dried Q Sepharose BB it was assumed that the residual weight percent (calculated from Table 1) is bromine since no other main elements are included in the manufacturing process.

Table 1. Original amount of carbon, nitrogen and bromine in dried Q Sepharose BB.

Batch Q Sepharose BB	Carbon			Nitrogen			Bromine
	Sample 1 (%w/w)	Sample 2 (%w/w)	Average (%w/w)	Sample 1 (%w/w)	Sample 2 (%w/w)	**Average** (%w/w)	**Average** (%w/w)
T-301065	44.7	44.8	44.8	2.1	2.1	**2.1**	**7.8**
T-301462	45.4	45.4	45.4	2.0	2.0	**2.0**	**6.8**
T-302192	45.9	46.0	46.0	2.0	2.0	**2.0**	**7.2**

Since all stability testing was performed with a volume of 40 mL sedimented Q Sepharose BB the total weight of the elements carbon, nitrogen and bromine in this sample volume was calculated. The results are shown in Table 2.

Table 2. Total mass of carbon, nitrogen and bromine in 40 mL sedimented gel.

Batch Q Sepharose BB	Dry weight. g/mL sed gel	C_0 Carbon ppm	T_m Carbon mg	C_0 Nitrogen ppm	T_m Nitrogen mg	C_0 Bromine ppm	T_m Bromine mg
T-301065	0.128	448000	2294	21000	108	78000	399
T-301462	0.122	454000	2215	20000	97.6	68000	332
T-302192	0.126	460000	2318	20000	101	72000	363

C_0= original amount of the elements in dried Q Sepharose BB (mg/kg)

T_m= Total weight of the element in 40 mL sedimented Q Sepharose BB (mg)

Calculations:

T_m= 40 x Dry weight x C_0 / 1000

3.1.2 Results, chemical stability of Q Sepharose BB

Storage stability data was obtained from analytical report 2. Table 3 and 4 shows the chemical stability results of Q Sepharose BB.

Table 3. Chemical stability results of Q Sepharose BB, carbon and nitrogen.

Q Sepharose BB T-301065													
C_m= 23.88 g			T_m= 2294 mg C							Tm= 108 mg N			
Storage solution	**Storing temperature (°C)**	**Storing time (h)**	**C_t (ppm C)**	**ppm agarose**	**M_t (µg C)**	**Loss C (µg/mL sed gel)**	**Loss agarose (µg/mL sed gel)**	**Loss carbon (%)**	**Loss agarose (%)**	**C_t (ppm N)**	**M_t (µg N)**	**Loss N (µg/mL sed gel)**	**%loss N**
1 mM HCl pH3	40	164.5	<0.8	<1,8	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0,06
1 mM HCl pH3	20	164.5	<0.8	<1,8	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0.06
0.1 mM HCl pH4	40	164.5	<0.8	<1,8	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0.06
0.1 mM HCl pH4	20	164.5	<0.8	<1,8	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0.06
0.01 M NaOH	40	167	29,01	64,8	1853	46,3	103,4	0,08	0,08	3,6	230	5,7	0,21
0.2 M NaOH	60	164.5	87,0	194,2	5557	138,9	310,1	0,24	0,24	11,46	732	18,3	0,68
0.2 M NaOH	20	164.5	17,5	39,1	1119	28,0	62,5	0,05	0,05	2,46	157	3,9	0,15
0.5 M NaOH	40	164.5	30,7	68,5	1959	49,0	109,3	0,09	0,09	3,85	246	6,1	0,23
0.5 M NaOH	20	164.5	17,2	38,4	1099	27,5	61,3	0,05	0,05	2,07	132	3,3	0,12
Q Sepharose BB T-301462													
C_m= 24.72 g			T_m= 2215 mg C							Tm= 97.6 mg N			
Storage solution	**Storing temperature (°C)**	**Storing time (h)**	**C_t (ppm C)**	**ppm agarose**	**M_t (µg C)**	**Loss C (µg/mL sed gel)**	**Loss agarose (µg/mL sed gel)**	**Loss carbon (%)**	**Loss agarose (%)**	**C_t (ppm N)**	**M_t (µg N)**	**Loss N (µg/mL sed gel)**	**%loss N**
1 mM HCl pH3	40	166.5	<0.8	<1,8	<52	<1,3	<2,9	<0,002	<0,002	<1	<65	<1,6	<0,07
1 mM HCl pH3	20	166.5	<0.8	<1,8	<52	<1,3	<2,9	<0,002	<0,002	<1	<65	<1,6	<0.07
0.1 mM HCl pH4	40	166.5	<0.8	<1,8	<52	<1,3	<2,9	<0,002	<0,002	<1	<65	<1,6	<0.07
0.1 mM HCl pH4	20	166.5	<0.8	<1,8	<52	<1,3	<2,9	<0,002	<0,002	<1	<65	<1,6	<0.07
0.01 M NaOH	40	167	27,3	60,0	1764	44,1	97,1	0,08	0,08	3,1	201	5,0	0,21
0.2 M NaOH	60	166.5	101,4	223,4	6565	164,1	361,5	0,30	0,30	13,22	856	21,4	0,88
0.2 M NaOH	20	166.5	27,0	59,5	1749	43,7	96,3	0,08	0,08	2,99	194	4,8	0,20
0.5 M NaOH	40	166.5	31,8	70,1	2059	51,5	113,4	0,09	0,09	3,78	245	6,1	0,25
0.5 M NaOH	20	166.5	19,4	42,8	1258	31,5	69,3	0,06	0,06	2,07	134	3,3	0,14
Q Sepharose BB T-302192													
C_m= 24.08 g			T_m= 2318 mg C							Tm= 101 mg N			
Storage solution	**Storing temperature (°C)**	**Storing time (h)**	**C_t (ppm C)**	**ppm agarose**	**M_t (µg C)**	**Loss C (µg/mL sed gel)**	**Loss agarose (µg/mL sed gel)**	**Loss carbon (%)**	**Loss agarose (%)**	**C_t (ppm N)**	**M_t (µg N)**	**Loss N (µg/mL sed gel)**	**%loss N**
1 mM HCl pH3	40	167	<0.8	<1,8	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0,06
1 mM HCl pH3	20	167	<0.8	<1.8	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0.06
0.1 mM HCl pH4	40	167	<0.8	<1,9	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0.06
0.1 mM HCl pH4	20	167	<0.8	<1.9	<51	<1,3	<2,8	<0,002	<0,002	<1	<64	<1,6	<0.06
0.01 M NaOH	40	167	28	62,5	1794	44,9	97,5	0,08	0,08	3,0	192	4,8	0,19
0.2 M NaOH	60	167	87,8	195,9	5624	140,6	305,6	0,24	0,24	11,64	746	18,6	0,74
0.2 M NaOH	20	167	23,3	52,1	1494	37,4	81,2	0,06	0,06	2,86	183	4,6	0,18
0.5 M NaOH	40	167	39,9	89,0	2556	63,9	138,9	0,11	0,11	24,21	1551	38,8	1,54
0.5 M NaOH	20	167	20,5	45,8	1314	32,8	71,4	0,06	0,06	2,51	161	4,0	0,16

[a] The blank sample has an inexplicably high amount of carbon, probably caused by contamination. The sample is not subtracted with the blank sample.

C_t= Total content of the element in the storage solution - blank (ppm)

C_m= Moisture content in the weighed amount vacuum suctioned gel that corresponds to 40 mL wet sedimented gel

M_t= Total weight of the element in the storage solution, mg (see analytical report 1 for moisture content in vacuum suctioned gel)

T_m= Total weight of the element in 40 mL wet sedimented Q Sepharose BB, mg

ppm agarose=C_t / %w/w carbon in Q Sepharose BB x100

Formulas:

$M_t = (C_m+40) \times C_t$

%loss= M_t / T_m x100

Table 4. Chemical stability results of Q Sepharose BB, total bromine and bromide

Q Sepharose BB T-301065 C_m= 23.88 g			**Total bromine, TBr** Tm= 399 mg Br				**Bromide, Br^-** Tm= 399 mg Br			
Storage solution	**Storing temperature (°C)**	**Storing time (h)**	**C_t (ppm TBr)**	**M_t (µg TBr)**	**Loss (µg TBr / mL sed gel)**	**%loss TBr**	**C_t (ppm Br^-)**	**M_t (µg Br^-)**	**Loss (µg Br^- / mL sed gel)**	**%loss Br^-**
1 mM HCl pH3	40	164.5	0,175	11,179	0,28	0,003	<1	<64	<1.6	<0,016
1 mM HCl pH3	20	164.5	<0.1	<6	<0,15	<0,002	<1	<64	<1.6	<0,016
0.1 mM HCl pH4	40	164.5	0,44	28,1	0,70	0,007	<1	<64	<1.6	<0,016
0.1 mM HCl pH4	20	164.5	0,17	10,7	0,27	0,003	<1	<64	<1.6	<0,016
0.01 M NaOH	40	167	188	12009	300	3,0	189	12073	302	3,0
0.2 M NaOH	60	164.5	1700	108596	2715	27,2	1640	104763	2619	26,3
0.2 M NaOH	20	164.5	810	51743	1294	13,0	762	48677	1217	12,2
0.5 M NaOH	40	164.5	1800	114984	2875	28,8	1840	117539	2938	29,5
0.5 M NaOH	20	164.5	1100	70268	1757	17,6	1180	75378	1884	18,9
Q Sepharose BB T-301462 C_m= 24.72 g			Tm= 332 mg Br				Tm= 332 mg Br			
Storage solution	**Storing temperature (°C)**	**Storing time (h)**	**C_t (ppm TBr)**	**M_t (µg TBr)**	**Loss (µg TBr / mL sed gel)**	**%loss TBr**	**C_t (ppm Br)**	**M_t (µg Br)**	**Loss (µg Br^- / mL sed gel)**	**%loss Br^-**
1 mM HCl pH3	40	166.5	0,14	9,1	0,23	0,003	<1	<65	<1,6	<0,019
1 mM HCl pH3	20	166.5	<0.1	<6	<0,15	<0,002	<1	<65	<1,6	<0,019
0.1 mM HCl pH4	40	166.5	0,38	24,6	0,61	0,007	<1	<65	<1,6	<0,019
0.1 mM HCl pH4	20	166.5	<0.1	<6	<0,15	<0,002	<1	<65	<1,6	<0,019
0.01 M NaOH	40	167	190	12297	307	3,7	193	12491	312	3,8
0.2 M NaOH	60	166.5	1600	103552	2589	31,2	1580	102258	2556	30,8
0.2 M NaOH	20	166.5	770	49834	1246	15,0	711	46016	1150	13,9
0.5 M NaOH	40	166.5	2100	135912	3398	40,9	2000	129440	3236	39,0
0.5 M NaOH	20	166.5	1200	77664	1942	23,4	1130	73134	1828	22,0
Q Sepharose BB T-302192 C_m= 24.08 g			Tm= 363 mg Br				Tm= 363 mg Br			
Storage solution	**Storing temperature (°C)**	**Storing time (h)**	**C_t (ppm TBr)**	**M_t (µg TBr)**	**Loss (µg TBr / mL sed gel)**	**%loss TBr**	**C_t (ppm Br)**	**M_t (µg Br)**	**Loss (µg Br^- / mL sed gel)**	**%loss Br^-**
1 mM HCl pH3	40	167	<0.1	<6	<0,15	<0,002	<1	<64	<1,6	<0,018
1 mM HCl pH3	20	167	0,14	8,9	0,22	0,002	<1	<64	<1,6	<0,018
0.1 mM HCl pH4	40	167	0,15	9,6	0,24	0,003	<1	<64	<1,6	<0,018
0.1 mM HCl pH4	20	167	0,11	7,0	0,18	0,002	<1	<64	<1,6	<0,018
0.01 M NaOH	40	167	163	10445	261	2,9	170	10894	272	3,0
0.2 M NaOH	60	167	1400	89712	2243	24,7	1340	85867	2147	23,7
0.2 M NaOH	20	167	860	55109	1378	15,2	828	53058	1326	14,6
0.5 M NaOH	40	167	2000	128160	3204	35,3	1930	123674	3092	34,1
0.5 M NaOH	20	167	1300	83304	2083	22,9	1220	78178	1954	21,5

C_t= Total content of the element in the storage solution - blank (ppm)

C_m= Moisture content in the weighed amount vacuum suctioned gel that corresponds to 40 mL wet sedimented gel

M_t= Total weight of the element in the storage solution, mg (see analytical report 1 for moisture content in vacuum suctioned gel)

T_m= Total weight of the element in 40 mL wet sedimented Q Sepharose BB, mg

ppm agarose=C_t / %w/w carbon in Q Sepharose BB x100

Formulas:

$M_t = (C_m+40) \times C_t$

%loss= M_t / T_m x100

3.2 Leakage of bromine

3.2.1 Introduction

Since bromine is used in the manufacturing process of Q Sepharose BB it is of importance to investigate the amount of bromine that migrates from the matrix and what form the bromine is in. The storage stability samples and additional samples were sent to an accredited laboratory, Analytica AB, for total bromine analysis and bromide analysis. Sodium bromide standards were also sent to investigate the performance of the analysis techniques Analytica AB use, ICP-SFMS and IC, and also to evaluate if there's any systematic difference between the total bromine analysis and bromide analysis.

3.2.2 Results and evaluation of bromine leakage

3.2.2.1 Estimation of the performance of the bromine and bromide analysis

Table 5 shows the result of the bromine and bromide analysis performed on the NaBr standards in milli-Q water (preparation of these samples, see Analytical report 1, section 3.3.7).

Table 5. Results, bromine and bromide analysis of NaBr standards in milli-Q water

ppm Br	Total bromine (ICP-SFMS) (ppm)	Bromide (IC) (ppm)
10	9,9	9,7
10	9,88	9,9
10	9,87	9,8
50	49,0	48,5
50	48,8	48,1
50	49,1	48,8
100	97	97,8
100	98	97,8
100	99	97,6
500	480	479
500	470	484
500	500	489
1000	990	1000
1000	1000	987
1000	980	979

Table 6 shows the result of the bromine and bromide analysis performed on the NaBr standards in 0.5 M NaOH.

Table 6. Results, bromine and bromide analysis of NaBr standards in 0.5 M NaOH

ppm Br	Total bromine, ICP-SFMS (ppm)	Bromide, IC (ppm)
1500	1430	1496
1500	1400	1523
1500	1410	1530
50	44,6	53
50	48,4	50
50	47,1	51

To investigate if there are any systematic differences at different bromine levels the average, standard deviation (SD), relative standard deviation (RSD) and 95% confidence interval were calculated according to Table 7.

Table 7. Comparison between bromine analysis (ICP-SFMS) and bromide (IC) analysis

ICP-SFMS	Average (ppm)	SD (ppm)	RSD%	Confidence limits (P=0.05)
10 ppm Br	9,9	0,02	0,18	9,88 ± 0,08
50 ppm Br	49,0	0,13	0,26	49,0 ± 0,5
100 ppm Br	98,0	1,00	1,02	98 ± 4,3
500 ppm Br	483,3	15,28	3,16	483 ± 66
1000 ppm Br	990,0	10,00	1,01	1000 ± 43
1500 ppm Br in 0.5 M NaOH	1413	15,28	1,08	1413 ± 66
50 ppm Br in 0.5 M NaOH	46,70	1,93	4,14	46,7 ± 8,3
IC	**Average (ppm)**	**SD (ppm)**	**RSD%**	**Confidence limits (P=0.05)**
10 ppm Br	9,8	0,1	1,02	9,80 ± 0,43
50 ppm Br	48,5	0,35	0,72	48,5 ± 1,5
100 ppm Br	97,7	0,12	0,12	97,7 ± 0,5
500 ppm Br	484,0	5,0	1,03	484 ± 21
1000 ppm Br	988,7	10,6	1,07	1000 ± 46
1500 ppm Br in 0.5 M NaOH	1516,3	18,0	1,18	1516 ± 77
50 ppm Br in 0.5 M NaOH	51,3	1,5	2,98	51 ± 6

There is no significant systematic difference between the ICP- SFMS and the IC method when NaBr were diluted in milli-Q water. Both methods show both high accuracy and low deviations inside the method. When dissolving NaBr in 0.5 M NaOH the total bromine analysis (ICP-SFMS) for 1500 ppm standards show a significant systematic difference, -87 ppm (6%) from the nominal concentration. All other analysis results, when dissolving NaBr in 0.5 M NaOH, show higher SD than milli-Q dissolved NaBr standards but no systematic differences.

3.2.2.2 Evaluation of the bromine leakage in the storage samples of Q Sepharose BB

Figure 1 shows the results and the differences between all storage solutions with >pH12. Figure 1 shows that there is a small trend that samples are containing more bromine than bromide.

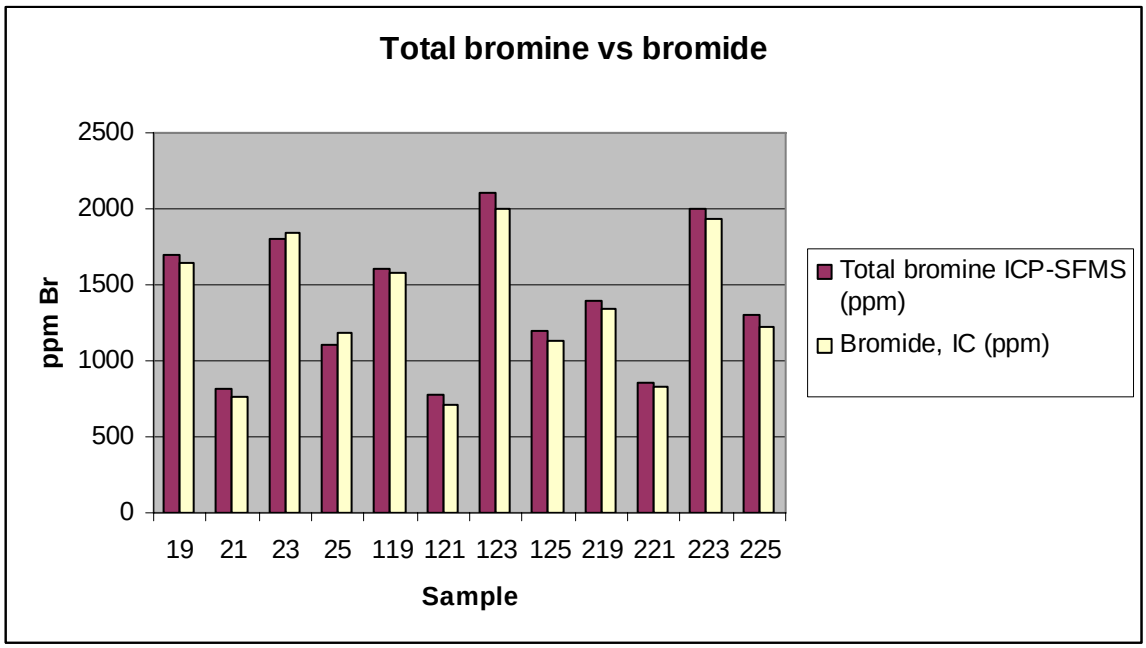

Figure 1. Bromine and bromide levels for Q Sepharose BB storage solutions >pH12.

Samples 23, 123 and 223 were analyzed a second time and Figure 2 shows that the trend is opposite compared to Figure 1. This indicates that the differences in bromide and bromine levels are random affects rather than due to differences between analyzing techniques or related to sample composition.

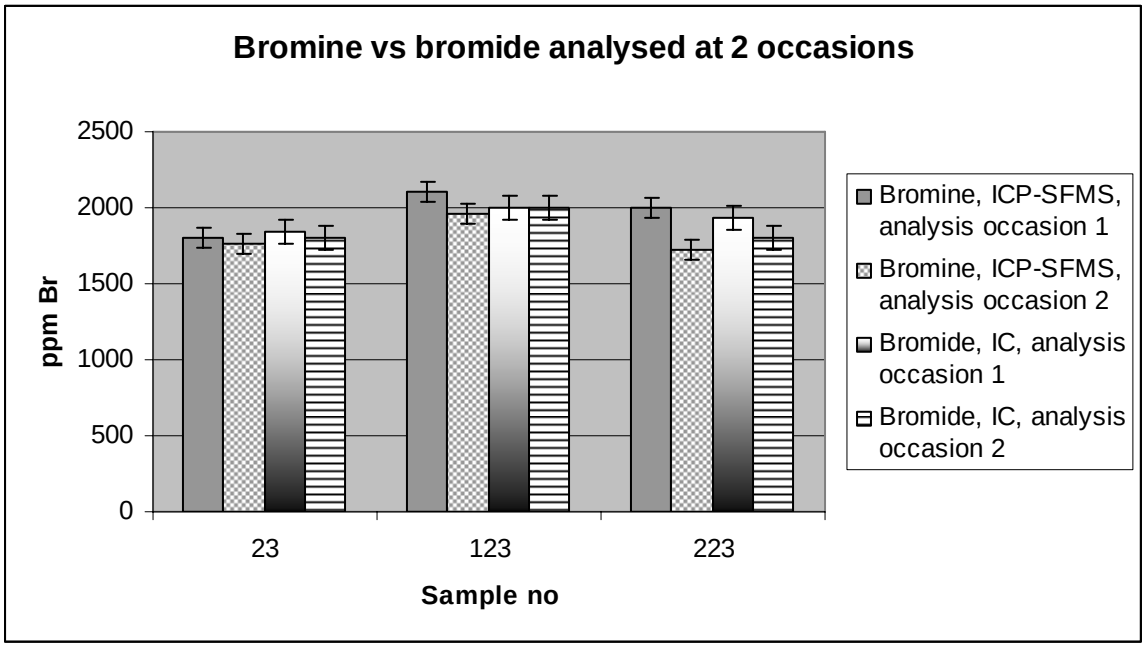

Figure 2. Bromide and bromine analysis of samples 23, 123 and 223 at 2 analysis occasions.

3.2.3.3 Removal of bromine in 0.5 M NaOH stored Q Sepharose BB

Since the bromine leakage is high when storing Q Sepharose BB in alkali solutions it is of interest to wash the matrix with a propriate buffer to remove excess bromine and analyse the amount of bromine after buffer wash. 20 mL pre-use washed Q Sepharose FF T-301065, T-301462 and T-302192 were washed in a G3 glass filter with 5 cv 0.5 M NaOH. At the 4th wash the glass filters were vacuum suctioned for one minute and transferred into storage flasks. 20 mL 0.5 M NaOH was added and the gels were stored for 168 h at 40^{o}C (incubator Nüve, id 534). After 168 h the gel mixtures were transferred into G3 glass filters and washed with 10 cv 0.1 M Tris/HCl pH 7.5 buffer. After the last wash the gels were vacuum suctioned for one minute and packed into three 11 mL PFE cells. The cells were extracted with milli-Q water as described in Analytical report 1, section 4. Table 8 shows the weight amount vacuum suctioned gel and mL extract obtained from the extractions.

Table 8. PFE of Q Sepharose BB, pretreated with 0.5 M NaOH for 168 h at 40^{o}C and wash with 10 cv 0.1 M Tris/HCl pH 7.5.

Batch Q Sepharose BB	Weight, vacuum suctioned gel (g)	Volume, wet sedimented gel (mL)	Volume extract (mL)
T-301065 (sample 320)	6.235	8.6	18.3
T-301462 (sample 321)	6.556	8.9	18.9
T-302192 (sample 322)	6.397	8.8	18.9

The samples were sent to Analytica AB for bromine and bromide analysis. Table 9 shows the result from the analysis. The bromine reduction is the percentage amount bromine that is removed with 10 cv 0.1 M Tris/HCl pH7.5 buffer from the Q Sepharose BB samples stored for 168 h with 0.5 M NaOH at 40°C.

Table 9. Calculation of reduction in bromine release when washing 0.5 M NaOH, 40°C stored Q Sepharose BB with 10 cv 0.1 M Tris/HCl pH 7.5 buffer.

Batch Q Sepharose BB	Bromine (ICP-SFMS) (ppm)	Bromide (IC) (ppm)	Total bromine (ICP-SFMS) content in extract (μg)	Bromide (IC) content in extract (μg)	Bromine extracted from Q Sepharose BB, (μg/mL wet sed gel)	Bromide extracted from Q Sepharose BB, (μg/mL wet sed gel)	Bromine release into storage solution 0.5 M NaOH 40°C (from Table 4) (μg/mL sed gel)	Bromine reduction, %
T-301065 (sample 320)	34.9	36.7	638	672	74	78	1800	95.7
T-301462 (sample 321)	20.7	21.7	391	410	44	46	2100	97.8
T-302192 (sample 322)	26.9	26.9	508	508	58	58	2000	97.1

3.3 Determination of the dry weight content in Q Sepharose BB batches up to 8 years old

3.3.1 Introduction

The manufactured Q Sepharose BB is stored in a shipping solution containing 20 %v/v ethanol. If the gel decomposes the dry weight content would decrease. The dry weight content of 8 batches Q Sepharose BB produced over a period of 8 years was determined.

3.3.2 Materials

Same materials as section 2.1.2 in Analytical report 1.

Table 10 shows the batches of Q Sepharose BB used in the experiment.

Table 10. Batches of Q Sepharose BB used in the experiment

Batch Q Sepharose BB	Manufacturing date
T-243424	1996-10-11
T-279882	1999-11-26
T-280981	2000-01-20
T-282557	2000-05-18
T-293719	2002-06-28
T-301065	2003-12-07
T-301462	2003-12-15
T-302192	2004-02-19

3.3.3 Chemicals

Same chemicals as section 2.1.2 in Analytical report 1.

3.3.4 Procedure

Same procedure as section 2.1.2 in Analytical report 1, except that the experiments were performed with a duplicate determination.

3.3.5 Results

Figure 3 shows the results of the dry weight determination of Q Sepharose BB batches.

Date for analysis: 2004-12-22

Batch Q Sepharose BB	Date for production	Storage time to analysis date (years)	Gel height 1 (cm)	Gel height 2 (cm)	Vc 1 (mL)	Vc 2 (mL)	Weight glass filter 1 (g)	Weight glass filter 2 (g)	Weight glass filter + gel 1 (g)	Weight glass filter + gel 2 (g)	Weight 1 (g)	Weight 2 (g)	Dry weight 1 (g / mL sed gel)	Dry weight 2 (g / mL sed gel)	Dry weight average (g / mL sed gel)
T-243424	1996-10-11	8,2	5,85	5,9	9,711	9,794	53,3734	53,3841	54,5618	54,581	1,1884	1,1969	0,122377	0,122207	**0,122**
T-279882	1999-11-26	5,1	6,05	6,6	10,043	10,956	53,7538	50,7105	55,0072	52,0693	1,2534	1,3588	0,124803	0,124023	**0,124**
T-280981	2000-01-20	4,9	5,3	5,3	8,798	8,798	50,4756	53,3065	51,5459	54,3656	1,0703	1,0591	0,121653	0,12038	**0,121**
T-282557	2000-05-18	4,6	5,05	5,9025	8,383	9,79815	53,5513	53,681	54,5891	54,9043	1,0378	1,2233	0,123798	0,12485	**0,124**
T-293719	2002-06-28	2,5	5,7	5,75	9,462	9,545	53,0876	53,747	54,2594	54,925	1,1718	1,178	0,123843	0,123415	**0,124**
T-301065	2003-12-07	1,0	4,6	4,9	7,636	8,134	50,4698	50,5037	51,4415	51,5397	0,9717	1,036	0,127252	0,127367	**0,127**
T-301462	2003-12-15	1,0	4,375	4,3	7,2625	7,138	48,3758	50,4857	49,2676	51,3507	0,8918	0,865	0,122795	0,121182	**0,122**
T-302192	2004-02-19	0,8	5,4	4,6	8,964	7,636	51,6399	51,7182	52,7793	52,6872	1,1394	0,969	0,127108	0,126899	**0,127**

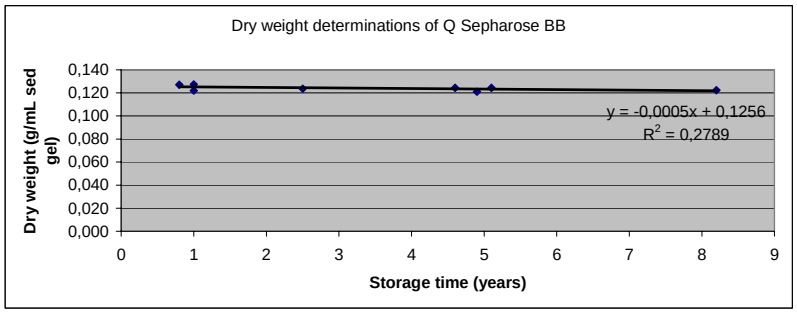

Figure 3. Results of the dry weight determination of Q Sepharose BB.

The results in Figure 3 show that there is no tendency towards a decreased dry weight content of Q Sepharose BB. These results clearly show that Q Sepharose BB is chemically stable in the shipping solution for at least 8 years.

3.4 Bromine content in Q Sepharose BB batches up to 8 years old

In order to obtain an estimate of the bromine levels present in the resin, the elements in Table 27 in Analytical report 2 were added and the remaining weight percent not accounted for determined. This remaining weight was assumed to be bromine, since no other elements are added in the manufacturing process.

Table 13 shows the calculated bromine content for all batches. As can be seen there is no trend towards a reduction in bromine content with age, thus indicating that the bromine is attached to the resin in a stable fashion.

Table 13. Estimated amount of bromine in manufactured Q Sepharose BB

Batch Q Sepharose BB	Manufacturing date	Carbon (%w/w)	Hydrogen (%w/w)	Nitrogen (%w/w)	Oxygen (%w/w)	Sulphur (%w/w)	Total (%w/w)	Estimated amount of bromine (%w/w)
T-243424	1996-10-11	44.8	7.1	2.1	36.95	2.15	**93.1**	**6.9**
T-279882	1999-11-26	45.4	7.2	2.0	36.45	2.0	**93.05**	**6.95**
T-280981	2000-01-20	46.0	7.3	2.0	35.6	1.85	**92.75**	**7.25**
T-282557	2000-05-18	45.15	7.5	2.35	36.05	2.35	**93.4**	**6.6**
T-293719	2002-06-28	44.85	7.3	2.25	36.35	2.2	**92.95**	**7.05**
T-302192	2003-02-19	44.95	7.45	2.3	37.05	2.2	**93.95**	**6.05**
T-301065	2003-12-07	44.65	7.25	2.2	36.5	1.95	**92.55**	**7.45**
T-301462	2003-12-15	44.8	7.4	2.3	36.25	2.15	**92.9**	**7.1**