CSS Adsorber Processing Aid in Beer

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PART 1 GENERAL INFORMATION

1.1 Applicants

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1.3 Nature of application

1.3.1 This application is to include permission in *Standard 1.3.3 – Processing Aids* of the Food Standards Code to use CSS – Adsorber as a processing aid in beer manufacture to remove undesirable, haze-creating proteins and polyphenols to enable enhanced shelf-life. The substances removed are sent to waste; there are no intended food uses for these substances.

1.3.2 The application relates only to beer. The supporting information for this application relates only to beer. Evidence supporting other uses in the food industry is not available from the applicants. Therefore, no other food use is covered by this application.

The use in beer is to selectively remove unwanted components – proteins (polypeptides) and polyphenols. The removed components have no value and they are not collected.

Some other food uses will be similar to the situation with beer in that the processing aid would be used to remove unwanted components of the food. However, other food uses may involve the collection of proteins or other components of the food as value-added products.

Extension of use to other foods increases the complexity of the application because it involves a wider group of foods used by a wider portion of the community. And although there are no detectable contaminants in beer using this system there is no evidence to support whether or not there are contaminants, and if there are, at what levels these contaminants might occur in other foods. Again this document does not include any information to support the extension of the use of CSS Adsorber for other foods or uses in the food industry other than beer.

1.3.3 This is a joint application by Lion Nathan – brewer, and GE Health Care Biosciences – resin manufacturer. The applicants have been assisted by Food Liaison Pty Ltd.

PART 2 SPECIFIC INFORMATION

2.1 Details of the CSS Adsorber processing aid

The name CSS Adsorber is used throughout this application. The name comes from the use in beer, and it means "Combined Stabilisation System". In this application and its appendices the name *CSS Adsorber* are used interchangeably with Trade Name *Q Sepharose® Big Beads* or *Q Sepharose® BB*.

A description of the CSS Adsorber is: Agarose, cross-linked and alkylated with epichlorohydrin and propylene oxide, then derivatised with tertiary amine groups whereby the amount of epichlorohydrin plus propylene oxide does not exceed 250% by weight of the starting quantity of agarose.

The description is based on another resin already approved in Standard 1.3.3-8: Regenerated cellulose, cross-linked and alkylated with epichlorohydrin and propylene oxide, then derivatised with tertiary amine groups whereby the amount of epichlorohydrin plus propylene oxide does not exceed 250% by weight of the starting quantity of regenerated cellulose.

These two resins are very similar in nature, the point of difference it the sugar base of the polymer: glucose in the case of regenerated cellulose and agarose (the sugar base of the approved additive, agar) in the case of CSS Adsorber.

The name CSS Adsorber is derived from the name of the system used to remove haze-causing components from beer - Combined Stabilisation System. The Adsorber is the *Q Sepharose® Big Beads.*

2.1.1 CAS Registry Details

CAS Registry Name: Agarose, polymer with (chloromethyl)oxirane, 2-hydroxy-3-(2-hydroxy-3-(trimethylammonio)propoxy)propyl ethers, sulfates salts

CAS Registry Number: 846053-13-2

2.1.2 Trade or Common Name

Trade or Common Name: Q Sepharose® Big Beads Food Grade (Trade Name). The names CSS Adsorber are use interchangeably in this application and in the appendices.

Q Sepharose Big Beads (BB) is a macroporous, strong cation exchanger with quarternary ammonium groups coupled to the matrix which consists of agarose highly cross-linked with epichlorohydrin. Because the resin is a product of a high molecular weight polygalactan and once crossed-linked, is insoluble in water, its molecular weight can not be determined. Any attempt to solubilise the gel would lead to its degradation.

The representation of the complex gel structure with a chemical formula is not possible owing to the fact that the detailed structure has not been elucidated and also to the fact that the subsituation can take place at many different hydroxyl groups. A structural representation appears in Figure 1.

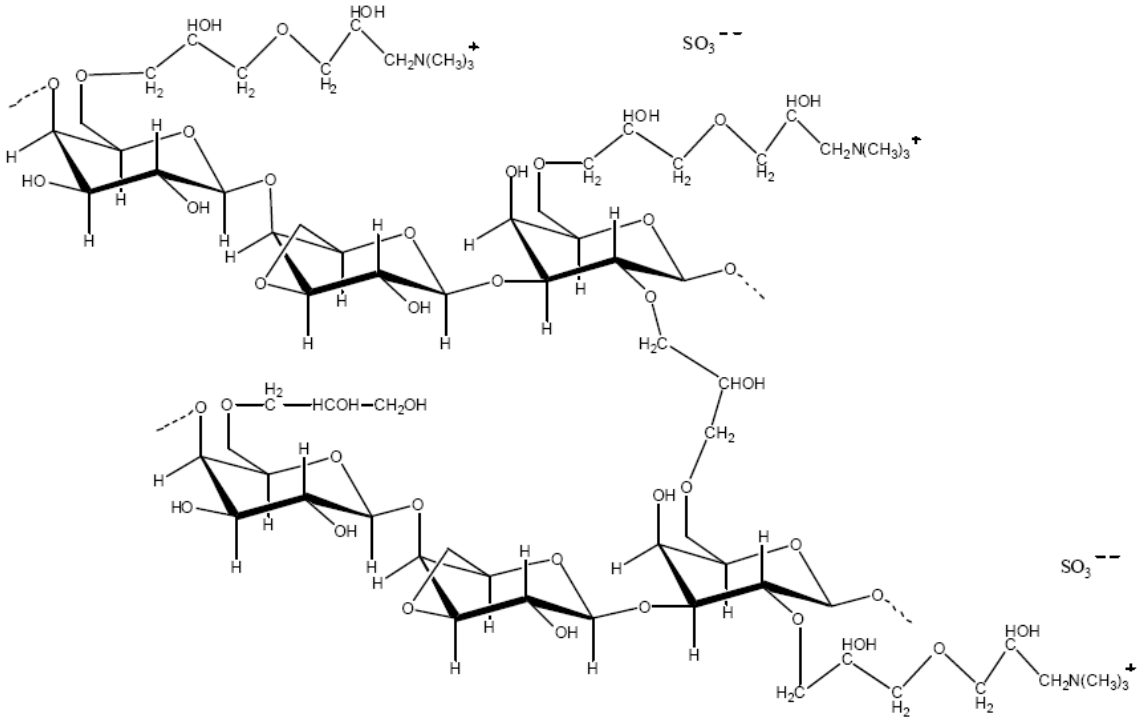


Figure 1 - Structural representation of a fragment of CSS Adsorber.

Further information on the identification of CSS Adsorber is provided in Appendix 1 - Identification - Structure of CSS Adsorber.

2.2 Purpose and efficacy of the additive

2.2.1 Purpose

CSS Adsorber is an ion exchange chromatography resin intended for repeated use in extracting individual proteins or substances present in similar low concentrations from beer.

CSS Adsorber is designed to enhance physical stabilisation of beer through the removal of haze forming polyphenols and proteins by adsorption onto polysaccharide gel beads. However, unlike conventional methods, there is no dosing of stabilisation aids into the beer flow. Haze forming compounds are removed by adsorption onto the surface and within the porous structure of the gel beads.

The adsorbent material is based on the macroporous, cross-linked polysaccharide agarose (polymer of galactose and 3,6-anhydrogalactose). The product is in the form of insoluble, porous, spherical beads, between 100 - 300μm in diameter. See Figure 2.

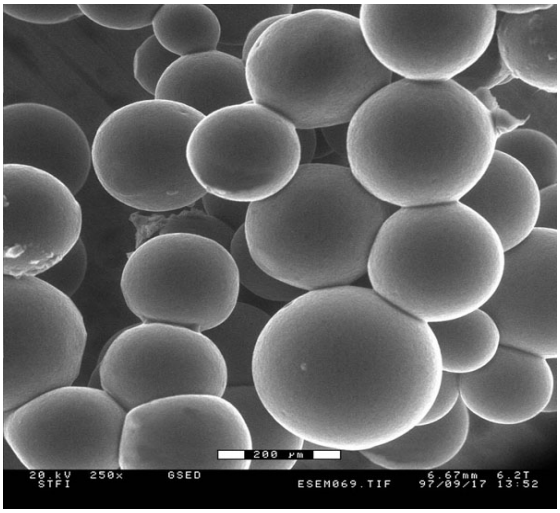


Figure 2 - CSS Adsorber Beads

The CSS unit consists of adsorption chambers containing a quantity of the adsorbent material immobilised within. It is shown in Figure 3. Beer passes through the chambers and after a short contact time with the adsorber, haze forming polyphenol and protein compounds are removed. The number and volume of adsorption chambers installed within the CSS is tailored to individual brewery needs and relates to the flow rate, total volume and degree of stabilisation required.

Figure 3 - Typical CSS Unit

Over the course of the stabilisation process, adsorption rates of polyphenol and protein materials gradually diminish as saturation of the adsorption sites within the bead bed occurs. In order to control and maintain consistent stabilisation of the beer, the supply is split prior to the adsorption chamber, see Figure 4.

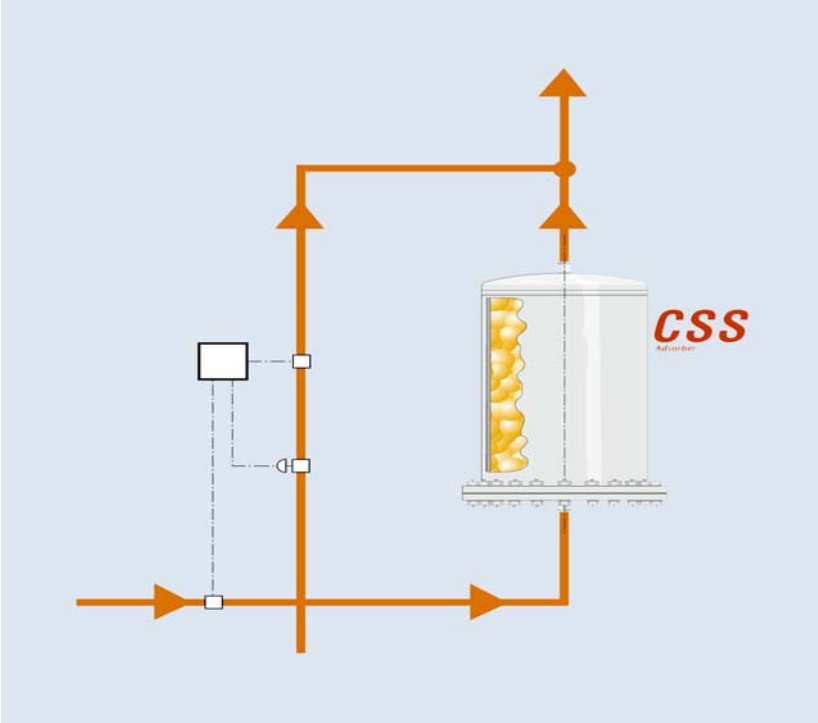


Figure 4 - CSS Flow Diagram

A pre-calculated portion of beer passes through the treatment chamber to be stabilised, with the remainder bypassing stabilisation. Controlling the stabilisation in this manner requires an intense stabilisation of one portion of beer, followed by the blending of this portion with unstabilised beer. Product consistency is achieved by the ratio control of stabilised to unstabilised bypass beer. A predetermined ratio curve is applied to compensate for the increasing level of saturation of agarose. Eventually, upon nearing saturation, the entire flow of beer will pass through the Adsorber chamber until complete saturation occurs and regeneration is required.

Regeneration is accomplished with a back flush of the chambers using NaCl (12%) followed by NaOH (4%). Regeneration with NaCl is designed to remove protein material whilst minimising denaturation and NaOH will thereafter remove any adsorbed material which remains. The final step is flushing with de-aerated water.

CSS Adsorber is used to extract selected polyphenols and proteins removed from the beer, which leads to enhanced stability of the beer.

The intended limitations proposed for this repeat-use CSS Adsrober resin for beer is in the range of -1.5°C to +0.5°C and a pH range 3-5. The pH range covers stabilising beer. Regeneration is also carried out about 20C and pH of approximately 14.

Intended use is typically in a column 200 cm in diameter with a resin bed height of 30 cm, which gives a column volume of 1000 litre resin. The volume of beer per cycle is at least 100,000 litres. Desorption requires minimum 1,000 litres and the other process steps at least 10,000 litres. A total of 100,000 litres, at a flow rate of 1500 litres per hour, passes through in about 66.7 hours. In each cycle about 18 kg of the unwanted proteins and polyphenols are extracted and sent to waste.

2.2.2 Efficacy

Results of previous work conducted by GE Health Care indicates the removal of both haze active polyphenols and proteins from the beer and several adsorption mechanisms for this have been proposed but are, as yet, unproven.

Polyphenol removal appears to be the fundamental mechanism behind this stabilisation. With respect to protein binding, agarose has been used within the field of protein isolation for many years, and the fact that proteins will bind to it is well known. However, the effectiveness of the CSS to adsorb both of these from beer under the operating conditions of a large brewery at temperatures of ~0ºC and pH of ~4 was unknown.

In 2005, Lion Nathan installed a commercial scale CSS unit for trial at the Tooheys Brewery, Sydney. The unit consists of three adsorption chambers, allowing for a flow rate of 600hL/hr, with a chamber volume of 9hL as shown in Figure 3.

Experiments involved 3 phases:

1. 9400 hL stabilisation
2. Comparison of beer stabilised by current means with stabilisation by CSS Adsorber.
3. Elucidation of the Adsorption Rates of Haze Active Compounds

2.2.2.1 Phase 1 – 9400 hL stabilisation

As shown in Figure 5 the 9400 hL stabilisation used for typical domestic beer.

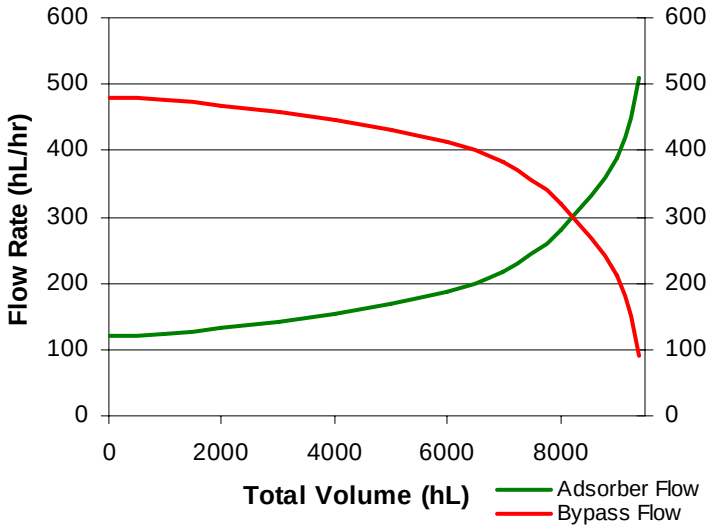


Figure 5 - 9400 hL beer stabilisation curve.

Comparisons of beer stabilised by the CSS and by alternative processes (SHG or SHG/PVPP combination) were made. Testing was carried out on a range of beer types, from low alcohol (2.7% abv) and full strength (5.0% abv) lagers to dark ale (4.4% abv). Haze testing included 0 week, 13 week, and 55ºC forced hazes.

Most of the early assessment on the degree of stabilisation achieved by the CSS was based upon forced haze data. This allowed for relatively quick responses to any issues which arose during the early stages of the trial. Curves were then tailored to the results of the forced haze results specific for each beer. Rough tailoring of stabilisation curves in this manner, specific to how each beer performed, ensured product quality and maintained operational efficiencies.

Published work on stabilisation by the CSS is very limited [1] [2] [3]. However, results of work, carried out by GE Health Care, on polyphenols and proteins indicates reductions in polyphenols and proteins.

Table 1 - Initial Trial Results shows the results from the initial brewery trial. Several beers (A to E) stabilised with SHG/PVPP are compared to the same beers stabilised by the CSS. Beers F and G used SHG alone. It can be seen the stabilisation of the beer with the CSS achieved comparable physical stabilisation to the control beers.

Beer	0 Week Haze (EBC)	13 Week Haze (EBC)	55ºC Forced Haze (EBC)
A	0.40	0.50	0.69
A CSS	0.43	0.50	0.69
B	0.53	0.72	1.36
B CSS	0.43	0.87	1.37
C	0.41	1.12	1.02
C CSS	0.41	1.59	1.72
D	0.50	0.92	1.04
D CSS	0.40	0.65	0.74
E	0.45	1.03	1.20
E CSS	0.42	0.88	1.13
F	0.40	0.42	0.52
F CSS	0.42	0.49	0.93
G	0.44	0.92	1.19
G CSS	0.39	0.92	0.94

Table 1 - Initial Trial Results

[1] Katzke, M., Nendza, R. and Oeschle, D., Die bierstabilisierung mit ionentauschern, Brauwelt, 1998, 138, 991-994

[2] Jany, A. and Katzke, M., CSS-A new beer stabilization process, *MBAA Technical Quarterly*, 2002, **39(2)**, 96-98

[3] Jany, A. and Katzke, M., Combined stabilisation systems (CSS): A new beer stabilisation process, *Proceedings of the 10th Convention of the Institute of Brewing and Distilling Africa Section (Pilanesberg)*, 2005, **10**, 141-144

2.2.2.2 Phase 2 – Direct Comparison of beer stabilised by SHG/PVPP with CSS Adsorber

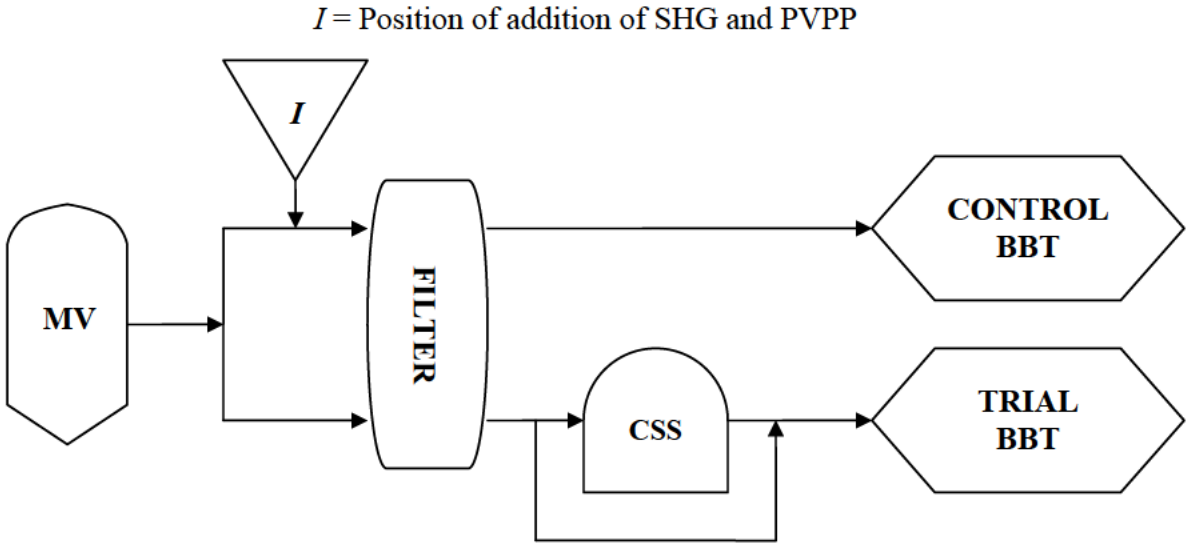


Figure 6 - SHGG/PVPP vs CSS Adsorber Trail

The next phase of the trial was a direct comparison of beer stabilised by SHG/PVPP (both described in Section 2.3) addition with that stabilised by the CSS as shown in Figure 6.

Maturation beer was stabilised with SHG (300mg/L) and single use PVPP (250mg/L) and transferred to a single bright beer tank (BBT). Beer was then taken from the same maturation vessel (MV), stabilised with an aggressive CSS stabilisation curve tailored for 'export' beer and transferred to a separate BBT. Samples were taken from each BBT for analysis. The trial was repeated three times, and the beer used, *Beer X*, was a 100% malt lager. Analysis was carried out on the physical properties of the samples (pH, alcohol, apparent extract, bitterness, hazes and head retention) as well as the chemical composition of the stabilised beer (concentrations of total polyphenols, anthocyanogens, the relative amounts of catechin, procyanidin B_3 and prodelphinidin B_3 and haze sensitive protein concentration).

Table 2 shows that there were no significant differences noted in pH, alcohol concentration, apparent extract, bitterness, 0 week haze or head retention, between the SHG/PVPP and the CSS stabilised samples.

Stabilisation of the beer with the CSS with this particular stabilisation curve showed good forced and 13 week haze results.

Sample	pH	Alcohol (%v/v)	Apparent Extract (°P)	Bitterness (IBU)	0 Week Haze (EBC)	55°C Forced Haze (EBC)	13 Week Haze (EBC)	Head Retention (Nibem)
Control Average	4.4	5.0	1.9	21.5	0.57	0.54	0.48	265
Trial Average	4.4	5.0	1.9	21.0	0.55	0.50	0.48	265

Table 2 - Physical Properties Comparison of beer stabilised by two systems

Polyphenol removal, as measured by total polyphenol and anthocyanogen concentrations, was comparable for both stabilisation processes, as shown in Table 3. However, HPLC analysis showed that the CSS stabilisation adsorbed each specific polyphenol in differing quantities. Catechin adsorption appears to be at a lesser scale, with the adsorption of the dimers procyanidin B_3 and prodelphinidin B_3 (the latter in particular) being the main changes. Sensitive protein adsorptions for both stabilisations were comparable.

Sample	Total Polyphenols (mg/L)	Anthocyanogens (mg/L)	Catechin	Procyanidin B_3	Prodelphinidin B_3	Sensitive Proteins (EBC)
Control Average	120	14.3	0.283	0.068	0.123	5.7
Trial Average	119	16.7	0.429	0.091	0.109	5.6

Table 3 - Chemical Composition Comparison of beer stabilised by two systems

2.2.2.3 Phase 3 –Elucidation of the Adsorption Rates of Haze Active Compounds

Having shown that the CSS does improve physical stability, the next stage of the trial was to elucidate the adsorption rates of haze active material. A 6000hL stabilisation test of a 100% malt lager was carried out. Samples were taken Pre CSS, Post CSS and at the Blend of stabilised and by-passed beer, every 1000hL. The 9400hL 'domestic' stabilisation curve was applied; see Figure 5 - 9400 hL beer stabilisation curve. Analysis of haze forming polyphenol content was carried out by total polyphenols, anthocyanogens and specific polyphenols by HPLC analysis.

Haze protein content was analysed by sensitive protein concentration. The analysis of proteins Z4, Z7 and LTP1 concentrations, which are known to be foam active 4 5 6, was completed by ELISA. SDS-PAGE immunoblotting allowed for the assessment of the extent of foam protein adsorption by the CSS. This included analysis of NaCl regenerate solutions.

The analyses carried out during the trials were as follows:

i. *Haze Measurement*. Turbidity analyses were carried out with the use of a Hach 2100N Turbidimeter (90º) (Hach, Loveland, CO, USA) at 0ºC, and measured in EBC formazin units (EBC).

ii. *Forced Haze*. Seven days storage at 55ºC, followed by 24 hours at 0ºC. Haze measurement then taken at 0ºC as described above.

iii. pH

iv. *Alcohol and Apparent Extract*. Analysis carried out using an Anton Paar DMA 4500 (Graz, Austria).

[4] Kaesgaard, P., and Hejgaard, J., Antigenic beer macromolecules, an experimental survey of purification methods, *Journal of the Institute of Brewing*, 1979, **85**, 103-111

[5] Lusk, L. T., Goldstein, H., and Ryder, D., Independent role of beer proteins, melanoidins and polysaccharides in foam formation, *Journal of the American Society of Brewing Chemists*, 1995, **53**, 93-103

[6] Sørensen, S. B., Bech, L. M., Muldbjerg, M., Beenfeldt, T., and Breddam, K., Marley lipid transfer protein 1 is onvolved with beer foam formation, *MBAA Technical Quarterly*, 1993, **30**, 135-145

v. *Total Polyphenols*. Analysis carried out as per EBC method[7].

vi. *Anthocyanogens*. Analysis carried as per methods of McFarlane[8].

vii. *Polyphenols by HPLC*. Analysis carried out as per Whittle *et al*[9].

viii. *Sensitive Proteins*. Analysis carried out as per the methods of Chapon[10].

ix. *ELISA* - Beer samples were diluted 1/1000 with phosphate buffered saline (PBS)/0.1% BSA/0.05% Tween 20.

Quantitative double-antibody sandwich-format ELISAs were used to quantify protein Z4, Z7 and LTP1 levels by methods described in Evans and Hejgaard[11]. ELISA's were completed by Dr Evan Evans at the University of Tasmania (Hobart, Tasmania, Australia).

x. *SDS-PAGE and Immunoblotting*. Beer samples were diluted (1:2) into SDS-PAGE sample buffer (5M Urea, 4% SDS, Tris buffer pH 8.0) containing 1% (v/v) 2-mercapto-ethanol. SDS-PAGE and immunoblotting were carried out using polyclonal antibodies (anti-2x Foam) developed essentially as described in Evans *et al*[12] and Robinson *et al*[13]. SDS-PAGE analysis was completed by Dr Evan Evans at the University of Tasmania (Hobart, Tasmania, Australia).

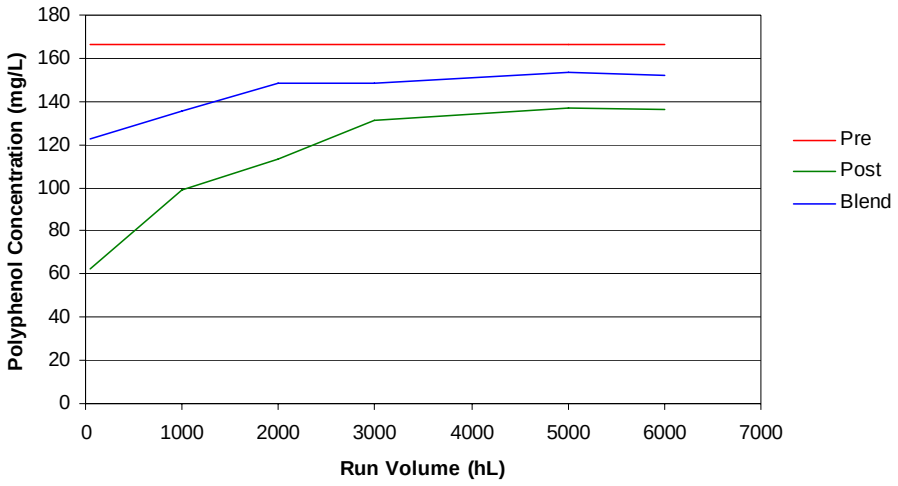


Figure 7 - Total Phenols Concentrations before Curve Alteration

[7] Analysis Committee of the EBC, Method 9.9.1., *Analytica EBC*, 1987, E.157

[8] McFarlane, W. D. and Bayne, P. D., Studies on the flavonoid compounds in wort and beer: III. Adsorption on polymeric resins, *EBC Proceedings of the 8th Congress (Vienna)*, 1961, 278-285

[9] Whittle, N., Eldridge, H., Barley, J. and Organ, G., Identification of the Polyphenols in Barley and Beer by HPLC/MS and HPLC/Electrochemical Detection, *Journal of the Institute of Brewing*, 1999, **105(2)**, 89-99

[10] Chapon, L., Nephelometry as a method for studying the relations between polyphenols and proteins, *Journal of the Institute of Brewing*, 1993, **99**, 49-56

[11] Evans, D. E. and Hejgaard, J., The impact of malt derived proteins on beer foam quality. Part I. The effect of germination and kilning on the level of protein Z4, protein Z7 and LTP1, *Journal of the Institute of Brewing*, 1999, **105**, 159-169

[12] Evans, D.E., Robinson, L.H., Sheehan, M.C., Tolhurst, R.L., Hill, A., Skerritt, J.S. and Barr, A.R., Application of immunological methods to differentiate between foam-positive and haze active proteins originating from malt, *Journal of the American Society of Brewing Chemists*, 2003, **61**, 55-62

[13] Robinson, L. H., Healy, P., Gibson, C. E., Eglinton, J. K., Ford, C. M. and Evans, D. E.. The identification of a malt haze active protein that can influence beer haze stability. Part I: The genetic basis of a barley malt haze active protein. Submitted: *Journal of Cereal Science*, 2005

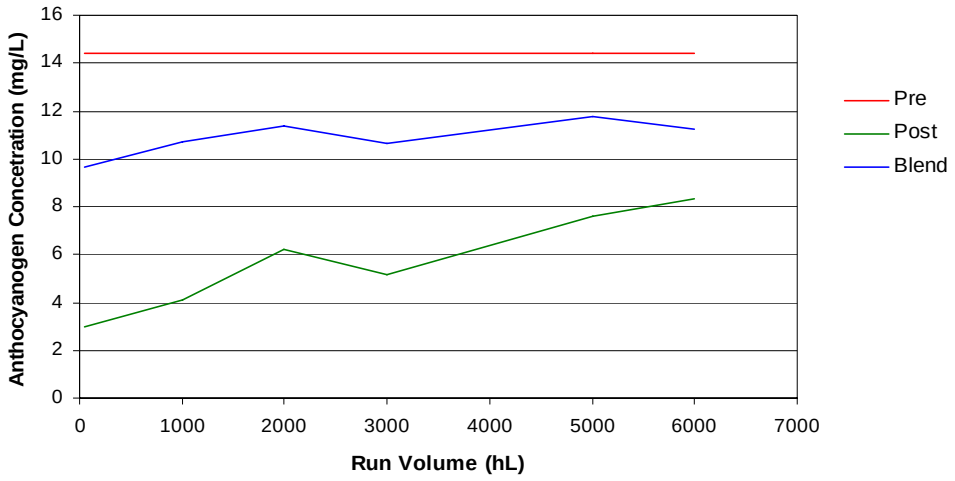


Figure 8 - Anthocyanogen Concentrations before Curve Alteration

Figure 7 and Figure 8 depict the total polyphenol concentrations taken from the three sample points throughout the extended stabilisation run. Polyphenol concentrations of the *Post* samples, in both sets of analysis, gradually increased over the course of the run. During the first 2000hL, the increase in *Post* sample polyphenol concentrations was mirrored in the *Blend* results. Ideally that of the *Blend* should remain constant. The data shows that the ratio of flow rates of stabilised to unstabilised beer was initially sub-optimal.

Small adjustments were then carried out to reduce the degree of stabilisation during the first 2000hL of subsequent trials, the results of which are shown in Figure 9 and Figure 10.

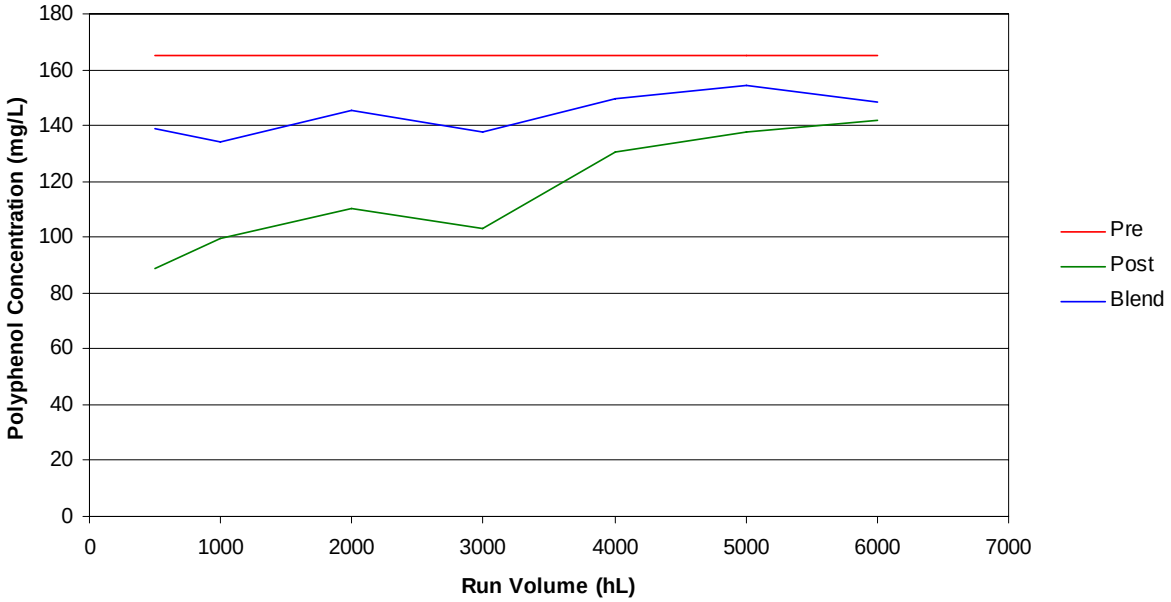


Figure 9 - Total Phenolic Concentrations after Curve Alteration

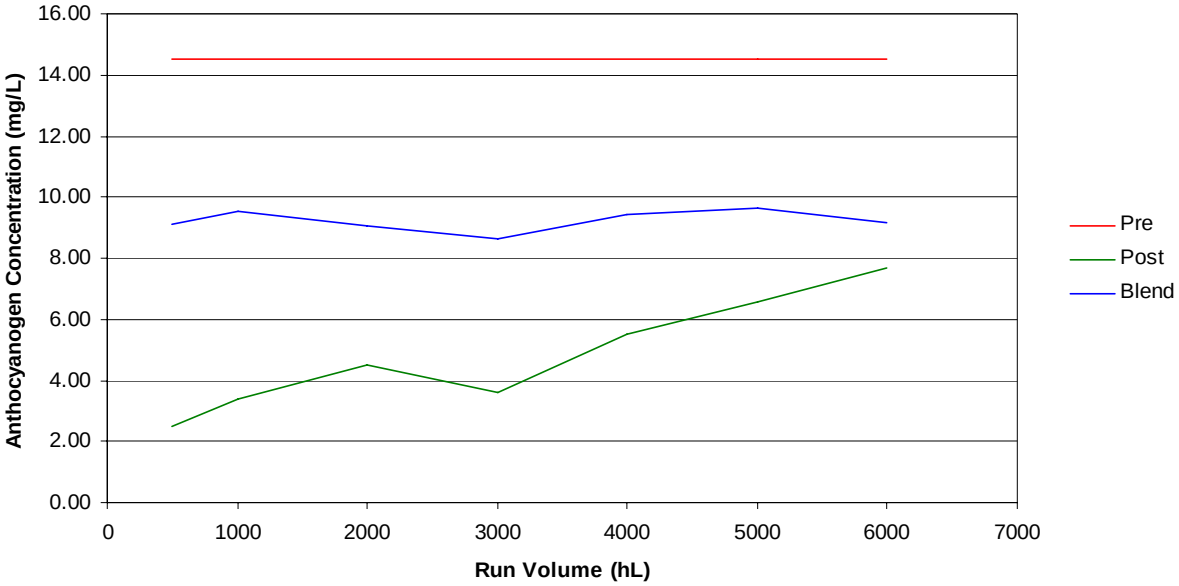


Figure 10 - Anthocyanogen Concentrations after Curve Alteration

During the first 1,000hL, see Figure 11, catechin concentrations increased dramatically. Procyanidin B_3 concentrations remained consistently low until 3000hL at which point they began to increase. The adsorption of prodelphinidin B_3 was the most consistent, and relative concentrations remained low throughout the entire run. These results concur with those depicted in Table 3, where the catechin concentrations for the CSS treated beer were proportionately higher than those of procyanidin B_3 and prodelphinidin B_3. Therefore, it appears that agarose preferentially adsorbs polyphenols of larger molecular size.

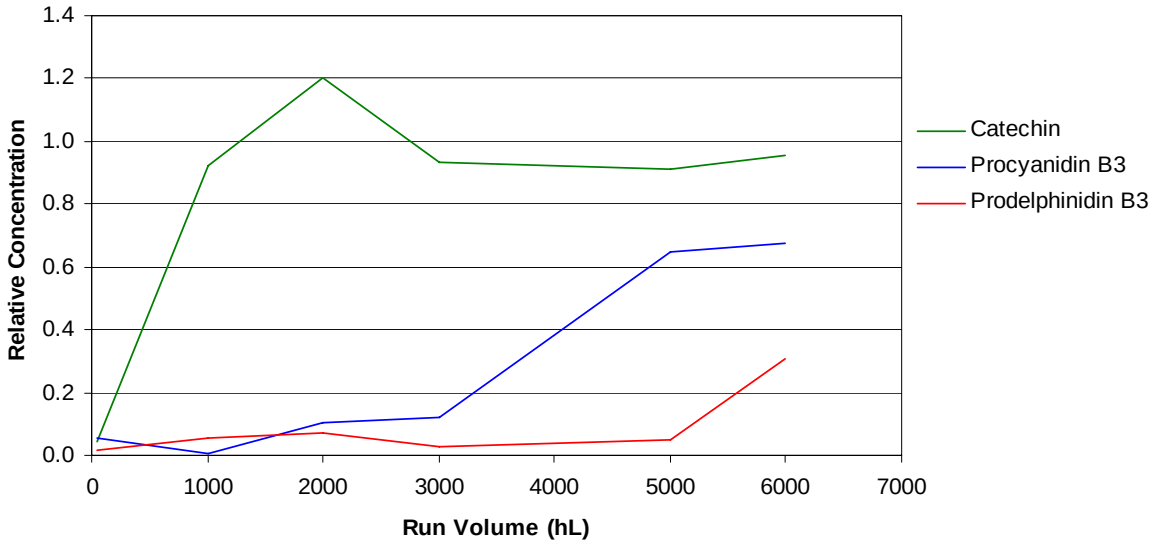


Figure 11 - Specific Phenolic Concentrations by HPLC

Catechin is less haze active than procyanidin B_3 and prodelphinidin B_3, the most haze active due to the presence of one more hydroxyl group within its structure[14] [15]. The latter two have higher levels of polymerisation and higher adsorption rates of these two compounds are advantageous to colloidal stability[16]. The relatively high and consistent adsorption rates of procyanidin B_3 and prodelphinidin B_3 during the test may begin to elucidate the mechanism by which the CSS improves physical stability[1] [2] [3]. (References on page 11)

A low level of sensitive protein material was removed during the trial as shown in Figure 12. Post stabilisation samples showed lower sensitive protein concentrations, with BBT samples slightly higher, which would be expected due to the CSS blending process.

CSS regeneration with NaCl removes protein material from the adsorber without denaturing the polypeptide material. Protein analysis of NaCl regenerate samples is therefore an ideal way in which to understand protein adsorption by the CSS. The foam protein spectrum, including the NaCl regenerate samples, produced by SDS-PAGE immunoblotting using foam protein antibodies, is shown in Figure 13. The data showed no significant differences between *Pre*, *Post* and *Blend* CSS samples. There were no significant protein concentrations of the main foam active bands of ~9,700kDa (protein LTP1) or ~40,000kDa (protein Z) in the NaCl regenerate samples.

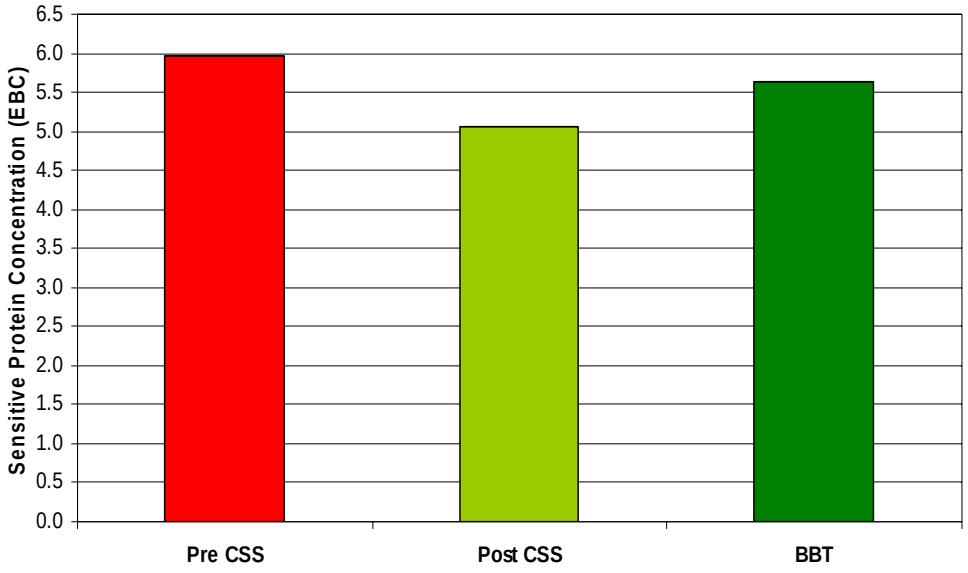


Figure 12 - Sensitive Protein Concentrations

[14] Doner, L. W., Bécard, G. and Irwin, P. L., Binding of flavonoids by polyvinylpolypyrrolidone, *Journal of Agricultural and Food Chemistry*, 1993, **41**, 753-757

[15] McManus, J. P., Davis, K. G., Beart, J. E., Gaffney, S. H., Lilley, T. and Haslam, E., Polyphenol Interactions 1. Introduction; Some observations on the reversible complexation of polyphenols with proteins and polysaccharides, *J. Chem. Soc. Perkin. Trans.*, 1985, **2 (11)**, 1429-1438

[16] Mulkay, P., and Jerumanis, J., Effects of molecular weight and degree of hydroxylation of anthocyanogens on the colloidal stability of beer, *Cerevisia*, 1983, **8(1)**, 29-35

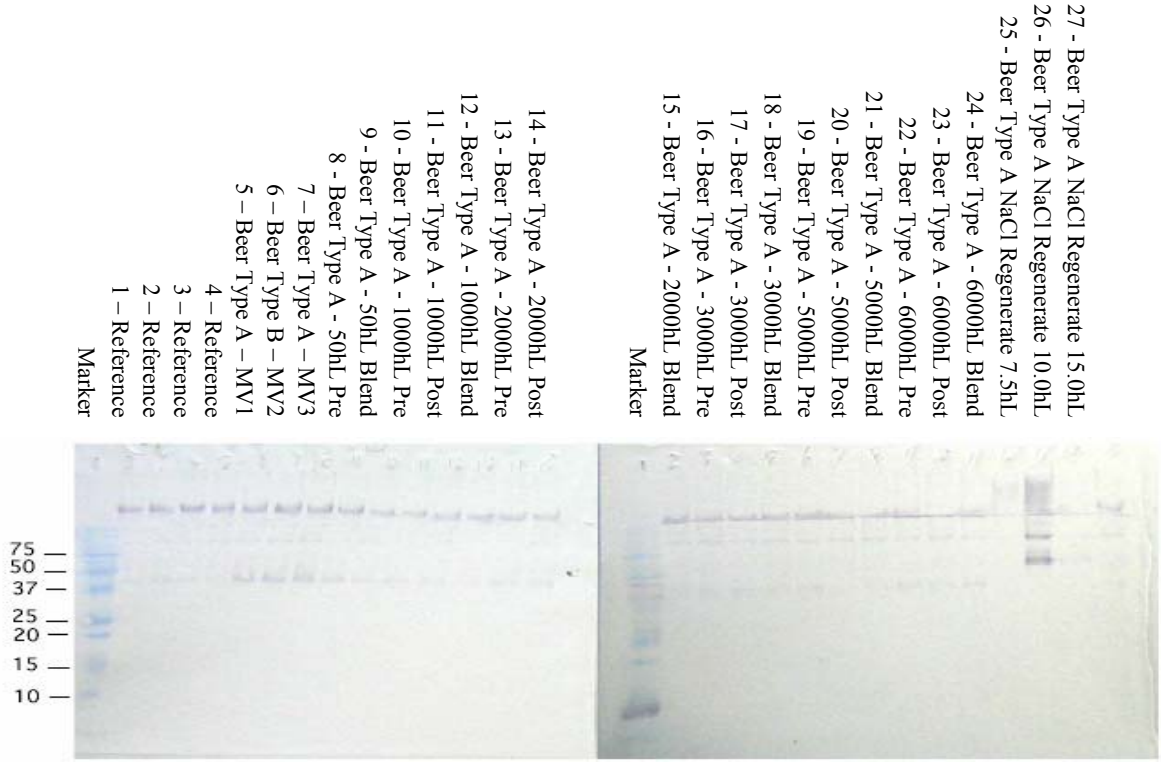


Figure 13 - Anti-2x Foam SDS-PAGE Immunoblot

Proteins Z4, Z7 and LTP1 are known to be involved in the production and stabilisation of foam[4] [5] [6]. (References on page 13) Quantitative analysis of these proteins was undertaken to further explore the effects of the CSS stabilisation of beer. Quantitative analysis was carried out, by ELISA[11], on *Pre*, *Post* and *Blend* CSS samples, as well as on NaCl regenerates these are shown in Table 4.

Concentrations of Z7 and LTP1 did not decrease with CSS stabilisation, and further to this neither of these polypeptide groups was present within the NaCl regenerate. Between the *Pre*, *Post* and *Blend* CSS samples there was also no significant decrease in Z4. 7µg/mL was found within the NaCl regenerate.

Protein	Concentration (µg/mL)			
	Pre CSS	Post CSS	Blend	NaCl Regenerate
Z4	54	54	52	7.0
Z7	5.2	5.3	4.9	<1
LTP1	2.0	2.2	2.1	<1

Table 4 - Protein Z4, Z7 and LPT1 Concentrations

The results shown in Table 4 therefore align with the foam stabilisation results of Table 2 and help explain why the beer stabilisation with the CSS does not compromise foam quality.

2.2.2.4 Summary of Efficacy

The trial of the CSS at the Tooheys Brewery, Sydney, has demonstrated that stabilisation of beer by the CSS does improve the physical stability of beer.

Polyphenol adsorption by agarose appears to be selective. Adsorption levels of catechin were the lowest. The adsorption of Procyanidin B_3 was greater. Prodelphinidin B_3 was removed to the greatest degree.

Haze sensitive proteins were adsorbed during stabilisation by the CSS.

The foam stability of the tested beers was maintained. The foam active proteins Z4, Z7 and LTP1 remained largely unaffected.

Therefore, it can be concluded that the CSS is a viable addition to the range of beer stabilisation options currently available to brewers.

2.3 Justification for the use of the additive

Beer is a complex mixture of soluble compounds derived from the malt, hops and fermentation which includes many groups of compounds including two known as polypeptides (proteins) and polyphenols which have both positive and negative effects in beer. Certain peptides play a positive part in beer foam and certain polyphenols resist flavour ageing, but other polypeptides and polyphenols complex with each other and form insoluble haze material which can limit the shelf life of beer.

In order to extend the shelf life of beer many breweries use techniques to reduce the concentration of either polypeptides or polyphenols or both. Several of these techniques are approved in the Joint Food Code of Australia and New Zealand and are widely used in Australasia.

Chill proof enzymes, generally described as papain (Standard 1.3.3-16), have the ability to preferentially cleave the peptide bonds of hydrophobic amino acids. This mechanism reduces the haze forming capability of beer. However, the process is not specific and hydrolysis of foam stabilising proteins also occurs, reducing foam quality.

Tannic Acid products (Standard 1.3.3-3) which are haze protein specific and relatively insoluble have been developed. These improve both physical and flavour stabilities in a single step, but have efficiency disadvantages.

The most popular adsorbent of haze proteins is silicagel, which is available in two forms: hydrogel (SHG), 60 – 70% moisture, and xerogel, <7% moisture. Silicagel interacts with protein proline residues to selectively adsorb haze forming polypeptides at an optimum of pH 4, with foam protein concentrations remaining intact.

There are several options available for the removal of haze forming polyphenols from beer.

Polyvinylpolypyrrolidone (PVPP), (Standard 1.3.3-6), is probably the most common stabilisation aid for the removal of haze active polyphenols used within the brewing industry at this time. Polyvinylpolypyrrolidone selectively binds to haze active polyphenols.

This application is to permit the use of the polysaccharide agarose in a matrix (CSS Adsorber) to absorb both polypeptides and polyphenols. Similar technology has been used in the pharmaceutical industry for many years for protein purification. Its use in the brewing industry is not to capture and purify components but rather reduce the concentration of certain polypeptides and polyphenols in beer to prolong shelf life.

The CSS adsorber is in the form of beads, which are held in a chamber and filtered beer is passed through. A portion of some specific polypeptides and polyphenols is adsorbed on to the surface of the beads, hence reducing their concentration in the resultant beer without affecting the concentration of other polypeptides which have a positive effect on the foaming characterisation of beer.

With time the beads of agarose require regeneration which is achieved by back flushing firstly with a salt (NaCl) solution followed 4% by caustic solution (NaOH).

The power of the CSS absorber is that it achieves a targeted reduction in both polypeptides and polyphenols with a single step. It has both technological and efficiency advantages over traditional processing aids.

Although there is a cost involved in setting up the equipment to use CSS Adsorber, the gain is that processes requiring two steps in current manufacturing of beer could be completed in one step with the CSS Adsorber System.

Results of trials conducted by Lion Nathan are shown in Section 2.2.2 and concluded that CSS Adsorber is a viable addition of the stabilisation options currently available to brewers.

2.4 Proposed Amendments to the Food Standards Code

2.4.1 Proposed Approval of CSS Adsorber in Standard 1.3.3

The suggested amendment to Standard 1.3.3 is to Clause 14 – *Permitted Processing Aids with Miscellaneous Functions*.

***Standard 1.3.3 – Processing Aids*, in the Table to Clause 14:**

Include CSS Adsorber as a processing aid in the manufacture of beer to remove selected, unwanted proteins and polyphenols by inserting in the Table to Clause 14 -

Substance	Function	Maximum Permitted level (mg/kg)
CSS Adsorber	Adsorbent to remove specific proteins and polyphenols during beer manufacture.	GMP

Alternate clauses in Standard 1.3.3 were considered and dismissed as discussed below. These clauses were:

Clause 6 - *Permitted decolourants, clarifying, filtration and adsorbent agents*, which states:

> "The processing aids listed in the Table to this clause may be used as decolourants, clarifying, filtration and adsorbent agents in the course of manufacture of any food provided the final food contains no more than the corresponding maximum permitted level specified in the Table."

The nature of this clause would need to be altered to limit the use on any of the listed substances to a particular use in food.

Clause 8 - *Permitted ion exchange resins*, which states:

> "The processing aids listed in the Table to this clause may be used as an ion exchange resin in the course of manufacture of any food provided the final food contains no more than the corresponding maximum permitted level specified in the Table."

Although CSS Adsorber is an ion exchange resin and there is an exchange of OH^- ions, this is not the purpose of the processing aid in beer production. The purpose of the use of CSS Adsorber is to remove specific, unwanted proteins and polyphenols. The exchange of OH^- ions is of no importance as it does not measurably influence the pH of the beer.

Again, the nature of this clause would need to be altered to limit the use on any of the listed substances to a particular use in food.

2.4.2 Proposed Specification for CSS Adsorber in Standard 1.3.4

The approved regenerated cellulose-based resin that is essentially the same as CSS Adsorber, except in the base sugar in of the polymer, has a specification included in the Schedule to Standard 1.3.4. It is likely that CSS Adsorber will also require an individual specification to be included in the Schedule. It is suggested that the specification be base on the existing specification for the regenerated cellulose based equivalent resin.

***Standard 1.3.4 – Identity and Purity*, in the Schedule**

Include a specification for CSS Adsorber –

Specification for CSS Adsorber:

(a) Agarose, cross-linked and alkylated with epichlorohydrin and propylene oxide, then derivatised with tertiary amine groups whereby the amount of epichlorohydrin plus propylene oxide does not exceed 250% by weight of the starting quantity of agarose;

(b) The resins are limited to use in aqueous process streams for the removal of proteins and polyphenols from beer. The pH range for the resins shall be no less than 2 and no more than 4, and the temperatures of water and food passing through the resin bed shall not exceed 2°C.

(c) When subjected to the extraction regime listed in the CFR Title 21 part 173.25(c)(4), but using dilute hydrochloric acid at pH 2 in place of 5% acetic acid, the ion exchange resins shall result in no more than 25 ppm of organic extractives.

2.4.3 Compliance with Specification

Appendix 3 – Compliance with Specification shows a certificate of analysis by ISEGA, independent laboratory in Germany confirm compliance with paragraph (c) of the above specification.

2.5 The need for CSS Adsorber as a processing aid in beer

This joint application is made by the manufacturer, GE Health Care, of the resin and an intended user of the resin, Lion Nathan. Lion Nathan has already invested time and money to evaluate the process using CSS Adsorber. They have concluded that its use will be of benefit to their products and will invest further resources to implement the process once approval is gained. Other brewers will also be in a position to use this system once approval is gained.

2.6 Nutritional implications

The use of CSS Adsorber as a processing aid in the manufacture of beer has no nutritional implications.

The amount of protein in beer is relatively low and only a portion of these are selectively removed. Beer is usually consumed for enjoyment and refreshment; it is not consumed as a source of proteins. So the removal of certain proteins from beer that affect the shelf life has no nutritional implications.

Polyphenols are not usually recognised as nutrients, so the removal of certain polyphenols has no nutritional implications

2.7 Dietary implications

2.7.1 Intake of Beer

The advantage of the use of CSS Adsorber in the manufacture of beer relates to the shelf-life of the beer by preventing the formation of haze during storage. It has no flavour or other sensory implications therefore it would not be expected to influence the amount of beer consumed.

Information on the potential for consumption of impurities from CSS Adsorber appears in Section 6.3.4

2.8 Advantage to the consumer

The advantage to the consumer is the same as it is for the manufacturer. The reduction of haze-forming substances will delay the onset of haze and increase the shelf-life of packaged beer, both in trade and when stored by the consumer. Whether this translates to an increase in the Best Before period of beer will be up to individual manufacturers using CSS Adsorber.

The formation of haze is usually associated with an off- flavour in the beer. Whether the two changes are linked or not is still the subject of research by the brewing industry.

PART 3 REGULATORY/LEGISLATIVE IMPLICATIONS

3.1 International standards

Codex standards do not regulate processing aids.

3.2 International legislation

CSS Adsorber is approved in:

- the USA – self-assessed as GRAS confirmed by FDA
- Germany – approved by the VLB (Berlin Brewing Institute)
- Russia – approved by the State Sanitary-Epidemiological Agency

Appendix 4 includes a copy of the confirmation of GRAS by the FDA and copies of certificates and translations of the German and Russian approvals.

3.3 Regulatory Impact Statement

3.3.1 Cost Implications

While the primary motivation for use of the technology is to achieve the improved physical stability of the beer, there are always two additional advantages for the brewer: a simplification by replacing two processes- silica gel/ PVPP- with a single process- CSS absorber, and an annual cost advantage between $50,000 and $500,000 depending on the equipment and size of the brewery.

3.3.2 Market Implications

Improved shelf-life due to a delayed onset of haze may improve competitiveness of treated beer in the market place.

3.3.3 Profit Implications

Long-term profit implications are small. Once the cost of installing the system has been recovered, the cost advantage is quite small and a small increase in profit is likely. The primary motivation for use of the technology is to achieve the improved physical stability of the beer

3.3.4 Price Implication

While there are cost savings for the brewer, they are not sufficient to have a significant effect on the price a drinker pays. For a best case situation a saving of less than 0.1 cents per bottle of beer might be achieved.

3.3.5 Trade Implications

CSS Adsorber is approved in the USA and Germany, export of beer processed with CSS Adsorber would be possible to these markets and probably to other European markets.

3.3.6 Employment Implications

There are likely to be no employment implications.

PART 4 ANALYTICAL PROCEDURES

Analytical Procedures used to determine impurities in CSS Adsorber appear in Appendix 1 – Analytical Reports 1, 2 & 3. Analytical procedures to determine the stability CSS Adsorber appear in Appendix 5 – Chemical Stability of CSS Adsorber.

PART 5 DETAILS OF REASONING

This application for CSS Adsorber, so called because of the name of the system in which it is used – Combined Stabilisation System, has been lodged jointly by Lion Nathan – Brewer and GE Health Care – Manufacturer of CSS Adsorber. CSS adsorber has the potential to replace two existing steps in the filtration process of beer by selectively and partially removing peptides (proteins) and polyphenols which react to form haze over time thus limiting the shelf life of the beer. The use of CSS Adsorber has been demonstrated to delay the onset of haze. Comprehensive safety analysis has been provided by GE Health Care.

CSS Adsorber has the Trade Name *Q Sepharose® Big Beads* or *Q Sepharose® BB.* It can be described as: Agarose, cross-linked and alkylated with epichlorohydrin and propylene oxide, then derivatised with tertiary amine groups whereby the amount of epichlorohydrin plus propylene oxide does not exceed 250% by weight of the starting quantity of agarose.

A similar resin is already approved in the Food Standards Code: Regenerated cellulose, cross-linked and alkylated with epichlorohydrin and propylene oxide, then derivatised with tertiary amine groups whereby the amount of epichlorohydrin plus propylene oxide does not exceed 250% by weight of the starting quantity of regenerated cellulose.

The only difference is the base sugar of the polymer. In the case of cellulose it is glucose and in the case of agarose polymer it is agarose. Agarose is the sugar base of the polymer agar, a generally approved food additive. It could therefore be concluded that CSS Adsorber is only a minor amendment to the Food Standards Code.

CSS Adsorber has the CAS Registry Number: 846053-13-2 and the CAS Registry Name: Agarose, polymer with (chloromethyl)oxirane, 2-hydroxy-3-(2-hydroxy-3-(trimethylammonio)propoxy)propyl ethers, sulfates salts.

The efficacy of CSS Adsorber has been evaluated by Lion Nathan and it has shown to successfully reduce haze forming compound and improve the shelf life of beer by delaying the onset of haze formation. Based on the efficacy of CSS Adsorber and the cost and potential savings, Lion Nathan has decided to invest in the process, therefore making this application with the resin manufacturer.

The proposed amendment to the Food Standards Code is to *Table to Clause 14 – Permitted Processing Aids with Miscellaneous Functions,* and limit its use to beer only. Although other uses for CSS Adsorber in aqueous systems is possible the applicants to do have evidence to support this and therefore consider the application should be limited to beer only and the specification should state use of CSS Adsorber at beer processing temperatures of -0.5°C ± 1°C and a pH of 3 - 4.

The use of CSS Adsorber has no nutritional or dietary implications. It provides an increased shelf-life of stored beer both in trade and in consumers' homes and thereby provides a benefit to consumers.

CSS Adsorber is currently approved for use in Beer in the USA, Germany and Russia.

Details of all reagents and the manufacturing process have been provided in the application. Comprehensive safety analysis conducted by GE Healthcare shows that CSS Adsorber does not impart any detectable contaminants to beer under normal processing conditions. It also

shows that no detectable contaminants under abuse conditions. However, an analysis of the potential for contaminants to be present at or just under the level of detection has revealed that the potential contaminants constitute an extremely low risk to consumers of beer.

PART 6 MANUFACTURING AND PUBLIC HEALTH

6.1 Manufacture

The manufacturing process uses an aqueous solution of agarose dispersed in toluene to give droplets of a certain size. After cooling and washing, the gel is cross-linked with epichlorohydrin and sodium hydroxide in the presence of sodium sulfate. The product is then washed and wet-seived, after which it is reacted with allylglycidyl ether in alkali. The product is washed repeatedly with 95% ethanol and with distilled water. This intermediate may be stored in 20% ethanol. The intermediate is reacted with bromine forming a bromohydrin followed by reaction with trimethylamine in alkali to produce the Q moieties. The product is washed repeatedly with distilled water, classified by wet-sieving and stored in 20% ethanol.

6.1.1 Reagents

The reagents used to prepare CSS adsorber are listed in Table 5.

Chemical name	CAS reg no	Function
Agarose	9012-36-6	Resin matrix
Toluene	108-88-3	Solvent
Ethylcellulose	9004-57-3	Emulsifier
Polyoxyethylene nonylphenyl phosphate ester sodium salt	68954-84-7	Emulsifier
Epichlorohydrin	106-89-8	Crosslinker
Sodium hydroxide, 50 %	1310-73-2	Reagent
Sodium sulphate	7757-82-6	Electrolyte modifier
Sodium borohydride	16940-66-2	Reducing agent
Acetic acid, 60 %	64-19-7	pH modifier
Allyl glycidyl ether	106-92-3	Reagent
Sodium acetate, trihydrate	6131-90-4	Buffering agent
Bromine	7726-95-6	Reagent
Sodium formate	141-53-7	Electrolyte modifier
Sodium bicarbonate	144-55-8	pH modifier
Trimethylamine, hydrochloride	593-81-7	Reagent
Ethanol	64-17-5	Solvent for washing and storage

Table 5 - Starting materials / reagents.

6.1.2 Manufacturing Process

The manufacturing process can be summarised as follows. An aqueous solution of agarose is dispersed in toluene to give droplets of 100-300 µm. After cooling and washing, the gel is cross-linked with epichlorohydrin and sodium hydroxide 50 % in the presence of sodium sulfate. The product is then washed and wet-sieved, where after it is reacted with allyl glycidyl ether in alkali.

The product is washed repeatedly with 95 % ethanol and with distilled water. The intermediate allyl sepharose may be stored in 20 % ethanol.

Finally the allyl sepharose is reacted with bromine forming a bromohydrin followed by reaction with trimethylamine in alkali. The product is washed repeatedly with distilled water, wet-sieved and stored in 20 % ethanol.
The flow sheet for the process and the manufacturing information is presented below.

The following schematic diagrams, presented in Figures 14-18, illustrate the reactions involved in the manufacture. It is to be noted that the reaction of the polysaccharide with epichlorohydrin is probably more complex than the reactions indicate. However since the full chemistry has not been elucidated a more detailed structure cannot be attempted.

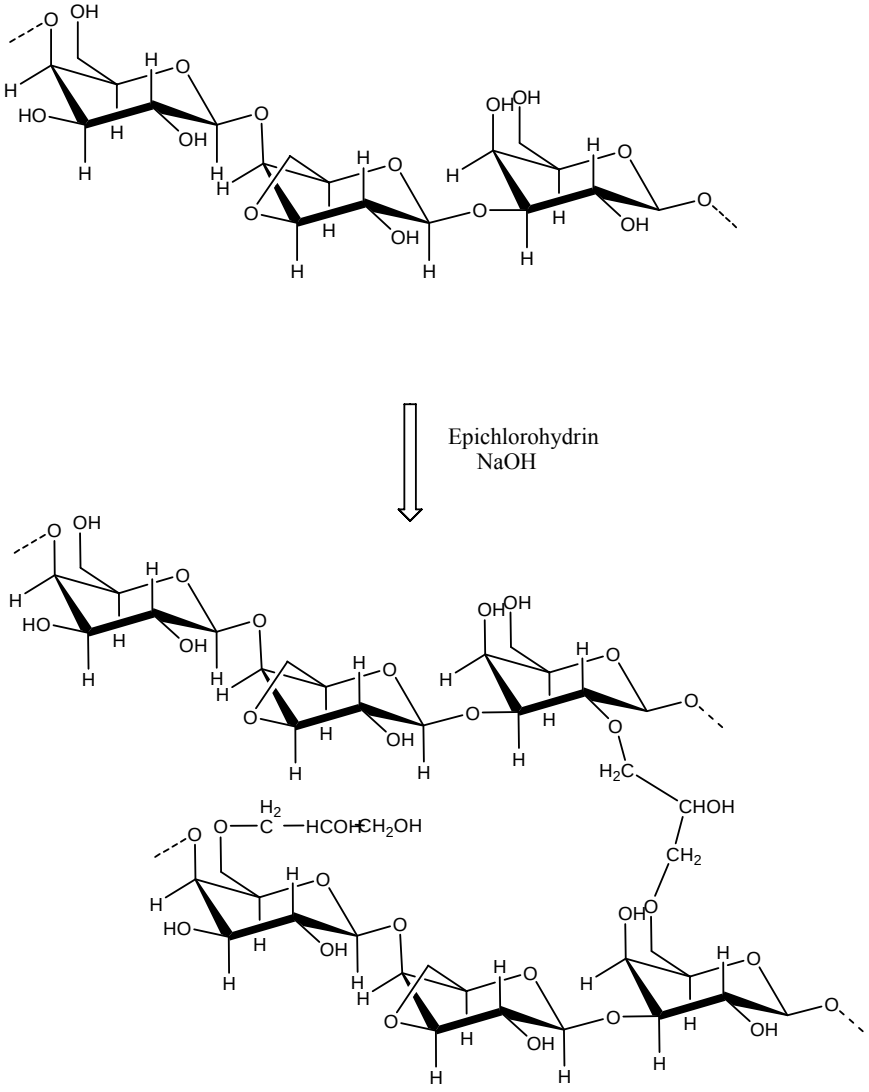


Figure 14 - Structural representation of a fragment of CSS Adsorber.

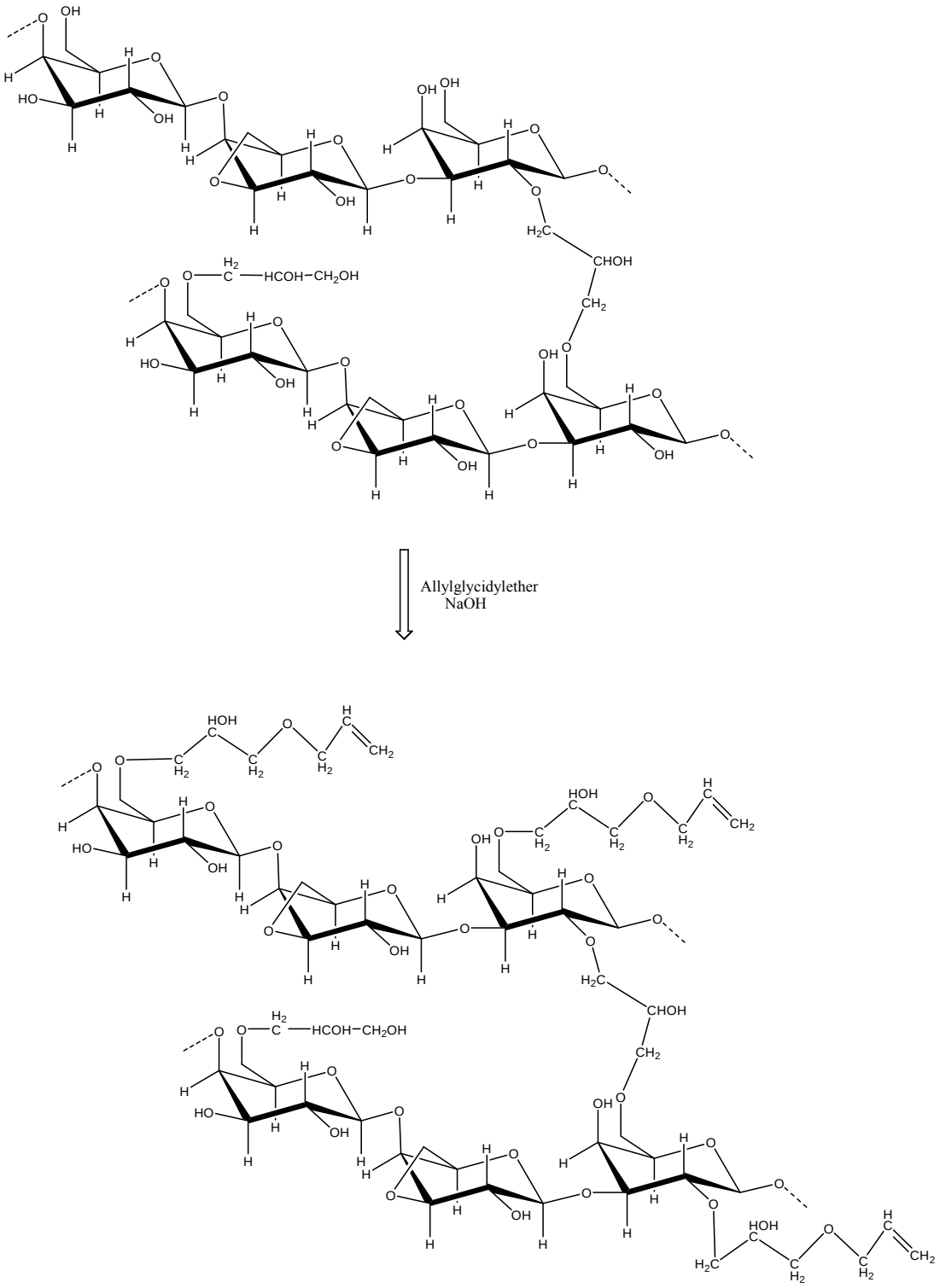


Figure 15 - Reaction between epichlorohydrin cross-linked agarose and allylglycidyl ether.

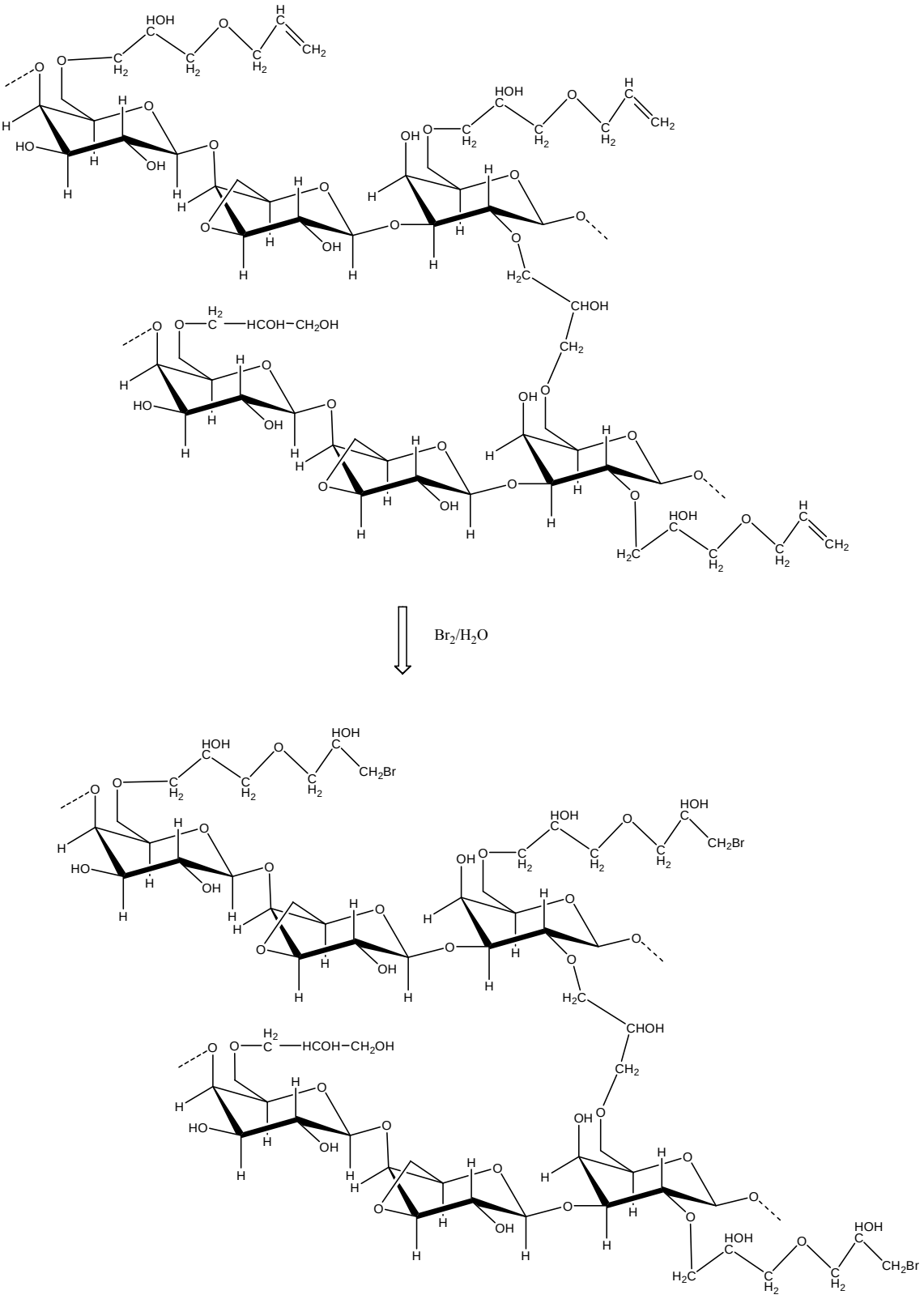


Figure 16 - Reaction between allylated epichlorohydrin cross-linked agarose and bromine.

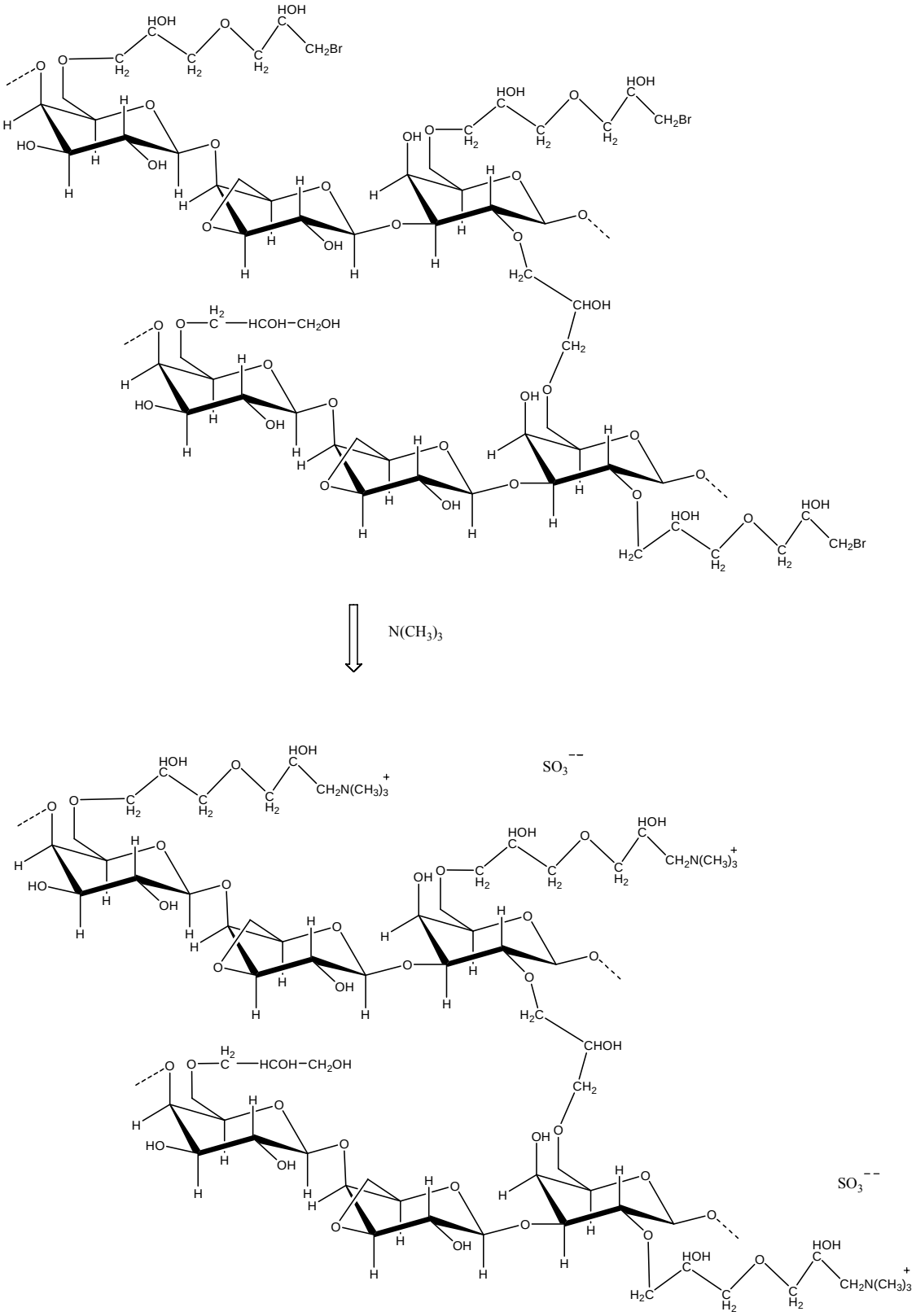
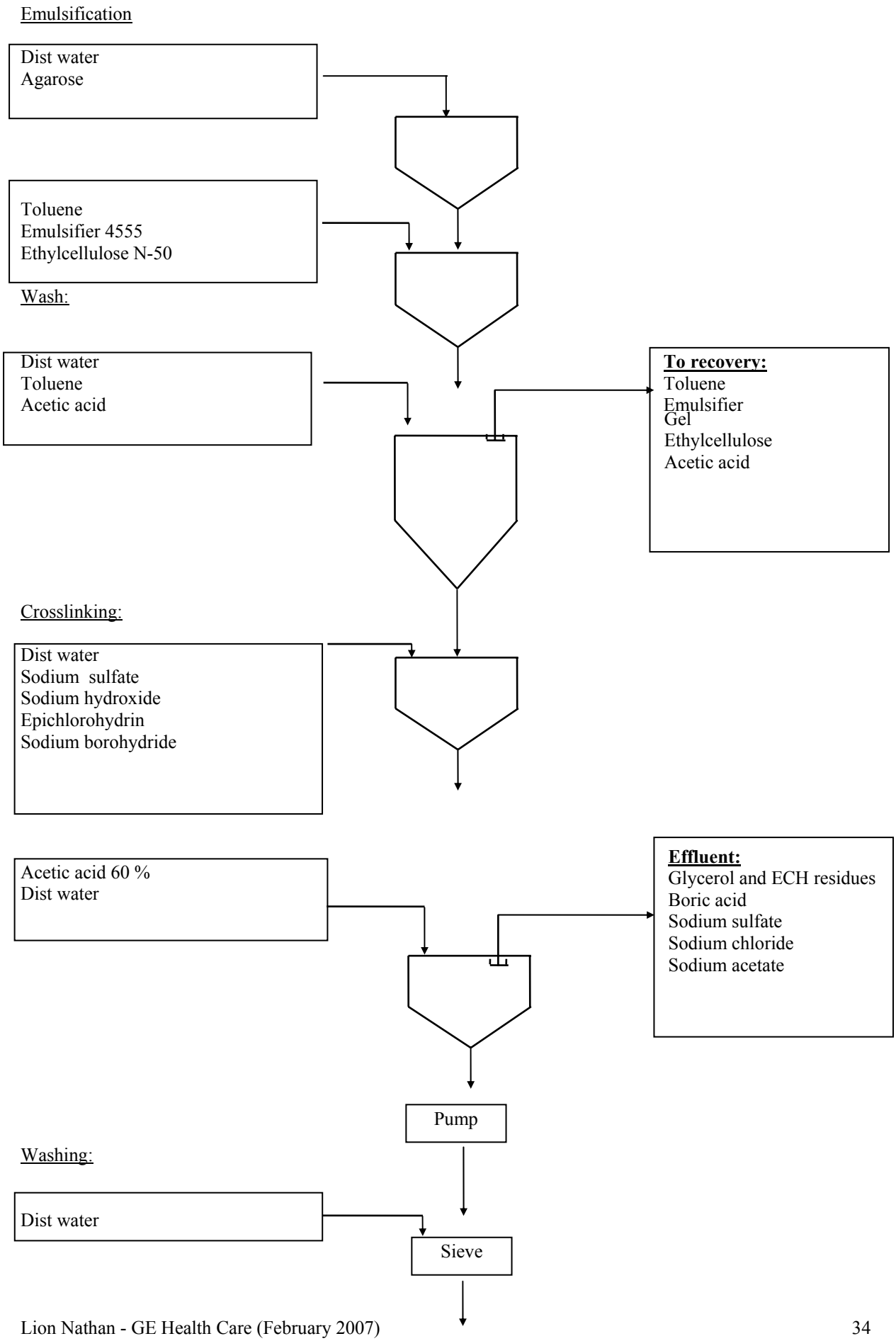
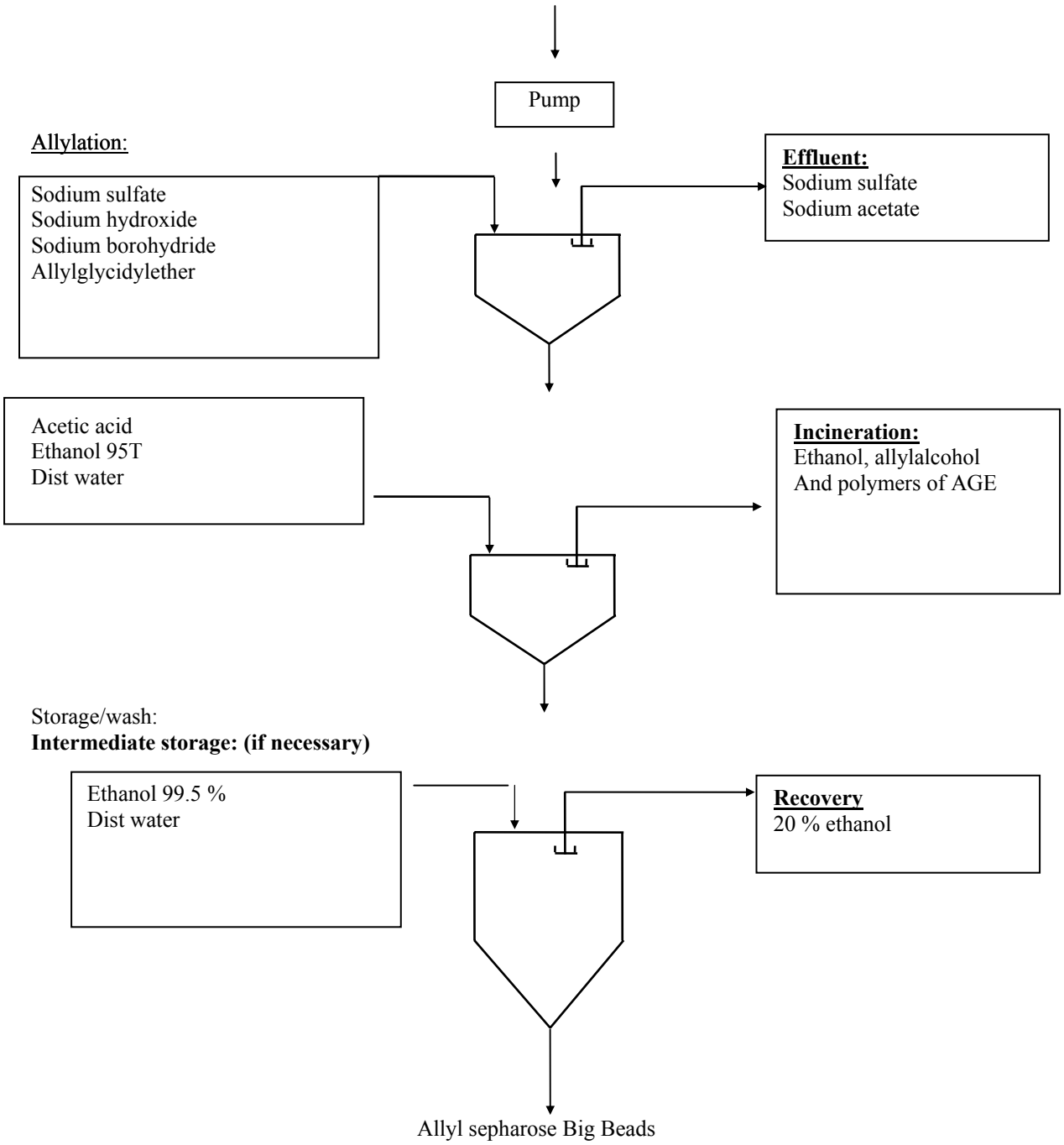


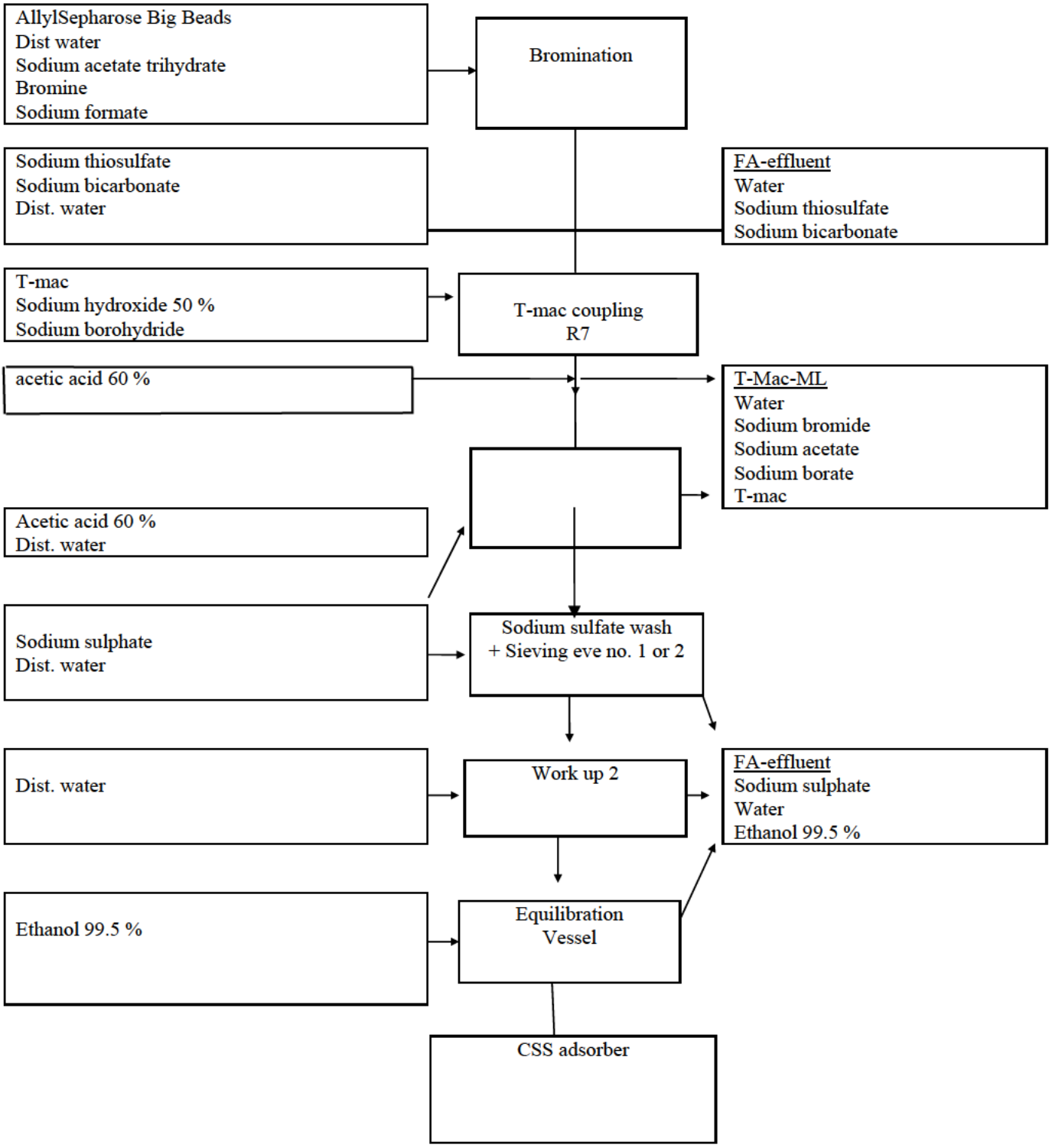
Figure 17 - Reaction between brominated allylated epichlorohydrin cross-linked agarose and trimethylamine.

6.1.3 Flow sheet for manufacture of Allyl Sepharose Big Beads




Pump
Allylation:
Sodium sulfate
Sodium hydroxide
Sodium borohydride
Allylglycidylether
Effluent:
Sodium sulfate
Sodium acetate
Acetic acid
Ethanol 95T
Dist water
Incineration:
Ethanol, allylalcohol
And polymers of AGE
Storage/wash:
Intermediate storage: (if necessary)
Ethanol 99.5 %
Dist water
Recovery
20 % ethanol
Allyl sepharose Big Beads

Flow sheet for manufacture of CSS Adsorber



6.1.4 Impurities from Manufacture

6.1.4.1 Identification of potential impurities

Based on the information on the manufacturing process and the possible side reactions that might occur, a list of impurities potentially present in the CSS Adsorber has been prepared. It is shown in Table 1. The safety status of each potential impurity is mentioned in Table 6 - Potential Impurities. They are further discussed in the section 6.3.

6.1.4.1.1 Soluble agarose fragments

Chemically modified agarose fragments may be slowly leached out of the resin on storage particularly when the resin is exposed to acid. No studies have been made on the molecular weight of these impurities but as the agarose is cross-linked, it is likely that they are high molecular weight.

6.1.4.1.2 Ethyl cellulose

Ethyl cellulose is used as an emulsifier in the early stages of the process and is a high molecular weight soluble polymer. It should be removed during the washings.

6.1.4.1.3 Polyoxyethylene nonylphenyl phosphate ester, sodium salt

This is also used as an emulsifier in the early stages of the process. It is a mixture of polymers and is water soluble and should be removed during the washings.

6.1.4.1.4 Epichlorohydrin and hydrolysis products

Epichlorohydrin is used to cross-link agarose in the initial stages of the process. It hydrolyses under alkaline conditions to form glycerol via the intermediate 2,3-epoxy-1-propanol (glycidol). 3-Chloro-1,2-propandiol may also be an intermediate in the hydrolysis. It is formed from epichlorohydrin under neutral or acidic conditions. All these compounds are believed to be removed during the washings.

6.1.4.1.5 Allyl glycidyl ether and hydrolysis products

Allyl glycidyl ether reacts with agarose via the epoxy-group to give an allyl ether. Unreacted allyl glycidyl ether may be hydrolysed by alkali to form the glycerol ether. Both compounds are expected to be removed during washing.

6.1.4.1.6 Sodium acetate

Acetic acid is used to neutralise the reaction mixtures and will form sodium acetate.

6.1.4.1.7 Sodium sulphate

Sodium sulphate is added in the first operation to modify the ionic balance during cross-linking. It is also used to exchange the counter-ions on CSS Adsorber in the final operations.

6.1.4.1.8 Sodium chloride

Sodium chloride is formed from epichlorohydrin during the cross-linking operation. It has not been analysed.

6.1.4.1.9 Sodium borate

Sodium borate is formed by hydrolysis of sodium borohydride added as a reducing agent to prevent discoloration of the resin. The borohydride is destroyed rapidly during the neutralization with acid of the reaction mixture.

6.1.4.1.10 Toluene

Toluene is used as a solvent for the emulsification of the agarose gel. Extensive washing of the gel removes final residues.

6.1.4.1.11 Bromine and bromine derivatives

Bromine is added to convert the allyl groups on the resin to bromohydrin moieties in preparation for the reaction with trimethylamine. Bromine is only slightly soluble in water and exists as a complex equilibrium. The active agent is the Br-Br species. When allowed to stand in alkaline solution, bromate and bromide ions are formed. Bromine reacts with aromatics, unsaturated aliphatic compounds, acids, amines and is generally an oxidising reagent. Some potential impurities from the bromination process are listed below.

Bromoacetic acid (sodium salt) could be formed by bromination of sodium acetate. This reaction generally requires a catalyst and is normally performed in non-aqueous media. However, even under the present conditions some bromination may occur. The bromoacetate may hydrolyse under the basic conditions of the amination operations to form sodium glycollate. It may also undergo amination to form betaine.

Sodium bromide may arise by several pathways. The main source will be the conversion of bromine to bromide under alkaline conditions.

Sodium bromate may form by conversion of elemental bromine under alkaline conditions.

All bromine derivatives formed during bromination are expected to be removed during subsequent washings. However, a certain amount of bromine remains attached to the resin, part of which is expected to leach out over the resin lifetime as bromide ions.

6.1.4.1.12 Sodium formate

Sodium formate is added as a buffer salt during the bromination of the allyl substituents.

6.1.4.1.13 Sodium bicarbonate

Sodium bicarbonate is used to modify the pH of the solution. It decomposes in dilute acetic acid to give sodium acetate and carbon dioxide.

6.1.4.1.14 Trimethylamine hydrochloride

Trimethylamine reacts with the bromohydrin intermediates to give the quaternary ammonium product. Residual trimethylamine would be present as the hydrochloride (pKa 9.3).

6.1.4.1.15 Ethanol

Ethanol is used as a solvent for washing and for the final storage formulation.

6.1.4.2 Determination of maximum levels of potential impurities

The maximum residual levels of the impurities that could potentially remain in the CSS Adsorber after the manufacturing process were evaluated by extraction of the CSS Adsorber followed by analyses of the extract. Before extraction, the CSS Adsorber was subjected to the recommended pre-use wash cycle as described in the instructions supplied with CSS Adsorber (5 column volumes water, 5 column volumes NaCl, overnight storage in NaCl and 5 column volumes water, see section 0).

Two types of quantification were performed:

(1) Worst-case calculation for individual compounds from elementary analysis; and
(2) Specific detection of certain substances.

The maximum impurity levels are shown in Table 6 - Potential Impurities. The basis on which all concentrations are reported is the CSS Adsorber as it is used, i.e. the wet resin containing 12±1% CSS Adsorber.

The details of the analyses are provided in the following analytical reports (see Appendix 2 – Analytical Reports):

(a) Analytical Report 1, Sample preparation, describes the procedures employed for preparing all types of samples as well as the extraction techniques used.

(b) Analytical Report 2, Elemental analysis, presents the quantification of different elements in extracts as well as the dried resin itself. It also reports the calculations made to convert the levels of different elements into worst-case estimations for individual compounds.

(c) Analytical Report 3, Specific analysis, describes the quantification of 5 specific substances in the extracts.

6.1.4.3 Extraction techniques

This work is reported in Appendix 1 – Analytical Report 1 - Sample preparation. The extractions were carried out using water as solvent and employing two different relatively mild techniques. When considering the choice of solvent and extraction techniques, it is important to keep in mind the particular nature of the agarose polymer of which the CSS Adsorber is composed. Agarose is a highly hydrophilic polymer. Furthermore it is a macroporous hydrogel, i.e. a highly swollen network in the rubbery state, with a very high internal mobility. The molecular diffusivities inside the network are of the same order of magnitude as in water.

The material thus has very different properties compared to a classic synthetic polymer in its glassy or crystalline state.

Experience with the resin has, over the years, also shown that contact time sometimes can be important factor when extracting substances. Therefore an extraction protocol involving long time was included as an alternative to the classic accelerated extraction method.

The two extraction protocols employed were:

(1) Pressurised fluid extraction (PFE) at 10 MPa and 40°C for 5 min; and
(2) Extraction at atmospheric pressure and 20 - 40°C for 160 h.

6.1.5 Impurity levels estimated from elemental analysis

This work is reported in Appendix 2 – Analytical Report 2 - Elemental analysis. The extracts were analysed for total amount of carbon (TOC analysis), nitrogen and bromine. Carbon, hydrogen, oxygen, boron, phosphorus and sulphur were also quantified directly in the resin, i.e. by direct combustion of dried resin samples.

The values for soluble agarose fragments, ethyl cellulose, glycerol, sodium acetate, sodium formate, sodium bicarbonate, ethanol, toluene trimethylamine, sodium glycollate and betaine were derived by assuming in turn that all the carbon detected in extracts of the resin originates from the substance in question and adjusting the level for the carbon content of the substance.

The values for sodium bromide, sodium bromate, bromine and bromoacetic acid were determined in the same way using the levels of bromine detected in the resin extracts.

The values for sodium sulphate, sodium borate and nonyl phenol ethoxylate phosphate ester come from elemental analysis of the dried resin.

6.1.6 Analysis of specific compounds

This work is presented in Appendix 2 (c) – Analytical Report 3 - Specific analysis. The values for allyl glycerol ether, 2,3-epoxy-1-propanol, allyl glycidyl ether, epichlorohydrin and 3-chloro-1,2-propandiol all come from specific detection work in the extract using different concentration techniques such as evaporation and solid phase microextraction (SPME) followed by gas chromatography.

IMPORTANT NOTE: None of the substances were detected, and the levels reported in Table 1 **and used in the following are the limits of detection for each analyte**.

Chemical name	CAS Reg no	Maximum residual [mg/kg in wet sedimented gel]	Safety status
Soluble agarose fragments (agar)	9012-36-6	7.1	Additive 406
Ethylcellulose	9004-57-3	5.5	JECFA ADI = unspecified
Polyoxyethylene nonylphenyl phosphate ester sodium salt	68954-84-7	10.9	potential toxicant
Glycerol	56-81-5	7.7	Additive 422
Sodium acetate	6131-90-4	10.2	Additive 262
Sodium formate	141-53-7	17.0	Permitted processing aid 1.3.3-18
Sodium sulphate	7757-82-6	14.7	Additive 514
Sodium chloride	7647-14-5	Trace amounts	Food
Sodium bicarbonate	144-55-8	21.0	Additive 500
Ethanol	64-17-5	5.8	Generally permitted processing aid
Toluene	108-88-3	3.3	Permitted processing aid 1.3.3-13
Sodium bromide	7647-15-6	0.76	Inorganic bromide is a permitted Agricultural Chemical
Trimethylamine	75-50-3	8.7	Permitted constituent of resins permitted for water for food use 1.3.3-11
Sodium glycollate	2836-32-0	12.2	potential toxicant
Betaine	07-43-7	5.8	potential toxicant
Bromine	7726-95-6	0.40	potential toxicant
Sodium bromate	7789-38-0	0.64	Permitted processing aid limited to 0.1 mg/kg to control germination in malting 1.3.3-14
Sodium borate	1303-96-4	25.2	Permitted constituent in package water to 30 mg/L 2.6.2-2(2)
Allyl glycerol ether	123-34-2	0.07	potential toxicant
Bromoacetic acid	79-08-3	0.70	potential toxicant
2,3-epoxy-1-propanol	556-52-5	0.5	potential toxicant
Allyl glycidyl ether	106-92-3	0.05	potential toxicant
Epichlorohydrin	106-89-8	0.05	Permitted constituent in other resins 1.3.3-8
3-chloro-1,2-propandiol	96-24-2	0.07	potential toxicant

Table 6 - Potential Impurities

6.2 Stability

6.2.1 General

Agarose gels show good stability at 45ºC from pH 4.5 to 9.0. However, prolonged treatment at lower pH results in degradation because of the lability of the 3,6-anhydro-galactosyl units. Thus a good yield of the disaccharide, agarobiose, is obtained when agarose is heated in 0.05% hydrochloric acid for 90 min[17]. In CSS Adsorber agarose has been stabilised by cross-linking and other substituents and CSS Adsorber exhibits improved stability. For normal production operations the gel has a working range of -2-40ºC in the pH interval 2-12.

Special attention has been directed to safety and migration levels working range and life-time of the CSS Adsorber have been limited to meet migration requirements. The normal lifetime of CSS Adsorber is at least 2 years. The resin reaches the end of its useful life not because it has degraded but because it over time loses its capacity to bind the target substances due to adsorption of different extraneous compounds from the food starting material. The CSS Adsorber contains residual bromo-compounds that are expected to partially leach out over the life-time of the resin in the form of bromide ions.

Some hydrolysis may occur during the cleaning / sanitation procedure performed at regular intervals between the between production cycles, as the cleaning takes place at high pH and temperature. However, the cleaning step is always followed by rinsing and equilibration steps before the next production cycle, which means that most of the degradation products would be expected to be washed away before the next cycle begins.

The functional groups on CSS Adsorber consist of quaternary ammonium moieties. These groups are known from the literature to undergo many reactions depending on the conditions[18]. The free base may decompose at elevated temperatures and high pH with the release of a tertiary amine (Hoffman degradation). CSS Adsorber would release trimethylamine. However, the conditions stipulated for the use of CSS Adsorber would largely preclude this type of reaction.

6.2.2 Stability Tests

Cross-linked agarose resins are stable except at very high and very low pH where they undergo hydrolysis. For normal production operations the resin has a working range of -2-40°C in the pH interval 2-12. A series of tests were performed under different conditions of temperature and pH to represent use at extreme pH to test stability.

The resin also contains residual bromo-compounds that are expected to partially leach out during hydolysis as bromide ions. This was experimentally verified and confirmed.

Resin samples were subjected to pH and temperature conditions resembling those used for cleaning/sanitation for a period of 160-170 hr, corresponding to 160-170 cleaning cycles. Since the cleaning cycle is performed every 5 cycles, and the lifetime is 750-1500 cycles, the

[17] *Preparation of agar oligosaccharides by acid hydrolysis and determination of their antioxidative effect*, Zhongguo Haiyang Yaowu, 21(1), 19-22, 2002. (Abstract only)
18 J.Goerdeler in 'Methoden der Organischen Chemie (Houben-Weyl)' Georg Thieme Verlag, Stuttgart, 1958, 631-640.

resin is expected to be subjected to 150-300 cleaning processes over its life-time. If the extent of hydrolysis is taken to be proportional to the exposure time, the equivalent of 300 cleaning processes would under the most severe conditions produce about 16 mg/kg soluble agarose fragments and 780 mg/kg bromide.

The resin is also subjected to pH 3 and 4 at 20°C and 40°C as well as pH 12 at 40°C, again for 160-170 hours. The highest values for an individual batch showed that at pH 3 and 4 less than 2.8 mg/kg agarose and 1.6 mg/kg bromide was released. The functional group loss was below 0.1%. At pH 12 up to 103 mg/kg of agarose fragments and 312 mg/kg of bromide was formed. The functional group loss was 0.21%.

The long-term stability of CSS Adsorber was investigated by analysing the agarose concentration in a number of samples that had been stored in buffer solutions under pH and temperature conditions similar to those encountered during normal use for periods of time up to five years. No reduction of agarose concentration could be detected for this time period, thus indicating that the polymer is stable to at least 2% by weight (the error margin of the test method) for at least five years. The bromine concentration in the same samples were also shown to be constant over time, indicating that at neutral pH very limited bromide release can be expected.

In order to examine the stability of the CSS Adsorber over time and under different conditions of temperature and pH, a series of tests were performed. The tests are presented in Appendix 4 – Chemical Stability of CSS Adsorber which provides details of the methodology and results for stability tests. The tests employed either the change in agarose concentration in the resin (dry weight) or the amount of extractives formed (total organic carbon (TOC) analysis) as indicators of stability. In some cases the change in functional group concentration was also evaluated through analysis of the nitrogen level in the solutions. Furthermore, the solutions were analysed for the amounts of bromide and total bromine released.

The precision of the dry weight test is about ±1 weight % whereas the TOC analysis has a detection limit of about 1 mg/kg and a quantitation limit of 3 mg/kg in the measured solutions. The nitrogen analysis was sensitive down to about 0.2 mg/kg in the test solutions. The bromine determination had a sensitivity of about 0.1 mg/kg whereas the detection limit in the bromide analysis was 1 mg/kg for the examined sample solutions.

It should be noted that in the following, all concentrations are reported against the basis of the wet resin containing 12±1% CSS Adsorber.

6.2.2.1 Fate of residual bromine in CSS Adsorber

Under the intended conditions of use of the CSS Adsorber, the expected form of bromine release would be bromide ions (see discussions in Section 6.1 - Manufacture and Section and subsection 6.1.5 - Impurities from manufacture). However, work was nevertheless carried out to experimentally verify the type of bromine compounds released from CSS Adsorber under different storage conditions.

Appendix 4 – Chemical Stability of CSS Adsorber presents data on bromine migration measured as amount of bromide ions as well as total bromine (elemental analysis). The results show that at the lower end of the working conditions typical of beer, i.e. pH 3-4, the levels of released bromine are up to 0.7 mg/kg and the bromide values are all below the

detection limit of 1.6 mg/kg. Theoretically this means that there could be up to 0.7 mg/kg of other (non-bromide) bromo-compounds released from the resin.

At the high end of the working conditions, i.e. pH 12, amounts of up to 307 mg/kg total bromine are released. However, the amount of bromide detected is always equal to or higher than the total bromine amount. Judging from the precision of the two analyses at the 100 mg/kg level, i.e. about 1 mg/kg, the worst-case assumption for the situation where total bromine equals detected bromide would be about 1 mg/kg of other (non-bromide) bromo-compounds. In some non-beer products the CSS Adsorber is used at the high end of the working conditions this is outside the requirements of this application.

At pH 13 and 14, i.e. under cleaning / sanitation conditions, the results directly from the cleaning solutions in some cases show total bromine levels of up to 3400 mg/kg. The detected bromide levels are at the most 160 mg/kg below the values for total bromine. However, measurements on the CSS Adsorber after subsequent rinsing and equilibration in all cases yield bromide values equal to or higher than the measured levels of total bromine. The bromide level also drops to 78 mg/kg or less.

From the above, it can be concluded that the maximum theoretical amount of non-bromide bromo-compounds released from the CSS Adsorber and of concern from a food safety perspective would be about 0.7 mg/kg. This amount would translate into an EDI of about 0.003 μg/kg in the daily diet which thus is more than 100-fold below the level of 0.5 μg/kg where toxicity testing is recommended by US FDA Guidance April 2002. Against this background, it is believed that the potential presence of non-bromide bromo-compounds released from the CSS Adsorber when used for beer stabilisation can safely be disregarded and the release of residual bromine taken to be essentially as bromide ions.

6.2.2.2 Long time storage tests

The long-term stability of the CSS Adsorber was investigated by analysing the agarose and residual bromine concentrations in a number of samples that had been stored in buffer solutions under pH and temperature conditions similar to those encountered during normal use for periods of time up to five years. The results showed that no reduction in agarose concentration with sample age could be detected, thus indicating that the polymer is stable to at least 2 weight% or so over several years. The bromine concentration in the CSS Adsorber was also shown to be constant over time, indicating that at neutral pH very limited bromide release can be expected even over very long times (up to 8 years).

6.2.2.3 Simulated cleaning / sanitation

The sensitivity of the CSS Adsorber to hydrolysis during cleaning / sanitation was investigated by subjecting samples to pH and temperature conditions resembling those used for cleaning for a period of 160-170 h. The amount of extractives formed was determined using TOC (see Analytical report 4, Table 3). The results show that the maximum quantity of agarose fragments formed (i.e. the highest values obtained for an individual batch) was164 mg/kg at pH 13.3 and 60°C. The highest amount of bromide released was 3400 mg/kg at pH 13.7 and 40°C. The functional group loss, examined by measurement of nitrogen release, was in all cases less than 1%.

Normal contact time during cleaning is less than 1 h, which means that the experiments simulated a total of more than 160 cleaning processes. Since cleaning is performed every 5 cycles and the resin lifetime is 750-1500 cycles, the CSS Adsorber is expected to be subjected to 150-300 cleaning processes over its life-time. If the extent of hydrolysis is taken to be proportional to the exposure time, the equivalent of 300 cleaning processes, i.e. 300 h exposure, would under the most severe conditions produce about 160 mg/kg soluble agarose fragments roughly equal to 0.6% agarose loss. The corresponding amount of bromide released would be 7800 mg/kg.

However, most of the extractives released during exposure are expected to be washed away during the rinsing and equilibration steps that invariably follow the cleaning. For instance, data in analytical report 4 shows that for bromide, a 96 - 98% reduction in extractives level is obtained on rinsing. Making the conservative assumption that only 90% of the extractives are removed during rinsing and equilibration leads to overall levels of 16 mg/kg agarose fragments and 780 mg/kg bromide that potentially could be released into food.

6.2.2.4 Simulated use at high and low pH

For completeness data from a range of conditions are shown although for stabilising beer the operating conditions are typically pH 4 and -1°C

Since the CSS Adsorber is the most sensitive to hydrolysis at extreme pH conditions, the CSS Adsorber was subjected to pH 3 and 4 at 20°C and 40°C as well as pH 12 at 40°C, again for 160-170 h. The amount of extractives formed was determined using TOC, TN and bromide analysis (see Analytical report 4). The results, using the highest values for an individual batch, show that at pH 3 and 4, only small amounts of extractables are formed. The levels of agarose and bromide are at or just above the detection levels of 2.9 and 1.6 mg/kg, respectively. The nitrogen analysis indicates a functional group loss below the detection limit of 0.1%. At pH 12, the extractable levels are higher. Up to 103 mg/kg of agarose and 312 mg/kg of bromide are released whereas the functional group loss is 0.21%.

If it is again assumed that the degree of hydrolysis is proportional to the exposure time, the data in Appendix 4 – Chemical Stability of CSS Adsorber can be used to estimate rates of hydrolysis at different pH and temperature.

The results of such an estimate are given in Table 7. The numbers Table 7 are not maximum values as presented above, but averages for the 3 batches studied.

Condition	Rate of agarose loss [%/1000h]	Rate of fragment formation [ppm/1000h]	Rate of functional group loss [%/1000h]	Rate of Br migration [ppm/1000h]
pH 3, 20° C	<0.01	<7.8	<0.4	0.9
pH 3, 40° C	<0.01	<7.8	<0.4	1.3
pH 4, 20° C	<0.01	<7.8	<0.4	1.2
pH 4, 40° C	<0.01	<7.8	<0.4	3.1
pH 12, 40° C	0.5	583	1.2	1700

Table 7 - Rates of hydrolysis, average values.

The data in Table 7 can then be used to estimate the overall amount of functional group loss as well as the corresponding agarose fragment formation, reduction in agarose concentration and amount of bromide released. As a rule of thumb, the acceptable functional group loss is 10%. At pH 3 and 4, the rates of hydrolysis are so low that even if the CSS Adsorber were continuously subjected to these conditions during the entire lifetime (2 years or 16000 h):

- the functional group loss would be below 0.4 x 16 = 6.4%;
- the amount of agarose fragment formation below 7.8 x 16 = 125 mg/kg; and
- the amount of bromide released no more than 3.1 x 16 = 50 mg/kg.

The probability that the resin is subjected to these extreme conditions for extended periods of time during normal use is low. This means that in reality, the expected release of agarose and bromide through degradation at low pH will be even lower than the values given above.

At pH 12, the rate of bromide migration is relatively high whereas the rate of functional group loss remains moderate. Therefore, the exposure limit under these conditions is defined so as to maintain a reasonable amount of bromide release rather than to avoid excessive loss of resin functionality. Defining the maximum permitted exposure time as 2000 h leads to:

- a functional group loss of 1.2 x 2 = 2.4 %,
- the formation of 583 x 2 = 1200 mg/kg agarose fragments; and
- the release of 1700 x 2 = 3400 mg/kg bromide.

The process condition limits, including exposure times at different pH, are specified in the instructions supplied with Q Sepharose BB described in 6.2.3. Although for beer the typical operating conditions are -1°C and pH 4. The operation conditions for beer would never be outside -3°C to +3°C and a pH range of 3.7 to 4.7 because of brewing processes outside the CSS Adsorber stabilising.

6.2.3 Instructions for use

In addition to the other information provided with the product, the following statements will be included in the documentation:

Intended use

The ion exchange chromatography resin is intended to be used for the separation of proteins or other compounds present in similar concentrations from beer.

Pre-use washing procedure

After packing into the column and before being used the first time, the resin should be washed using the following steps:

1. 5 column volumes water
2. 5 column volumes 1M NaCl
3. Leave in 1M NaCl for 12 h
4. 4 - 5 column volumes water

Process conditions and limitations

The agarose matrix is sensitive to hydrolysis at extreme pH. In order to avoid excessive degradation the exposure time at high pH should be limited.

For continuous use, the process conditions for beer should be between pH 3 and 5 and temperatures below 40°C. In beer, use is likely to be at or around -1°C.

Cleaning at 20°C may take place at up to pH 14; cleaning at 40°C conducted at up to pH 13.7 and cleaning at 60°C can be performed at pH up to 13.3.

6.3 Safety Data

The following safety assessment is divided into two parts. The first is concerned with CSS Adsorber and the impurities that are approved in the Food Standards Code as food additive or processing aids, and thus are of limited safety concern. The second part deals with unregulated substances

6.3.1 Safety information of CSS Adsorber and impurities

The regulatory status of agarose, ethylcellulose, nonyl phenyl ethoxylate phosphate ester, sodium acetate, sodium sulphate, sodium chloride, glycerol, ethanol, toluene, trimethylamine, sodium formate, sodium bicarbonate, sodium bromide, glycolic acid and betaine are detailed in section 6.1 – Manufacture.

It is found that of these substances, agarose and ethylcellulose are recognized as direct food additives. Nonyl phenyl ethoxylate phosphate esters, toluene, sodium bromide, glycolic acid and trimethylamine are regulated in the US Code of Federal Regulations. Betaine is approved for use in dietary supplements whereas glycerol, sodium acetate, sodium sulphate, sodium chloride, sodium bicarbonate and ethanol are either foods or approved food additives or processing aids. To summarise, all substances are approved for use in or with food which is a clear indication that they are of little toxicological concern.

The estimated daily exposure through the use of the CSS Adsorber for all substances except agarose and sodium bromide is below 0.5 μg/kg or 1.5 μg / person / day. For agarose, the estimated daily exposure calculated from stability testing is 12 μg/kg which is to be compared with the approved level of agar in food of 0.25% or 2500 mg/kg. For sodium bromide, the estimated daily intake is 0.76 mg/kg bw/day which can be compared with the acceptable daily intake value of 400 μg /kg bw/day set by the European Agency for the Evaluation of Medicinal Products.

In view of the benign nature of these substances and the very low concentrations in which they are present, the exposure to the substances through the use of the CSS Adsorber should be of no concern with respect to human health.

6.3.2 Safety assessment of potentially toxic and/or carcinogenic impurities

A more comprehensive safety assessment of sodium borate, allyl glycerol ether, 2,3-epoxy-1-propanol, allyl glycidyl ether, epichlorohydrin, 3-chloro-1,2-propandiol, bromide, bromoacetic acid and sodium bromate is presented in the Part III, CTP.

With regard to allyl glycidyl ether, epichlorohydrin and 3-chloro-1,2 propanediol, these substances have already been evaluated for carcinogenic potency by the FDA and thus been given an Unit Cancer Risk (UCR) number. 2,3-epoxy-1-propanol (glycidol), is a genotoxic substance that has been shown to induce tumours in life-long studies in rats and mice. Tumour potency was shown to be highest in male rats with an estimated UCR value of 0.025 $(mg/kg\ bw/day)^{-1}$.

The UCR, the estimated daily exposure through the use of the CSS Adsorber and the estimated upper bound life-time cancer risk for these three substances are given in Table 8. The very low lifetime cancer risks estimated from these UCR and EDI values suggest that there is no concern for human health with the potential exposure to these impurities.

Table 8. UCR, EDI and life-time cancer risk for carcinogenic potential impurities

Substance	UCR $(mg/kg\ bw/day)^{-1}$	EDI (mg/kg bw/day)	Life- time cancer risk
Allyl glycidyl ether	0.0042	9.08 x 10-9	0.038 x 10-9
Epichlorohydrin	0.0027	9.08 x 10-9	0.025 x 10-9
3-chloro-1,2 propanediol	0.0087	12.7 x 10-9	0.11 x 10-9
2,3-epoxy-1-propanol	0.025	90.8 x 10-9	2.3 x 10-9

Bromoacetic acid can be formed in waters that contain bromide and it may also be a by-product of drinking water disinfection where coastal waters contain significant levels of bromine. The estimated maximum daily intake of bromoacetic acid from the use of the CSS Adsorber under abuse situations is 0.127 ng/kg bw.

There are limited data available on the toxicity of bromoacetic acid. Acute oral toxicity studies in rats have shown LD50 values below and above 50 mg/kg. Studies on repeat dosing in animals are essentially absent. Some reproductive toxicity data is available with evidence of developmental toxicity at 100 mg/kg bw in rats and with a NOAEL at 50 mg/kg bw. Based on this NOAEL and the use of an uncertainty factor of 1000, an oral reference dose (RfD) of 50 µg/kg bw is obtained. Such a RfD is thus more than 5 orders of magnitude higher than the estimated maximal daily exposure to bromoacetic from the use of the CSS Adsorber.

Of perhaps more relevance when assessing the potential risk associated with exposure to bromoacetic acid is it documented genotoxic activity. Several reports are avilable on the positive effects of bromoacetic acid in Salmonella typhirurium strains TA 100 and TA 98. Few other recognized genotoxicity assays have been performed and no carcinogenicity studies are available. In the absence of such studies and recognizing the genotoxic potential of bromoacetic acid, it is not unreasonable to assess the risk of inadvertant bromoacetic acid exposure from a threshold exposure of 1.5 µg/day (approximately 0.02 µg/kg bw/day) i.e. the threshold of regulation at the FDA for food-contact materials. The estimated maximimum daily intake of bromoacetic acid from the use of the CSS Adsorber is more than two orders of magnitude lower than this threshold dose of 1.5 µg/day. There should thus be no concern from a health perspective with the estimated exposure to bromoacetic acid.

The estimated maximum daily intake of sodium bromate from the use of the CSS Adsorber is 0.116 ng/kg bw. Bromate ions in the form of potassium bromate are approved for use as a processing aid in the malting of barley (Standard 1.3.3-14). Maximum permitted level allowed is 0.5 mg/kg.

The US EPA have made a "chronic health hazard assessment for noncarcinogenic effects" and a "carcinogenicity assessment for lifetime exposure" of the bromate ion (BrO3-). In assessing the noncarcinogenic effect they established an RfD of 0.004 mg/kg bw/day of BrO3 -using an uncertainty factor of 300. In assessing the carcinogenicity of a life time exposure to the bromate ion, the Agency reviewed three life-long studies in rats with exposure of potassium bromate via the drinking water. The weight-of-evidence judgment was that the bromate ion is a likely human carcinogen by the oral route of exposure. An excess cancer risk of 1 in a million was estimated to occur at a drinking water concentration of 0.05 µg/L. Assuming a daily intake of 2 L of water, this would translate into a daily oral dose of 100 ng or 1.4 ng/kg bw for a body weight of 70 kg.

This "acceptable daily exposure" as well as the RfD value of 4000 ng/kg bw/day are both well above the estimated maximal daily intake of bromate ion (0.099 ng/kg bw/day or 0.116 ng/kg/day of sodium bromate) that originates from the CSS Adsorber.

The Australian Drinking Water Guidelines gives the guideline value for bromate based on health concerns as 0.02 mg/L[19].

There should thus be no concern from a health perspective with any exposure to the bromate ion originating from the CSS Adsorber.

Bromine can leach from the CSS Adsorber resulting in an estimated maximal daily intake of 0.0727 ng/kg bw/day. In US, bromine is registered for use in water filters to purify drinking water aboard U.S. Naval ships and offshore oil well platforms. It also is used as a general disinfectant and sanitizer in indoor, non-food contact areas such as commercial establishments, hospitals and households, to control bacteria and fungi.

The US EPA established a food additive tolerance for 1.0 ppm residual bromine in potable water aboard Naval surface ships in 1976 and confirmed in 1993 that this level provides an adequate margin of safety to protect the public health. A daily intake of 2 L of water at the 1.0 ppm threshold level would result in a daily exposure to bromine of 2 mg (0.03 mg/kg bw/day for a person weighing 70 kg). This level of acceptable bromine exposure is thus more than 5 orders of magnitude greater than the estimated daily intake of bromine originating from the CSS Adsorber.

Allyl glycerol ether is an alkaline hydrolysis product of allyl glycidyl ether. Very limited information is available regarding the toxicity of allyl glycerol ether. A literature search identified only one report and that addressed the genotoxicity of this and other industrial chemicals.

Allyl glycerol ether was tested for its ability to cause gene mutations in bacteria, gene conversion in yeast and chromosomal aberrations in rat liver cells in vitro. The highest

[19] Australian Government – National Health and Medical Research Council – Natural Resource Management Ministerial Council – National Water Quality Management Strategy – Australian Drinking Water Guidelines 6, 2004.

concentrations investigated were essentially according to present recommendations. No evidence for any genotoxic activity was obtained.

Although there is only limited information available regarding the toxicity of allyl glycerol ether, the negative outcome in the in vitro test for genotoxic activity is reassuring. Importantly, the estimated total daily dietary concentration of allyl glycerol ether is 0.00025 ppb. This value is 2000-fold below the level of 0.5 ppb where toxicity testing is recommended by the US FDA Guidance April 2002.

It can therefore safely be concluded that the potential exposure to allyl glycerol ether from the use of the present CSS Adsorber (ion exchange chromatography resin) should be of no concern with respect to human health.

Sodium borate, another potential impurity in the CSS Adsorber, is predominantly present as boric acid in dilute aqueous solutions at physiological pH. Therefore when assessing the toxicity and the potential risk with exposure of minute amounts of sodium borate it is relevant to consider toxicity data from both sodium borate and boric acid.

The major relevant toxicity information on sodium borate/boric acid stems from the National Toxicology Program studies on boric acid. The NTP have conducted genetic toxicity testing, a full program of reproduction and developmental studies, repeat dose toxicity studies in mice and a carcinogenicity study in the same species. The other important data set comes from an older publication in Toxicology and Applied Pharmacology where information on repeat dose toxicity is presented for rats and dogs (both chemicals) and a carcinogenicity study in rats (also both compounds). This comprehensive data set with many high quality studies facilitate an adequate risk assessment of low dose exposure to sodium borate.

Boric acid/sodium borate was shown to have no genotoxic activity in a relevant set of in vitro genotoxicity studies. No carcinogenic activity was observed in life-long studies conducted in mice and rats. It can therefore be concluded that sodium borate confers no carcinogenic risk.

Repeat dose toxicity studies, the two carcinogenicity studies as well as the study on general reproductive performance in mice showed testis to be the most sensitive target organ. Reduced sperm count as well as tubular atrophy was observed at dose level from 150 mg/kg and above.

Tubular atrophy was observed in mice, rats and dogs but the NOEL's differed between species being in the range of 25-100 mg/kg. This difference in NOEL could well reflect the large spacing/interval of the doses in the various studies rather than a true difference in sensitivity or target organ exposure.

Developmental toxicity studies in rats and rabbits showed clear evidence of toxicity to the embryo/foetus. Malformation was also observed. The NOEL for developmental toxicity was determined to125 mg/kg based on embryo/foetal deaths and cardiovascular malformations in rabbits.

By using the FDA recommended safety factor of 1000, an ADI value of 25 mg/kg body weight per day is obtained. This value should be compared to the estimated daily exposure to sodium borate from the use of the CSS Adsorber i.e. 0.0046 mg/kg bw/day. It can therefore be safely concluded that the potential exposure to sodium borate from the use of the present CSS Adsorber should be of no concern with respect to human health.

6.3.3 Overall conclusion.

Overall, it can be concluded, that of the potential impurities stemming from the CSS Adsorber, several are already regulated in the Food Standards Code while the others may be potentially toxic. The regulated substances are of little safety concern. In assessing the exposure and the risk (inherent toxicity and potency) of the toxicants, it is clearly shown that there are sufficient safety margins to allow the conclusion that there should be no concern with respect to human health with the use of the CSS Adsorber.

The regulatory status and safety of all starting materials and impurities/potential impurities were reviewed and assessed. GE Healthcare concludes that all identified chemicals are either safe for this intended use or present no safety concern because the calculated exposures are so low. GE Healthcare has briefly summarised its findings below and in the following attachments in Appendix 6 – Toxicology Profiles

Agarose, the major starting materials for this resin, is not regulated as such, but agar from which agarose is obtained is generally permitted food additive included in Standard 1.3.1, Schedule 2. The use of agar as an additive is limited by GMP and not a specific level. GE Healthcare compared its starting material, agarose, to agar's Food Chemicals Codex specifications and found that agarose complied with those specifications except for a slightly higher gelation temperature.

GE Healthcare concludes that agarose is safe for this intended use because it meets food grade specifications and the calculated EDI for soluble agarose fragments is 0.6 μg/kg bw/day. Compared to the lowest maximum use level of agar directly added to food, these values provide a sufficiently wide safety margin for this chemical.

Several of the processing materials and/or impurities identified are either approved in the Food Standards Code as food additives, processing aids, or as foods. Most of these ingredients are water soluble and if remaining in the resin after the extensive washings, would be found at levels that do not represent safety concerns.

Many of the other substances in the resin are either permitted food additives or processing aids; however some are not mentioned in Food Standards Code, though they do have food use approval in the USA. These substances are present in extremely low concentrations after their extensive washings of the resin prior to its use. They should not present a concern with respect to human health:

Permitted Food: Sodium chloride - salt

Permitted Food Additive in Schedule 2, of Standard 1.3.1:

INS	Additive
262	Sodium acetate
522	Glycerol
500	Sodium bicarbonate
514	Sodium sulphate

Permitted Processing Aids

Processing Aid	Clause in Standard 1.3.3	Maximum Residue
Ethanol	3 – Generally Permitted	GMP
Triethylamine	8 – Ion Exchange Resins	Permitted component - GMP
Toluene	13 – Extraction Solvents	1 mg/kg
Sodium formate	18 – Microbial nutrients & adjuncts	GMP

Substances not mentioned in the Food Standards Code:

Substance	Notes
Betaine	Found in foods like beetroot, broccoli, and spinach. Permitted in the USA as a Dietary Supplement.
Ethylcellulose	Similar additives methyl cellulose (461) and methyl ethylcellulose (465) are generally permitted additives. JECFA has issued an ADI of "not specified".
Nonyl phenol ethoxylate phosphate ester substances	Permitted in USA - 21 CFR 178.3400 Approved emulsifiers in food contact materials.
Sodium glycollate	Permitted in USA - 21 CFR 175.105 Approved as an indirect food additive.

6.3.4 Estimated maximum potential daily intake of processing aid and impurities under abuse situation

6.3.4.1.1 Food consumption factors

The processing aid (agarose resin) is to be used for partly separating haze causing substances like individual polyphenols and proteins from a liquid food like beer. The separated beer substances are wasted and not used for further food processing. In so far, the extracted substances do not play a role in this application and for the calculation of potential intake rates.

The intended limitations for this repeat-use agarose resin are a temperature range 0-60°C and a pH range 3-14. Intended use is typically in a radial column with a resin bed height of 12 cm. Depending on the length of the column, the resin volume is typically 140 – 260 litres per column. The flow rate of beer through one column is 5,000 – 22,000 litres per hour. The average contact time of the agarose resin with the beer is 30 – 120 seconds.

Typically, 1 ml of resin will stabilise 1 litre of beer during 1 production cycle. With minimum 500 cycles, 1 ml of agarose will stabilize minimum 500 litre beer during its useful lifetime. This is equal to a use of 0,002 ml agarose resin per 1 litre of beer. These figures are the base for the calculations of potential intake rates.

> Example: Given a column with 200 litre agarose resin, the batch volume of the treated beer per cycle is 200,000 litres. With an intended lifetime of the agarose resin of minimum 500 cycles, the minimum volume of treated beer is 100,000,000 litres.

In lack of information about the individual annual food intake by Australian respectively New Zealand consumers, we assume a daily food intake of 3000 g per person taken from US figures.

6.3.4.1.2 Food Starting Material - Beer

The average beer consumption in Australia is 91.5 litres per capita and year; in New Zealand it is 72.8 litre per capita and year. In the following, the Australian beer consumption data were used as base for the calculations.

The beer consumption in Australia is 91.5 litres / person / year. This is equal to 0,25 litres or 250 g per day and person.

Based on a total daily food intake of 3000 g / person / day, the fraction of food in contact with the processing aid would thus constitute 250 / 3000 = 0.084 or 8.4% of the daily diet.

6.3.4.1.3 Estimated daily intake of the processing aid and impurities

In the following, the estimated daily intake of the different impurities and the CSS Adsorber via the food starting material (beer) assuming 100% migration over the lifetime is detailed.

The calculations are based on the impurity levels determined for the CSS Adsorber in section 6.1.1 As can be seen, the EDI levels are very low.

It was attempted to determine CEDIs for the compounds. However, no information could be found on any of the substances in FDAs EDI/CEDI data base.

6.3.4.1.3.1 Soluble agarose fragments

Based on the levels of soluble agarose fragments extracted from the fresh resin and the amounts of fragments formed during normal use as well as during exposure to high and low pH discussed in Section 6.2 - Stability, the EDI of soluble agarose fragments via the treated beer would be no more than
[(7 + 16 + 1200) ppm / 500 000] x 0.084 = 0.205464 ppb.

(Note: According to the stability study, 16 ppm agarose fragments could be found at pH 3-4; 1200 ppm at pH 12. One (1) litre agarose is used for processing 500,000 litre of beer (1 litre agarose per 1000 litre beer * 500 cycles)

6.3.4.1.3.2 Sodium bromide

The EDI of sodium bromide was calculated from the amounts extracted from the fresh resin and the quantities formed during normal use as well as during exposure to high and low pH discussed in section E. The maximum EDI from the food starting materials would be
[(1 + 780 + 3400) ppm / 500 000] x 0.084 = 0,702408 ppb.

6.3.4.1.3.3 Other impurities

The maximum EDI of these substances via the food starting materials is calculated from the amount present in the resin (C_{resin}) according to the following formula:
$EDI_{feed} = 0.084 \times (C_{resin} / 500\,000)$

The EDIs calculated in this manner from the impurity levels presented in Section 6.1.1 are given in Table 9.

	CAS Reg no	EDI [ppb dietary conc.]			Total EDI
		From CSS Adsorber μg/kg	*Analysis mg/kg*	*from beer μg/kg*	**[μg/kg b.w./day]**
Soluble agarose fragments	9012-36-6	3.59	1223	0.203018	0.0101509000
Ethylcellulose	9004-57-3	0.0161	5488.636364	0.000911114	0.0000455557
Polyoxyethylene nonylphenyl phosphate ester sodium salt	68954-84-7	0.032	10909.09091	0.001810909	0.0000905455
Glycerol	56-81-5	0.0226	7704.545455	0.001278955	0.0000639477
Sodium acetate	127-9-3	0.0299	10193.18182	0.001692068	0.0846034091
Sodium formate	141-53-7	0.0499	17011.36364	0.002823886	0.0001411943
Sodium sulfate	7757-82-6	0.0431	14693.18182	0.002439068	0.0001219534
Sodium bicarbonate	144-55-8	0.0616	21000	0.003486	0.0001743000
Ethanol	64-17-5	0.017	5795.454545	0.000962045	0.0000481023
Toluene	108-88-3	0.00968	3300	0.0005478	0.0000273900
Sodium bromide	7647-15-6	12.3	4181	0.702408	0.0351204000
Trimethylamine	75-50-3	0.0255	8693.181818	0.001443068	0.0000721534
Sodium glycollate	2836-32-0	0.0358	12204.54545	0.002025955	0.0001012977
Betaine	107-43-7	0.17	57954.54545	0.009620455	0.0004810227
Bromine	7726-95-6	0.0117	3988.636364	0.000662114	0.0000331057
Sodium bromate	7789-38-0	0.0188	6409.090909	0.001063909	0.0000531955
Sodium borate	1303-96-4	0.739	251931.8182	0.041820682	0.0020910341
Allyl glycerol ether	123-34-2	0.00205	698.8636364	0.000116011	0.0000058006
Bromoacetic acid	79-08-3	0.0205	6988.636364	0.001160114	0.0000580057
2,3-epoxy-1-propanol	556-52-5	0.0147	5011.363636	0.000831886	0.0000415943
Allyl glycidyl ether	106-92-3	0.00147	501.1363636	8.31886E-05	0.0000041594
Epichlorohydrin	106-89-8	0.00147	501.1363636	8.31886E-05	0.0000041594
3-chloro-1,2-propandiol	96-24-2	0.00205	698.8636364	0.000116011	0.0000058006

Table 9 - EDIs of CSS Adsorber and Impurities

6.3.5 Potential for contamination under normal processing conditions for beer

Under normal conditions of use in a brewery no contaminants are detected and this is the case under abuse situations. All the previous material relates to hypothetical maximum amounts based on the levels of detection of each of the substances.

In order to effectively filter beer there a narrow set of process conditions for both temperature and pressure. The typical temperature range for filtration, the process step immediate prior to the CSS stabiliser, is -0.5°C ± 1°C. Too warm a temperature allows proteins to re-dissolve, too cold a temperature will cause freezing. Too little pressure will cause the carbon dioxide to come out of suspension and because beer is filtered into large tanks pressure is kept to an operational minimum. The operating pressure of the CSS Unit is less than 300 pKa. The pH of beer is typically between 3.90 and 4.4.

The actual contact time between beer and the CSS absorber will vary depending on the beer being treated. For beer that will be packaged in clear glass bottles and then exported (the maximum stability requirement) would have contact time of less than 2 minutes. A standard beer in brown glass for domestic production would have a contact time of less than 45 seconds.

These normal beer processing conditions of temperatures (-1°C), pressures (300kpa), pH (4.2) and contact times (2mins) should be compared with the conditions used to "abuse" the samples for analysis for impurities where the temperatures (20°C & 40°C), pressures (10MPa), pH (3.4&12) and contact times (1-6 hours) were much higher.

A further issue that is likely to decrease the amount of substances transferred to the beer during processing is the cleaning regime. This is severe and is likely to remove any potential contaminants before beer is processed.

The cleaning regime is:

Process	Duration/Conditions
2M NaCl solution	20 minutes
Rest	5 minutes
4% NaOH	20 minutes at 40°C
Rest	5 minutes
Carbonated water	20 minutes at 1°C
Hot water	30 minutes at 80°C
Beer	

1.

PART 7 STATUTORY DECLARATION

STATUTORY DECLARATION

Statutory Declarations Act 1959

I, David John Panasiak, Food Regulation Consultant with Food Liaison Pty Ltd, Queanbeyan, New South Wales make the following declaration under the *Statutory Declarations Act 1959*:

1. The information provided in this application fully sets out the matters required.
2. The information provided in this application is true to the best of my knowledge and belief.
3. No information has been withheld that might prejudice this application, to the best of my knowledge and belief.

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[redacted]

Declared at Queanbeyan, New South Wales on 9th day of February 2007

Before me, [redacted]

* A statutory declaration must be made before a prescribed person under the *Statutory Declarations Act 1959*, available online at http://www.frli.gov.au/.