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The Rapid Quantitative Determination of Alginates by Poly(hexamethylenebiguanidinium chloride) Complexation in Industrial Liquors Extracted from Brown Seaweed

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SUMMARY

Further development of the poly(hexamethylenebiguanidinium chloride) assay method previously reported by these authors for the determination of alginates has resulted in a rapid procedure to determine this polysaccharide in liquors obtained by alkali extraction of brown seaweeds. The method involves neutralisation of the liquor followed by a simple alginate precipitation procedure employing the organic cation poly(hexamethylenebiguanidinium chloride) which possesses a UV absorption at 235 nm and can therefore be used in the quantitative step of the assay. Results produced by the assay for determination of alginate in liquors are in good agreement with another recognised method. The method is insensitive to the presence of numerous substances in the liquors in addition to alginate. In contrast to many other existing methods, alginate materials with various D*-mannuronic acid/*L*-guluronic acid ratios show essentially identical responses to the assay.*

INTRODUCTION

The polysaccharide alginate is commercially important due to its solution properties, notably the ability to form gels in the presence of calcium ions (Cottrell & Kovacs, 1977, 1980). Alginate occurs as a

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major structural component of brown algae (seaweed), but the micro-organisms *Azotobacter vinelandii* (Linker & Jones, 1966; Deavin *et al.*, 1977) and *Pseudomonas aeruginosa* (Carlston & Mattews, 1966; Gorin & Spencer, 1966) produce a polymer of similar structure. The polysaccharide exists as a linear co-polymer of β-D-mannopyranosyluronic acid and α-L-gulopyranosyluronic acid residues which are arranged in three types of block sequences (Haug *et al.*, 1966, 1967*a*, *b*, 1974; Penman & Sanderson, 1972; Grasdalen *et al.*, 1979). These involve homopolymeric blocks, as well as heteropolymeric blocks containing both hexuronic acids. Enzymic evidence indicates that the heteropolymeric blocks in alginate do not exist as a strictly alternating sequence of both hexuronic acids, as had been considered originally (Boyd & Turvey, 1978).

Most polysaccharides tend to show consistency in their carbohydrate composition. However, the relative amounts of the two hexuronic acids vary widely for algal alginates obtained from different species of brown algae (Haug *et al.*, 1974) and for bacterial alginates produced under different fermentation conditions (Haug & Larsen, 1971). Since the gelation of alginate solutions is due to enhanced binding of Ca^{2+} ions with the poly-α-L-guluronic acid blocks of the molecule (Grant *et al.*, 1973; Morris *et al.*, 1973, 1978), variation in uronic acid composition affects the gelling properties of the polymer (Smidsrod, 1974). Alginates with a high L-guluronic acid content tend to form strong, brittle gels, while samples of the polysaccharide with high D-mannuronic acid content form more elastic gels.

The variation of chemical composition in alginate samples also complicates attempts at the quantitative determination of this polymer in solution. Indeed, many of the reagents which can be used for alginate determination, such as carbazole and 3-hydroxybiphenyl, are unsatisfactory since they do not react in an identical manner with both D-mannuronic acid and L-guluronic acid (Bitter & Muir, 1962; Blumenkrantz & Asboe-Hansen, 1973). Recently, we reported the use of poly(hexamethylenebiguanidinium chloride) [$PHMBH^+Cl^-$] as a reagent for the rapid quantitative determination of alginates (Kennedy & Bradshaw, 1984). The procedure consists of the addition of a known excess of $PHMBH^+Cl^-$ (Fig. 1) to alginate solutions to precipitate the sample quantitatively and thereafter measuring the UV absorption of residual $PHMBH^+Cl^-$ in the supernatant. Further development of the $PHMBH^+Cl^-$ method has resulted in a procedure suitable for the assay of alginate in liquors extracted from brown seaweed, a task which is generally difficult due to the presence of other chemicals and impurities.

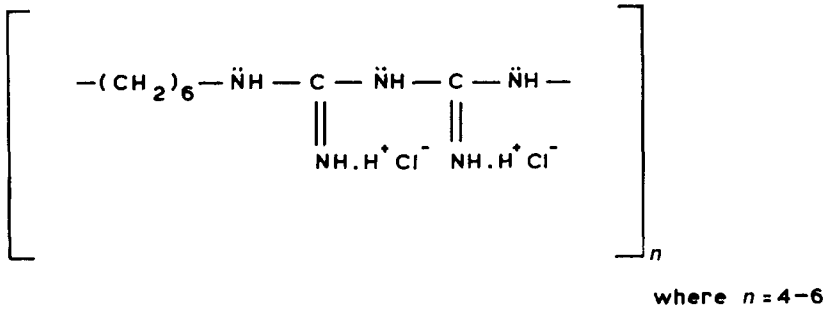

Fig. 1. Structure of poly(hexamethylenebiguanidinium chloride).

We have also investigated further the suitability of the $PHMBH^+Cl^-$ method for alginate samples with different hexuronic acid compositions.

EXPERIMENTAL

Materials and general methods

All solid alginate samples and alginate liquors were obtained from Alginate Industries Ltd (now Kelco/AIL International Ltd), Girvan, Scotland, UK. The sodium alginate sample designated XM 225 was of high purity (alginate content 89·0%; dry solids content 89·2%) and used in the calibration of the assay procedure. The sodium alginate content and dry solids content, respectively, are shown in parentheses for various samples used which were derived from various seaweed origins: *Ascophyllum nodosum* (87·5%, 90·2%), *Laminaria hyperborea* (89·6%, 91·0%), *Lessonia flavicans* (89·3%, 91·3%) and *Durvillea potatorum* (84·2%, 87·1%). All solid samples for use in quantitative assessments were pre-dried at 61°C *in vacuo* over P_2O_5 for 24 h.

The poly(hexamethylenebiguanidinium chloride) was obtained under the trade name Vantocil 1B from ICI Ltd, Pollution Control Division, Hyde, Cheshire, England, UK, as a 20% (w/v) solution. Protein contents of alginate liquor samples were assessed using the method of Lowry *et al.* (1951).

Standard assay procedure

To duplicate aliquots (5 ml) of sodium alginate solutions (1–5 mg ml^{-1}), aliquots (10 ml) of 0·3% $PHMBH^+Cl^-$ in 1% sodium acetate were

added with continuous magnetic stirring. This stage can be performed conveniently in pyrex test tubes (6 in × ¾ in) using magnetic followers of 12 mm diameter. Agitation of the solutions was continued for a further 5 min to allow formation of the alginate–$PHMBH^+$ precipitates, which were recovered from the supernatant by low speed centrifugation (3000 rpm, 5 min). The supernatants were retained and the UV absorbance, after 100-fold dilution, measured at 235 nm against a distilled water blank. The calibration curves were constructed by plotting supernatant absorbance against sodium alginate concentration. The standard solutions were prepared allowing for the non-alginate material known to be present in the sample.

Effect of neutralisation on the response of sodium alginate to the assay

Aliquots of sodium alginate solution (5 ml; 5–25 mg) were prepared at pH 11·5 and allowed to stand at room temperature for 1 h. Acetic acid (1% w/v, 1·5 ml) was then added to each solution to neutralise to pH 6·5. The sodium alginate solutions were then precipitated by $PHMBH^+Cl^-$ and the supernatants analysed for UV absorbance using the standard assay method. As a control, the procedure was repeated with aliquots of sodium alginate solution whose pH had remained unaltered at 6·5. Distilled water (1·5 ml) was added to these solutions to allow for the volume of acetic acid added. The UV analysis of the supernatants from this experiment is shown in Table 1.

TABLE 1
The Effect of Neutralisation of Sodium Alginate Solutions at Alkaline pH on the Response to the $PHMBH^+Cl^-$ Assay

Sodium alginate concentration (mg ml^{-1})	*Supernatant absorbance[a,b] (235 nm)*	
	Control	*Neutralised*
1	0·879	0·893
2	0·644	0·648
3	0·478	0·486
4	0·344	0·332
5	0·202	0·200

[a] After 100-fold dilution.
[b] Average of two values.

Effect of addition of known amounts of sodium alginate to the estimation of alginate content in liquors

To aliquots (2·5 ml) of alginate liquor (see below), various volumes (0·5–2·5 ml) of standard sodium alginate solution (5 mg ml^{-1}) were added and the volume made up to 5 ml with distilled H_2O. Acetic acid (1% w/v, 0·4 ml) was then added to neutralise the samples. The total sodium alginate content of the prepared solutions was analysed by the $PHMBH^+Cl^-$ assay using the standard procedures. Again, allowance for additional volume was required for the standard sodium alginate solutions used to calibrate the experiment. As a control for the experiment, the response of 2·5 ml of alginate liquor diluted to 5·0 ml with distilled H_2O was also measured. Results are shown in Table 2.

TABLE 2
The Response to the $PHMBH^+Cl^-$ Assay by Known Amounts of Sodium Alginate Added to Alginate Liquors

Sodium alginate added (mg)	*Sodium alginate assayed*[a,b] *(mg)*
2·5	2·6
7·5	7·8
12·5	12·4

[a] Includes allowance for deduction of sodium alginate present in liquor sample.
[b] Average of two values.

Assay procedure for alginate liquors

Samples of liquors (~ pH 10) were obtained as slurries and prepared for analysis by filtering through a cotton wool bed and then adding formaldehyde or polyacrylamide, or a combination of these additives. The former additive was used as a preservative and the latter as a settling aid to remove insoluble materials. The liquors were then allowed to settle overnight and the supernatant decanted off, for analysis, from any residue which had been deposited.

Quadruplicate aliquots (5 ml) of alginate liquor were neutralised to pH 6·5 by addition of acetic acid solution (1% w/v, 0·4 ml). The liquor samples were stirred continuously and $PHMBH^+Cl^-$ solution (0·3%,

10 ml) added. The procedure was then continued using the standard assay method outlined above. It was necessary to add distilled water (0·4 ml) to the standard sodium alginate solutions to allow for volume difference. Results of the analysis of the alginate liquor samples are shown in Table 3.

TABLE 3
Comparison of the Analysis of Alginate Liquors for Sodium Alginate Content by the $PHMBH^+Cl^-$ and Neutral Equivalent Methods

$PHMBH^+Cl^-$ analysis	*Sodium alginate concentration[a] (mg ml^{-1})*						
	1a	*1b[b]*	*2a*	*2b[b]*	*3a[b]*	*3b[b]*	*4[b]*
1	2·39	2·65	3·14	3·02	3·86	3·84	2·94
2	2·32	2·65	3·18	3·02	3·90	3·82	2·94
3	2·32	2·58	3·08	2·98	3·80	3·82	2·90
4	2·34	2·70	3·08	3·06	3·94	3·84	2·94
Average	2·34	2·65	3·12	3·02	3·80	3·83	2·94
Neutral equivalent analysis[a]	2·50	2·66	3·21	3·29	3·65	3·69	2·70

[a] Average of three values.
[b] Polyacrylamide (20 ppm) added to liquors during settling.
HCHO (400 ppm) present in all liquors except 3a.

Determination of alginate in liquors extracted from brown seaweed by the neutral equivalent method (Alginate determination was made by a method based on the work of Achwal (1981)

To 1 litre of alginate liquor, pre-filtered with heavyweight nylon cloth, sodium hypochlorite (0·1 M, 10 ml) was added, mixed and the solution allowed to stand for 30 min. To the liquor sample, a mixture of calcium chloride solution (20% w/v, 15 ml)–acetic acid solution (50% w/v, 3 ml) was added slowly, under vigorous agitation. The calcium alginate precipitate formed was allowed to harden for 15 min, filtered through a nylon cloth and finally squeezed as dry as possible.

A 3-in-diameter sinter-glass (No. 1 porosity) filter funnel, fitted with a screw-clip, was placed upside-down in a beaker of water. Water was sucked into the stem until full and the screw-clip closed. The funnel was then placed over a stand and the calcium alginate precipitate transferred quantitatively to it. Hydrochloric acid (1 M) was added to cover the calcium alginate and the precipitate teased with an ebonite rod. After

5 min contact time, the screw-clip was opened and the liquid allowed to run off slowly. The leaching process was continued by topping up the funnel with acid and running off the liquid, until a negative calcium test was obtained (to 1 ml of the drainings, an equal volume of ammonium oxalate (saturated) solution was added — the mixture was allowed to stand for 2–3 min; a fine white suspension indicated the presence of calcium). When the sample was free from calcium, the precipitate was washed with deionised water until free from acid (to 0·5 ml of drainings, 2–3 drops of Tropaeolin 00 indicator were added — a red colour indicated the presence of acid; a yellow colour indicated freedom from acid).

The sample was then transferred to a beaker by washing with a small amount of deionised water. After addition of a few drops of phenolphthalein indicator, the mixture was stirred mechanically with the aid of a glass stirrer and titrated with sodium hydroxide solution (0·5 M) until a permanent pink colour was achieved.

Using the assumption that the equivalent weight of sodium alginate is 216, the polysaccharide content of the liquor is calculated as follows:

$$\text{sodium alginate content} = \frac{T \times 0{\cdot}216 \times M}{10} \%\,(\text{w/v})$$

where T = titre volume (ml) and M = molarity of NaOH solution.

RESULTS AND DISCUSSION

There is an apparent lack of reliable analytical methods in the literature which are suitable for the direct quantitative estimation of polysaccharides in liquors resulting from extraction of plant materials or through fermentation procedures. Although the commercial methods of producing sodium alginate from brown seaweed are proprietary, reports suggest that the fundamental steps, including alkali extraction, developed for the original processes are still employed. In this present work we have been interested in a method for the determination of the alginate content of liquors obtained by alkali extraction of brown seaweed. Commercially, this analytical stage is important, since it provides an estimate of the quantity of purified alginate to be expected at the end of the process. In addition, it provides a monitor over the efficiency of the extraction process. Further, detection and quantitation of any alginate in waste liquors from the process is also important.

From the above, it follows that the quality control operation associated with algal alginate production is extensive and that time-consuming assay methods are undesirable for this purpose. The $PHMBH^+Cl^-$ assay procedure for alginate determination which we reported recently has many useful features (Kennedy & Bradshaw, 1984). In particular, assay response is easily reproducible and insensitive to salt concentration, pH and temperature variation within defined limits. Further, the working range of the assay can be altered by simply adjusting the excess quantity of $PHMBH^+Cl^-$ which is added during the precipitation stage of the assay. The upper concentration range of the assay is 0·1–0·5% polysaccharide and this was required for the estimation of the sodium alginate content of liquors. In addition, the short time required to perform the $PHMBH^+Cl^-$ assay makes it suitable as a quick analytical procedure for alginates. Despite the obvious advantages of the $PHMBH^+Cl^-$ method it was necessary to carry out further development work to allow the assay to be used for the specific application of alginate liquor samples.

The wide variety of materials present in brown seaweed was expected to be one of the obstacles to the successful use of the $PHMBH^+Cl^-$ method. In particular, the presence of protein and polysaccharides, other than alginate, was considered to be the most likely source of interference to the assay. Besides alginate, the most commonly found polysaccharides in brown seaweed are laminaran (Black & Dewar, 1973) and a group of polymers related by the presence of L-fucose (Medcalf, 1978). Although the laminaran content of *Ascophyllum* species can vary between 1 and 7% in brown seaweed (Chapman, 1970), the neutrality of its structure suggested that it would not interfere directly with the $PHMBH^+Cl^-$ assay method. However, the L-fucose containing polysaccharides possess acidic sulphate groups and could therefore interfere with the quantitative determination of alginates in liquor samples. The possible presence of these sulphated polysaccharides was examined using IR spectroscopy. An absorption at 1100 cm^{-1} would have been indicative that this interfering polymer was present (Miller & Wilkins, 1952). However, the IR spectra of both the freeze-dried alginate liquor and the alginate–$PHMBH^+$ complex showed no absorption at this frequency and it was assumed that interference from sulphated polysaccharides would not be present.

Since protein would be present in the cellular material of brown seaweed, it was considered that residual quantities of the substance might be included in the alginate extract liquors. Analysis indicated that the protein content of the alginate liquors was 1·9%, with respect to alginate. Our previous work has shown that protein can be precipitated

by $PHMBH^+Cl^-$ above its isoelectric point value (Kennedy *et al.*, 1981). Since the assay method is performed at pH 7, any protein with an isoelectric point value above 7 could give rise to a small interference in the determination of the alginate content of liquor samples using $PHMBH^+Cl^-$. If avoidance of this type of interference was required, treatment of the sample with some suitable protease to degrade the protein could be used (Holding & Pace, 1981).

To be suitable for the determination of alginates in commercial liquors, it was necessary for the $PHMBH^+Cl^-$ assay response to be independent of certain changes in the chemical composition of the polysaccharide such as uronic acid composition and calcium content. The effect of Ca^{2+} on the response of sodium alginate to the $PHMBH^+Cl^-$ assay was studied by adding small amounts (Table 4) of the divalent

TABLE 4
Response of Alginate to the $PHMBH^+Cl^-$ Assay in the Presence of Low Levels of Calcium Ions

Sodium alginate concentration (mg ml^{-1})	*Supernatant absorbance[a,b] (235 nm)*			
	Calcium concentration (% w/w)[c]			
	0	*0·2*	*0·4*	*1·0*
1	0·916	0·922	0·928	0·942
2	0·723	0·729	0·724	0·718
3	0·524	0·521	0·519	0·526
4	0·345	0·340	0·351	0·350
4·5	0·253	0·257	0·258	0·271

[a] After 100-fold dilution.
[b] Average of two values.
[c] Weight basis relative to sodium alginate.

metal ion to standard solutions of the polysaccharide. Since Ca^{2+} displays a greater affinity than Na^+ for the carboxylic acid groups in alginate, variation in the stoichiometry of the alginate–$PHMBH^+$ precipitate might have occurred giving rise to interference to the assay. Any interference would have been evident from deviation of the UV absorbance of the supernatants obtained from precipitation of the alginate solutions containing added Ca^{2+} from the values for the control samples (no Ca^{2+} present). The results indicate that the low levels of Ca^{2+} studied, which are several factors higher than that expected in the liquors, do not cause interference to the $PHMBH^+Cl^-$ assay. However, quantities of Ca^{2+} sufficient to cause gelling of alginates would be

expected to cause difficulties with the precipitation stage of the assay procedure.

Although most polysaccharides show consistency in their sugar composition, the variation of the D-mannuronic acid (D-Man A)/L-guluronic acid (L-Gul A) ratio is an inherent feature of both algal and bacterial alginates. Thus, the ability to produce the same response with alginate samples of different uronic acid composition is an important requirement for any new method developed for the assay of this polysaccharide. Preliminary evidence suggested that response to the $PHMBH^+Cl^-$ assay was independent of the uronic acid composition of the alginate sample (Kennedy & Bradshaw, 1984). This characteristic of the $PHMBH^+Cl^-$ assay has been investigated further by comparing the responses of various sodium alginate samples from different brown seaweed origins. The sodium alginate samples used were all purified products containing small, but similar, amounts of non-alginate material (see 'Experimental' section). As shown, sodium alginate samples from various seaweed sources, *Ascophyllum nodosum, Lessonia flavicans, Durvillea potatorum* and *Laminaria hyperborea*, produced more or less identical responses to the standard $PHMBH^+Cl^-$ assay (Table 5). Data for the XM 225 sample are also included for comparison.

TABLE 5
Comparison of the Responses of Various Sodium Alginate Samples from Different Brown Seaweed Origins to the $PHMBH^+Cl^-$ Assay

Sodium alginate concentration (mg ml^{-1})	*Supernatant absorbance[a,b] (235 nm)*				
	Ascophyllum nodosum	Lessonia flavicans	Durvillea potatorum	Laminaria hyperborea	*XM 225 sample*
5	0·866	0·859	0·861	0·849	0·840
10	0·670	0·672	0·642	0·660	0·637
15	0·501	0·490	0·516	0·458	0·463
20	0·340	0·317	0·337	0·296	0·295
25	0·199	0·184	0·195	0·159	0·184

[a] After 100-fold dilution.
[b] Average of two values.

A variety of commercial sodium alginate products are available on the open market which possess a range of D-Man A/L-Gul A ratios. The response of two of these samples, Manucol DH and Manugel DJ, to the $PHMBH^+Cl^-$ assay is shown in Fig. 2 along with data for the XM 225 sample. The exact sodium alginate content of the commercial samples

was unknown and therefore the responses were compared on a dry weight basis only. As illustrated (Fig. 2), the two commercial sodium alginate samples showed more or less identical responses. Since the Manucol DH and Manugel DJ samples possessed D-Man A/L-Gul A ratios of 0·9–1·1 and 0·3–0·4, respectively, further evidence was obtained which indicated that the $PHMBH^+Cl^-$ assay response for alginates was independent of uronic acid composition. Interestingly, the XM 225 sodium alginate showed the largest response (lowest set of absorbance data) to the assay, indicating that this sample was of higher purity than the two commercial materials.

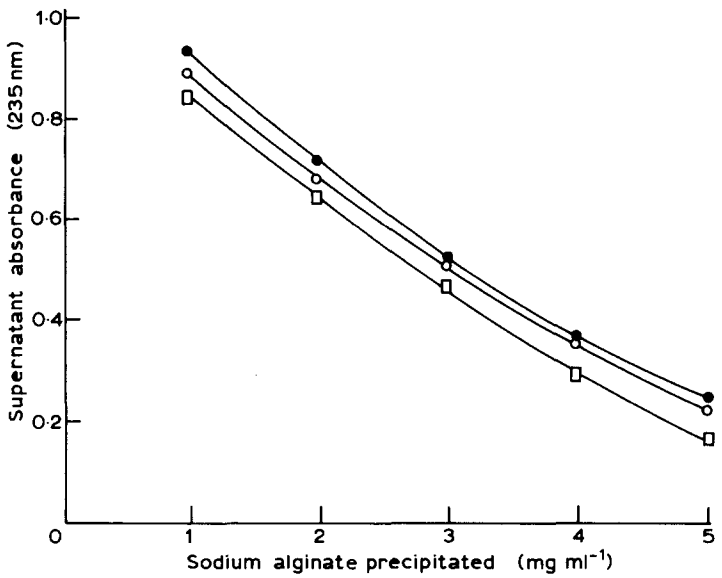

Fig. 2. Comparison of the response of various sodium alginates to the $PHMBH^+Cl^-$ assay. Values for absorbance of supernatant (diluted 100-fold) with variation of sodium alginate (5–25 mg; 1–5 mg ml^{-1}) precipitated by $PHMBH^+Cl^-$ (30 mg) are shown for Manucol DH (○), Manugel DJ (●) and the sample designated XM 225 (□).

The above results show that alginate samples with different D-Man A/L-Gul A ratios will produce similar responses to the $PHMBH^+Cl^-$ assay which is indicative of the specificity of the method for the amount of carboxylic acid groups on the polymer molecule rather than the spatial arrangement of these charged residues. In more general terms, this suggests that variation in the sugar composition of an acidic polysaccharide would not affect the response to the $PHMBH^+Cl^-$ assay, provided that the overall charge per repeating unit of the polymer remained unaltered. Thus, alteration of one uronic acid for another in a

polysaccharide would not be expected to have any effect on assay response. However, alteration of a neutral sugar for a uronic acid, or vice versa, in a polysaccharide would affect assay response.

Development of the $PHMBH^+Cl^-$ method for application to alginate liquors involved testing the possible interference to the assay caused by the chemicals used in the extraction of the polysaccharide from seaweed plants. Previous work had shown that the UV absorption of $PHMBH^+Cl^-$ at 235 nm (the assay measurement wavelength) can vary outside of the pH range 4–8 (Kennedy & Bradshaw, 1984). Since neutralisation of the algal alginate liquors was therefore necessary before analysis, it was pertinent to demonstrate that sodium alginate which had been readjusted from alkaline pH would show a similar response to the $PHMBH^+Cl^-$ assay as an identical sample which had remained at neutral pH. As shown (Table 1), the data indicate that the readjusted sodium alginate solutions gave a similar response to the control samples and therefore the neutralisation step of liquors prior to analysis was not expected to produce misleading results.

The standard $PHMBH^+Cl^-$ procedure developed previously was used for the assay of the sodium alginate content of liquor samples, except that a neutralisation step was included prior to the addition of reagent (Kennedy & Bradshaw, 1984). Values for the sodium alginate content of liquor samples were obtained by correlation of the UV absorbance of the supernatants with a typical calibration curve (Fig. 3). Only purified sodium alginate samples were used for the calibration which is of a reproducible nature. Results are quoted on a dry weight basis and thus it was necessary to check periodically the moisture content of the standard sodium alginate samples.

As a first step, the self-consistency of the $PHMBH^+Cl^-$ assay for liquor applications was tested by observing the response of liquor samples with added amounts of a standard sodium alginate. Results showed that good agreement was achieved between the quantities of polysaccharide added and assayed (Table 2). To test the reproducibility of the $PHMBH^+Cl^-$ assay, analysis of some alginate liquor samples was performed in our Birmingham laboratory and at a different location several hundred miles away using the same experimentor (Table 6). This provided the opportunity to assess the possible effects of local variations such as nature of the water on the assay method. The analysis reveals that good agreement was obtained, indicating that the $PHMBH^+Cl^-$ assay can be reproduced at different locations. A delay of 1 week occurred between the two sets of analysis, thus inferring that a moderate length of time could elapse without interfering with the estimation of the

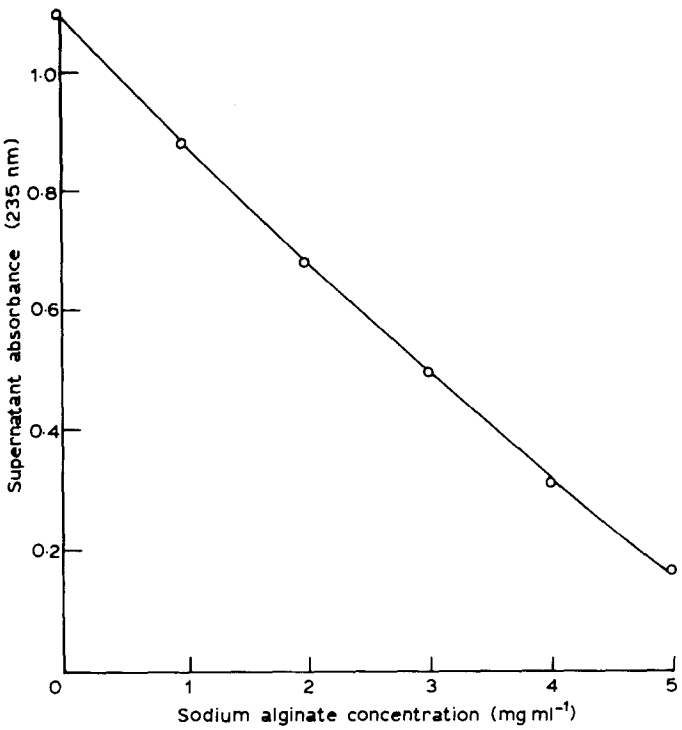

Fig. 3. Typical calibration curve for the response of sodium alginate to the standard $PHMBH^+Cl^-$ assay. Values shown are for absorbance of supernatant (diluted 100-fold) with variation of sodium alginate (5–25 mg; 1–5 mg ml^{-1}) precipitated by $PHMBH^+Cl^-$ (30 mg).

TABLE 6

Analysis of Identical Alginate Liquor Samples at Different Laboratories with the $PHMBH^+Cl^-$ Assay

Sample[b]	*Sodium alginate concentration*[a] *(mg ml^{-1})*	
	Birmingham laboratory	*Alternative laboratory*
A	2·78	2·74
B	3·04	2·90
C	2·62	2·60

[a] Average of four values.

[b] Samples derived from *Ascophyllum nodosum* origin.

sodium alginate content of alginate liquors. However, it would be unwise to allow the alginate liquors to stand for longer periods of time prior to analysis.

The results from the analysis of the alginate liquors, which were extracted mainly from an *A. nodosum/D. potatorum*, by the standard $PHMBH^+Cl^-$ procedure are shown in Table 3. Each analysis was performed in quadruplicate and these data, as well as the average of the four estimations, are shown in Table 3. For comparison, the estimation (triplicate analyses) of the sodium alginate content by the neutral equivalent method is also shown (Achwal, 1981). This particular method was chosen because it gives an absolute value and, further, it will give the same response with alginates of different D-Man A/L-Gul A ratio. The principle of the method involves precipitation of alginate as the calcium salt, conversion to the H^+ form (alginic acid) with dilute acid and finally titration of the newly formed acidic groups with alkali. From the quantity of alkali required, the equivalence of acid and hence the amount of alginate can be calculated (see 'Experimental' section).

As shown (Table 3), reasonable agreement was reached between the $PHMBH^+Cl^-$ and neutral equivalent methods for the sodium alginate content of the liquors. The percentage difference for sodium alginate content estimated by these two methods varied between 0·8 and 8·2%, whilst the average deviation was 4·8%. Such a difference would not be unexpected for the analysis of crude liquors by two alternative methods. The only liquor sample to be analysed which did not come from an *Ascophyllum nodosum/Durvillea potatorum* origin was liquor 4, which was produced from *Lessonia flavicans* seaweed. A variation of 8·2% was observed between the two analysis methods for the sodium alginate content of this particular sample. Further observation of the data reveals that good reproducibility was achieved with the $PHMBH^+Cl^-$ assay. For the quadruplicate data, the average deviation between the lowest and the highest value was 2·5%. Small amounts (20 ppm) of settling aid (polyacrylamide based) are normally added to alginate liquors during the production process. However, the analysis of liquors 1 and 2 suggests that no significant interference occurs to the $PHMBH^+Cl^-$ assay from the presence of this substance in liquor samples.

With regard to practical aspects the $PHMBH^+Cl^-$ method possesses two valuable assets (Kennedy & Bradshaw, 1984). First, the assay has a simple procedure with a short analysis time and is clearly more suitable than the neutral equivalent method in these respects. Secondly, the method has good reproducibility which is evident from the repeat analysis on one particular sample and the agreement between analysis performed at different locations. In addition, the $PHMBH^+Cl^-$ method

has been shown to be insensitive to a wide variety of additives present in alginate liquors and neutralisation of the samples is the only necessary pretreatment required before analysis. Application to alginate liquors of different brown seaweed origin is possible since samples of the polysaccharide with various D-Man A/L-Gul A ratios show similar responses to the assay. However, the assay need not be confined to the analysis of algal alginate liquors; application to other types of algal and microbial acidic polysaccharide liquors should be possible. The most likely difficulty which may arise with the assay is in the precipitation stage. However, provided that the correct procedure is followed precisely, this difficulty should not occur. In particular, it is important to ensure that the supernatant from precipitation is free from any slight turbidity, thus avoiding any interference to the measurement of UV absorption.

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