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A rapid quantitative determination of pectin and carboxymethyl cellulose in solution using poly(hexamethylenebiguanidinium chloride)

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There is a need for an effective wet method for the determination of pectin or carboxymethyl cellulose in solution. The poly(hexamethylenebiguanidinium chloride) complexation method has proved to be of value in this respect. Fears that heterogeneous distributions of the carboxyl groups might prevent a meaningful assay result from being obtained have proved groundless. In fact investigations of the complexation of such polysaccharides by poly(hexamethylenebiguanidinium chloride) have established that the assay procedure can be considered as a means of characterizing pectins or other carboxylated polysaccharides in terms of average charge density, i.e. equivalent weight, or, if the latter is known, determining polycarboxylate concentrations in dilute aqueous media.

INTRODUCTION

Poly(hexamethylenebiguanidinium chloride) [PHMBH$^+$Cl$^-$] is a cationic polymeric precipitating agent which undergoes cross-linking reactions with a wide range of acidic polysaccharides (Kennedy & Bradshaw, 1984, 1986, 1987) in aqueous solution. The PHMBH$^+$Cl$^-$ assay was originally developed for isolation and purification of xanthan gum by precipitation (Kennedy *et al.*, 1981). Further application of PHMBH$^+$Cl$^-$ as a precipitating agent resulted in the development of a rapid method for the assay of acidic polysaccharides. The principle of PHMB$^+$Cl$^-$ assay consists of the addition of a known excess of PHMBH$^+$Cl$^-$ to acidic polysaccharide solutions in order to precipitate the sample quantitatively and thereafter measuring the u.v. absorption of residual PHMBH$^+$Cl$^-$ in the supernatant. PHMBH$^+$Cl$^-$ is a low molecular weight polymer with an average molecular weight of 1200.

Pectin is a linear poly-D-galacturonic acid which has a considerable commercial application in food and industrial uses due to its ability to form gels with divalent cations, particularly calcium (Fishman & Jen, 1986; Silva, 1990). Carboxymethyl cellulose (CMC) is obtained by direct carboxymethylation of cellulose and has been used as constituent in a wide variety of products and formulations. Thus, it is important for both users and producers to know the amount of such polysaccharides existing in the products and formulations.

In this work use was made of the simple and rapid PHMBH$^+$Cl$^-$ assay for the quantitative determination of pectin in aqueous solution and industrial liquors and pure and crude CMC in aqueous solution.

EXPERIMENTAL

Materials

All pectin samples were kindly provided by HP Bulmer Limited, Hereford, UK. All CMC samples were kindly provided by Courtaulds Research PLC, Coventry, UK. Sodium alginate and xanthan were kindly provided by Kelco/AIL International Limited. Poly(hexa-

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methylenebiguanidinium chloride) was kindly provided under the trade name Vantocil 1B by ICI PLC, Pollution Control Division, Hyde, UK), as a 20% solution.

Standard assay procedure

To duplicate aliquots (1·0 ml) of polysaccharide solutions (1·0–5·0 mg/ml), were added aliquots (2·0 ml) of PHMBH$^+$Cl$^-$ (0·3%) in water or in aqueous sodium acetate (1%) with continuous stirring. Each solution was then stirred for a further 5 min, when the CMC$^-$PHMBH$^+$ precipitates were collected by centrifugation (3000 rpm, 5·0 min.). The supernatant solution was diluted 100-fold, and the u.v. absorption was determined at 235 nm. The calibration curves were constructed by plotting supernatant absorbance against pectin or CMC concentration. Pectin solutions had their pH adjusted to 6·5.

Increasing concentration of PHMBH at fixed concentration of pectin

The mixtures of PHMBH-pectin (5 ml) were in each case centrifuged and the supernatants (1 ml) diluted 100-fold for the optical readings at 235 nm. Pectins employed in these experiments contained 35% (PLM 35) and 70% (PCF) esterified galacturonate residues, respectively.

RESULTS AND DISCUSSION

The PHMBH$^+$Cl$^-$ assay has been shown to precipitate carboxylated polysaccharides such as xanthan, alginate, etc., but not neutral polysaccharides. The PHMBH$^+$Cl$^-$ assay has several advantages (Kennedy & Bradshaw, 1984), in particular, the response is easily reproducible and insensitive to variation of salt concentration, pH, temperature, within defined limits. The precipitation of the polysaccharide by the PHMBH$^+$Cl$^-$ occurs due to the electrostatic nature of the complexation reaction between the negative charge of the acidic polysaccharide and the positive charge of the precipitating reagent.

Acidic polysaccharides possessing various charge densities per repeating unit show different responses to the PHMBH$^+$Cl$^-$ assay (Fig. 1). Sodium alginate, polygalacturonate, and CMC (BDH, DS (degree of substitution) = 0·7–0·8), have greater numbers of acidic groups per molecule than has oxidized tamarind gum, the results for which are off-scale in Figs 1 and 6 included in this paper.

CMC with DS ranging from 0·63 to 2·1 gave as expected good responses in the PHMBH$^+$Cl$^-$ assay. Within the DS range 0–1, CMC samples gave similar response slopes. However, a large increase in the slope was observed for CMC with DS greater than 1·0 (Fig. 2). This expected behaviour, is due to the greater amount of carboxylated units with increasing DS and the DS giving more than one group per glucose residue. The percentages of CMC were obtained by calculating the tangent to each slope and comparing them to that tangent obtained from standard commercial CMC (BDH), which was taken as 100%. These percentages were then plotted versus DS (Fig. 3). At a DS of 1·0 and below there is little to choose between the points (in the box), but above this area of DS the response to DS alters. Thus CMCs with different DS values showed slightly different responses to the PHMBH$^+$Cl$^-$ assay up to a DS of 0·94. However a large difference in the

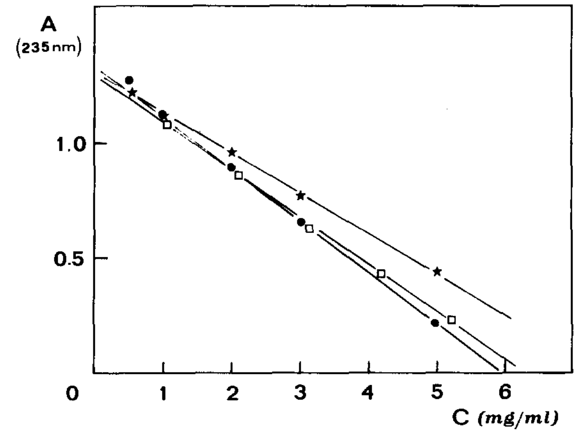

Fig. 1. Response of polycarboxylates of different chemical structure and charge density (★) CMC DS = 0·7–0·8, (□) polygalactunonate, and (●) alginate, to the PHMBH$^+$Cl$^-$ assay.

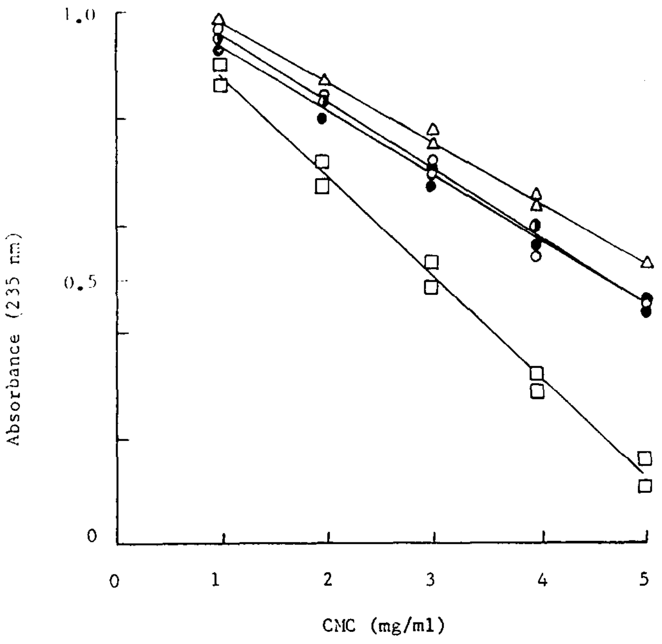

Fig. 2. Response of CMC with different DS values to the PHMBH$^+$Cl$^-$ assay; CMC DS = 0·66 (△), 0·78 (●), 0·94 (○), 1·73 (□).

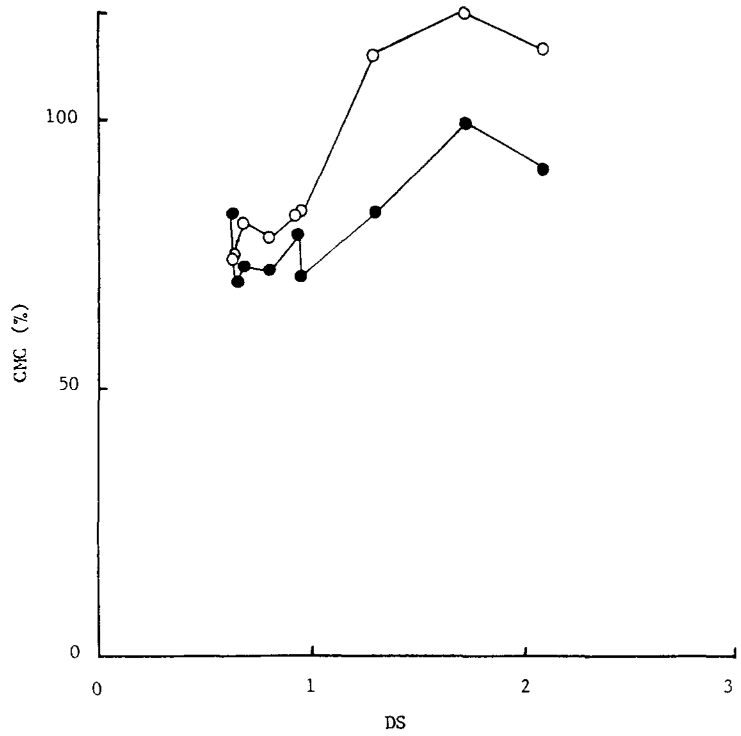

Fig. 3. Response of CMC with different DS values to the PHMBH$^+$Cl$^-$ assay calculated by (○) slope and (●) PHMBH$^+$Cl$^-$ calibration curve.

response to the PHMBH$^+$Cl$^-$ assay was observed for CMC samples with DS values greater than 1·0.

The quantitative analyses of CMC with different molecular weights by the PHMBH$^+$Cl$^-$ give similar slopes (Fig. 4). This behaviour could be due to similar charge densities per CMC molecule, since the CMC samples have the same DS. It has been reported that the molecular size is not important in determining the response to the assay (Kennedy & Bradshaw, 1986), since cross-linking occurs in the precipitation of polysaccharides with PHMBH$^+$Cl$^-$ forming insoluble white salts.

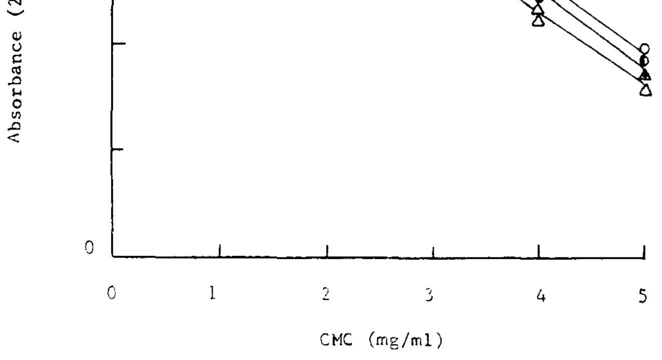

Fig. 4. Response of CMC samples with different MW to the PHMBH$^+$Cl$^-$ assay: (△) MW = 1·11 × 10^6, (●) MW = 3·16 × 10^6, (○) MW = 2·51 × 10^6.

Pectin samples with different degrees of esterification gave different responses to the PHMBH$^+$Cl$^-$ assay. Pectin with 70% (PCF) methyl ester content (high-methyl ester pectin) showed the lowest response to the PHMBH$^+$Cl$^-$ assay while pectin with *c*. 0% showed the

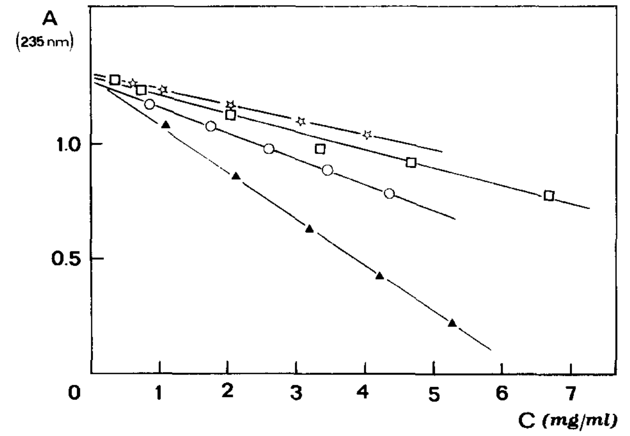

Fig. 5. Response of pectins with different degrees of methoxylation (DE) and equivalent weight (Eq. W.). (★) DE = 69 (Eq. W. = 679), (□) DE = 47 (Eq. W. = 461), (○) DE = 35 (Eq. W. = 386), and (▲) DE *c*. 0 (Eq. W. = 205), to the PHMBH$^+$Cl$^-$ assay.

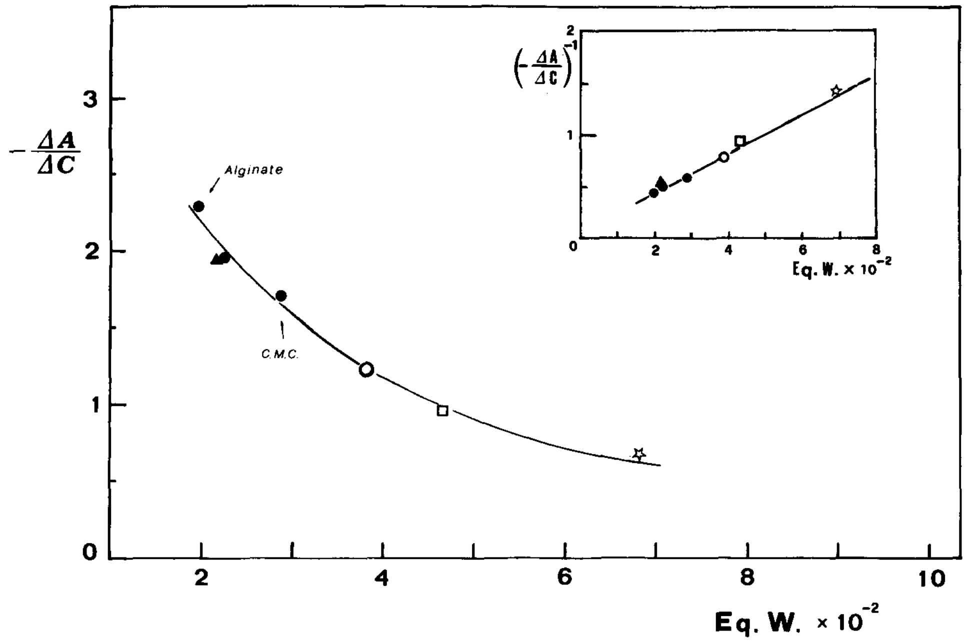

Fig. 6. Dependence of the slopes from Figs 1 and 5 on polycarboxylates equivalent weight (Eq. W.). The pectin sample (●) with DE = 6 (Eq. W. = 207) has been considered in addition to those of Fig. 5. The insert is the inverse of the slope $(-\Delta A/\Delta C)^{-1}$ against polysaccharide equivalent weight. For key see Fig. 5.

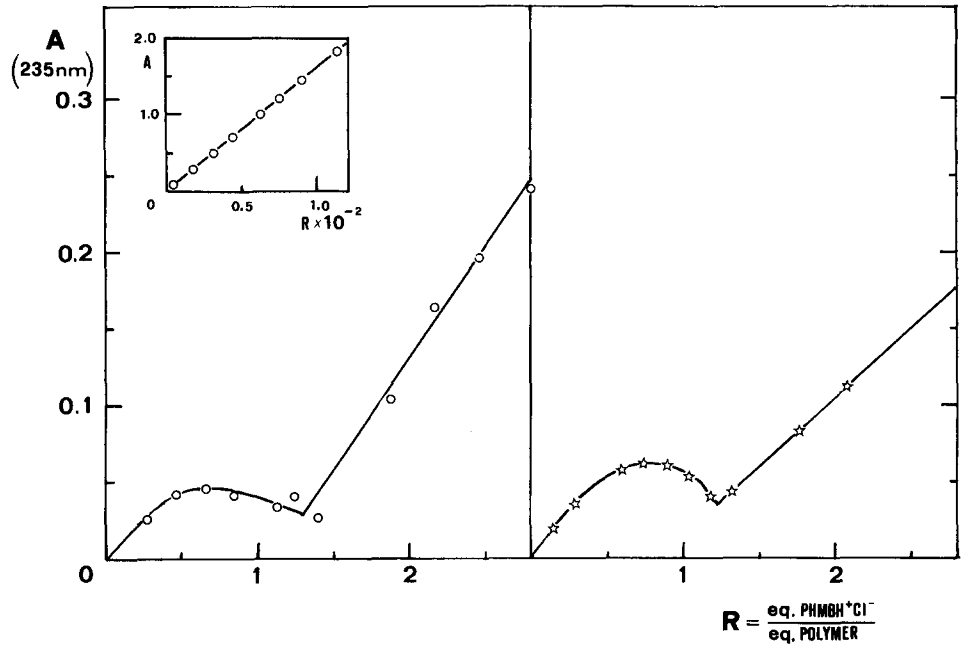

Fig. 7. Response of fixed concentration of pectins with DE = 35 (○) and DE = 69 (☆) for increasing concentrations of $\text{PHMBH}^+\text{Cl}^-$. The insert corresponds to the maximal values of A in the spectra of Fig. 8.

highest response (Fig. 5). This expected behaviour could be explained in terms of the different charge densities of the pectin molecules with different degrees of esterification. The higher the esterification, the less charge per molecule.

The dependence of the slope of the lines drawn in Figs 1 and 5 on polycarboxylate equivalent weight (Eq. W.) suggests a linear relationship between the inverse of the slope and polysaccharide equivalent weight (Fig. 6). The linear portions of the plots in Fig. 7 corresponds to quantitative pectin precipitation with sharp phase separation.

The peculiar curved portions relative to PHMBH/pectin equivalent concentration ratios less than *c.* 1·3

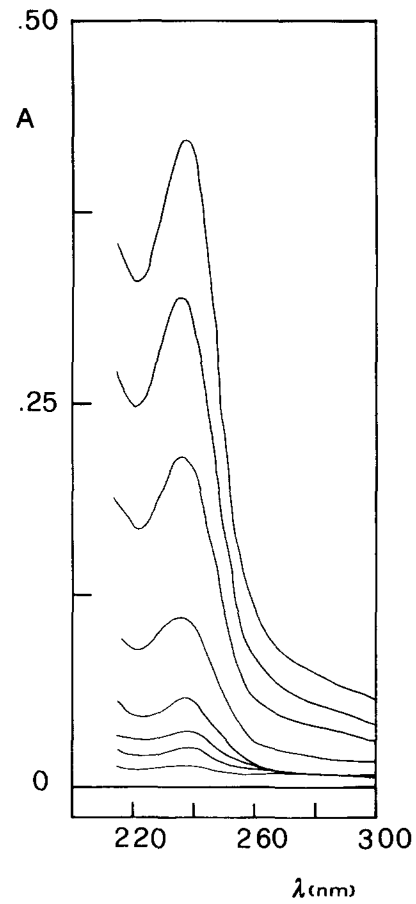

Fig. 8. $PHMBH^+Cl^-$ spectra recorded at a fixed concentration of pectin for increasing concentrations of $PHMBH^+Cl^-$.

could be traced to a changing stoichiometry of the PHMBH-pectin complexes. The original spectra recorded as explained for the insert of Fig. 7 is shown in Fig. 8. The bands of PHMBH are identical to those recorded for PHMBH in water, with no additional spectral features beyond 300 nm. Therefore, interaction with pectin does not change the spectral properties of PHMBH.

This work therefore leads to the conclusion that polysaccharides of a type but carrying different amounts of free carboxylate anion groups can be satisfactorily measured by the use of complexation with poly(hexamethylenebiguanidinium chloride). The method can yield an accurate equivalent weight value for polycarboxylates (Fig. 6), i.e. DS values in the case of samples of known w/v % concentration, or the polymer concentration when the equivalent weight (DS) is known.

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