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Effects of Chromatography Mobile Phase Properties in Analysis of Pectins

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After removal of soluble sugars and other compounds by washing, citrus peel is largely composed of pectin, cellulose, and hemicelluloses. One of the major components, pectin, can be modified using pectinesterases, which reduce the degree of methylation (DM), and DM can be determined by size exclusion chromatography using conductivity to measure the relative charge on the pectin molecules using mobile phases with low molecular salt ions present. Results herein indicate that maximum sensitivity was observed with decreasing salt ion concentrations in the mobile phase. Mobile phase containing dodecyl sulfate ions showed higher sensitivities for the standard curves as compared to formate ions. This higher sensitivity could be due to associations of dodecyl sulfate ions with the pectin molecules, resulting in a greater apparent conductivity detector response. Data indicate the importance of establishing mobile phase ion type and concentrations for degree of methylation determination when using this approach.

Pectin is a complex polysaccharide composed of at least five different sugar moieties where 80% to 90 % of its dry weight is anhydrogalacturonic acid (AGA). The majority of the AGA is found in the homogalacturonan (HG) regions of pectin as unbranched polymers of AGA in which a variable proportion of the AGA residues contains a methyl ester at the C6 position (Ridley et al., 2001; Vincken et al., 2003). Pectin's functional properties and reactivity toward calcium and other cations is largely dependent on the amount of unmethylated galacturonic acid subunits and their distribution pattern within the HG stretches (Powell et al., 1982; Willats et al., 2001). Thus, degree of methylation (DM) is an important parameter for characterization of pectin. Methods of analysis, such as the titrimetric method (Codex, 1981), require time-consuming steps of purification and sample preparation, and relatively large sample sizes. Recently a contactless conductivity detection has been developed for determination of orange pectin DM using size exclusion chromatography (SEC) to separate high molecular weight pectins from low molecular weight salts with detection by differential contactless conductivity and differential refractometer in series (Luzio and Cameron, 2010). More recent work indicates that the concentration and type of mobile phase has an effect on the standard curve for analysis of DM. This study reports the effect of using two ammonium salts, ammonium dodecyl sulfate and ammonium formate, at varying concentrations in the mobile phase when determining pectin DM.

Material and Methods

Materials. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Chemicals and reagents. Buffer for the mobile phase was 1 mM ammonium formate pH 6.5 (no. 09735, >99 % purity, Fluka BioChemika, Steinheim, Switzerland) in high purity deionized-distilled water. Pectins tested were ≥85% DM (P-4516), 55% to 70% DM (P-9436), 20% to 34%DM (P-9311), and polygalacturonic acid (P-7151). Pectins were dissolved at room temperature at a concentration of 0.5% in mobile phase.

DM analysis by contactless conductivity. DM analysis by SEC using a contactless conductivity detector in combination with a refractive index detector was previously described (Luzio and Cameron, 2010). Standard curves were developed, as described previously, for each mobile phase concentration tested. Two different ions were tested in the mobile phase, ammonium formate and ammonium dodecyl sulphate. The mobile phase was varied from 0.1 mM to 2 mM ammonium formate a flow rate of 0.6 $mL\cdot min^{-1}$ and from 0.1mM to 1 mM ammonium dodecy sulfate. Pectin solutions were micro-centrifuged at high speed before injection. Data were collected at 1.25-s intervals. Results were processed using the software provided by the manufacturer (DNDC for Windows v. 5.90.03, and Astra for Windows v. 4.90.07, Wyatt Technologies). Processed data were exported to a spreadsheet and adjusted for inter-detector delay volume and baseline.

Results and Discussion

Conductivity is affected by mobility, molecular size and shape, molecular charge, temperature and is proportional to concentration. High molecular weight ions such as pectin have lower mobility, which lessens the signal response relative to low molecular weight salt ions. In addition, higher DM pectins have lower charge density which also lowers response relative to lower DM pectins. Conductivity can also be affected by concentration of buffer present in the mobile phase and the molecular size and charge of the buffer ions. Further, pectin can undergo dissociation in the presence of varying concentrations of mobile phase ions and could be affected by the types of mobile phase ions being used and their concentrations.

Two mobile phase ions were tested at varying salt concentrations, ammonium formate from 0.1 to 2 mM and ammonium

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dodecyl sufate from 0.1 to 1 mM. Mobile phase concentrations were kept low to prevent saturating the conductivity detector. In the case of ammonium dodecyl sulfate, buffer was kept below the critical micelle concentration of 6 mM for this ion. The same cation, ammonium ion, was used in both mobile phases so that affects for anion could be compared. Ammonium ion was selected, since ammonium salts of pectin exhibit less aggregation as compared to other cations (Fishman et al., 2001). As shown in Figure 1, the slope of the standard curve had larger negative values for lower salt concentrations for both formate and dodecyl sulfate. Larger negative slopes indicate greater sensitivity at lower salt concentrations. Most likely this is due to less shielding by the salt ions in the mobile phase as the salt ion concentration decreases. Dodecyl sulfate exhibited greater sensitivity, larger negative slopes, as compared to formate ion at all concentrations tested. This may be due to dodecyl sulfate ions associating with pectin molecules that have some hydrophobicity due to the ester groups present on the molecule. Such associations would increase the negative charge on the pectin molecules and thereby increase their response to the conductivity detector.

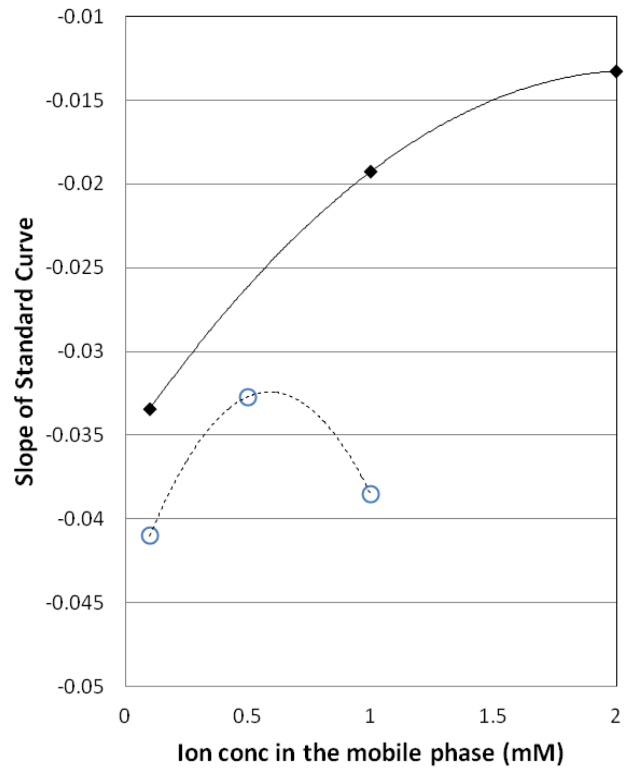

Fig. 1. Slopes of standard curves for determination of degree of esterification of pectin at different concentrations of salts in mobile phase. Ammonium dodecyl sulfate ❍ ----- ; ammonium formate ♦ —— .

Conclusion

A method was examined for determination of pectin DM using SEC to separate high molecular weight pectins from low molecular weight salts with detection by differential contactless conductivity and differential refractometer in series in mobile phases with either ammonium formate or ammonium dodecyl sulfate with varying ion concentrations. These data show the importance of using the same mobile phase for all standardization determinations and that mobile phase ion concentrations should be minimized when maximum sensitivity is required. This method may also be useful when surfactants such as ammonium dodecyl sulfate are required to dissociate the pectins for DM determinations by SEC conductivity analysis.

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