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Study of high-molecular weight compounds in sugar using gel-permeation chromatography with an evaporative light scattering detector[1]

Luis San Miguel Bento*, Susana Sá

RAR-Refinarias de Açucar Reunidas, Porto, SA, Portugal

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Abstract

Raw sugars and other sugar process materials are studied by GPC (Gel Permeation Chromatography) using a Superose 12 column. As eluent was used a solution of 30% acetonitrile with 0.005 M ammonium acetate. As detector was used to spectrophotometric Diode Array Detector (DAD) and an Evaporative Light Scattering Detector, in series. By this arrangement both chromophoric and non chromophoric compounds are detected simultaneously. High sensitivity of both detectors allows a rapid detection of high molecular weight compounds without pre concentration of samples. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Sugar; Chromatography; Light scattering

1. Introduction

Compounds with high molecular weight (HMW) are present in sugar refining materials as raw sugars, syrups and liquors. These compounds can be originated from sugar cane as starch, or can be formed during sugar extraction or refining. Dextran can be formed after the cutting of sugar cane because of infection by *Leuconostoc* bacteria. Some sugar colourants can also polymerise originating HMW compounds. The problems related with these HMW compounds in sugar refining are summarised in Table 1.

The presence of HMW compounds in sugar liquors increase their viscosity. Resulting from this fact filterability decreases, reducing production rate and increasing sweet waters. Also, crystallisation, centrifugation, carbonation and ion-exchange resin operations are affected by viscosity.

HMW compounds in white sugar are related with floc formation in soft drinks and other beverages. Also, it is observed that HMW compounds have a higher affinity to sugar crystals during the crystallisation process. As some colourants are associated with HMW polysaccharides, this fact is detrimental for sugar quality as crystal colour and haze can increase with the presence of HMW compounds in sugar liquors. Also, as polysaccharides have optical activity, their presence in sugars and sugar solution can obscure the sugar content analysis when polarimetry is used. This fact is important, as raw sugars and sugar cane are paid normal attention in polarisation analysis.

These facts support the importance of the measurement and characterisaton of HMW compounds in sugar materials during sugar extraction and refining.

To study HMW materials in sugar solutions Godshall and Clark (1988) used gel permeation chromatography (GPC), a Sephacryl S-500 column, with a spectrophotometer detector associated in series with an RI (refraction index) detector. In these tests sugar solutions were previously concentrated through dialysis.

In the study presented here, GPC is used to separate HMW material in cane sugar products. These compounds were detected using a diode array detector (DAD) and an evaporative light-scattering (ELS) detector in series. Bento et al. (1997) used this technique to study HMW compounds in cane and beet sugar products.

With this arrangement, can one detect compounds, such as polysaccharides, not detected by spectrophotometry at 280 nm or higher wavelengths.

2. Light-scattering detector

An evaporative light-scattering detector is a sensitive detector for non-volatile solutes in a volatile liquid stream. Eluent enters the detector at the top of the evaporation

* Corresponding author

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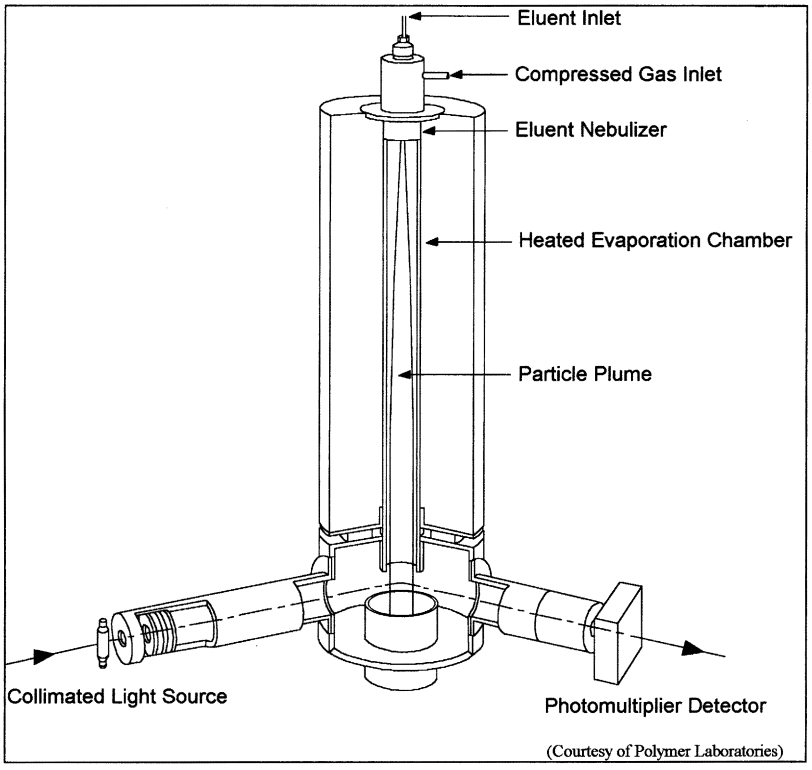

Fig. 1. ELS detector.

chamber (Fig. 1). The eluent/solute is fed into the nebulizer assembly where an air-operated venturi jet atomises the eluent into a uniform dispersion of droplets which then pass as a continuous stream into the evaporator. When pure eluent is being evaporated, only its vapour passes through the light path and the amount of light scattered to the photodetector is small and gives a constant response. When non-volatile solute is present, a particle cloud passes through the light, causing light to be scattered. This scattered light enters the optical aperture of the detection system and generates a signal response from the photomultiplier The signal is amplified and gives a voltage output result. The quantity of light detected is dependent on the solute concentration and solute particle size distribution (PL-EMD960 Operator's Manual).

Table 1
HMW compounds in sugar refining

Problem	Location
Increase viscosity	Filtration
	Crystallisation
	Centrifugals (purging)
	Ion-exchange resins
	Carbonation
Floc formation	Soft drinks
	Other beverages
They are 'crystallophilic'	Colour in sugar crystals
They have optical activity	Polarisation measurements
	Payments cane/raw

3. Materials and methods

3.1. Equipment

Pharmacia FPLC system equipped with a liquid chromatography Controller LLC-500 Plus and High Precision Pump P-500.

Column (Pharmacia): 300 mm length and 10 mm internal diameter, packed with Superose 12, cross-linked, agarose-based medium, with an average particle size of 10–11 μm, and an exclusion limit of ca 2×10^6 g/mol (globular proteins).

Detectors: Lichograph diode array detection system (DAD) L-4500 Merck–Hitachi, continuous absorption measurement in the UV/VIS range.

Evaporative light scattering detector (ELS): PL-EMD 960 Polymer Laboratories.

Other equipment used: Laboratory centrifugal (MSE, Basket 300); ultrasounds (Bandelin, Sonorex TK30); Nylaflo membranes (Gelman Sciences).

3.2. Reagents

Acetonitrile, gradient grade, for chromatography and ammonium acetate (Merck).

3.3. Eluent and sample preparation

With the ELS detector, an eluent with a low boiling point must be used for evaporation inside the detector. In this study we used distilled water with 30% acetonitrile, containing

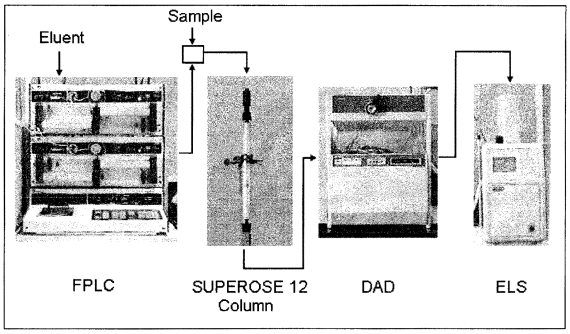

Fig. 2. Experimental apparatus.

0.005 M ammonium acetate. The eluent, at pH 7.0, was filtered through a 0.20 μm membrane and then degassed for 15 min with ultrasound.

Samples were diluted in eluent and adjusted to pH 7.0. Solutions were filtered through a 0.02 μm membrane and then degassed for 5 min with ultrasound.

3.4. Run method

Before each run, the system was stabilised for a period of 30 min with eluent at 0.30 ml/min. Run time of 80 min started with sample (200 μl) injection. Eluent flow rate was 0.30 ml/min. Effluent was analysed in DAD and ELS detectors placed in series (Fig. 2).

Measurements with DAD detector ranged from 240 to 450 nm.

Conditions at the ELS detector were: air flow at 5 l/min and air temperature 80°C. For security reasons a flow of nitrogen was fed continuously into the detector case.

Table 2

	A	B	C
Retention times (min)	20–30	30–48	48–57
Molecular weight (kDa)	> 250	12–250	2.5–12
Raw cane sugar (%)	2.8	56.7	40.5
Affined sugar (%)	9.2	70.3	20.5
Carbonated liquor (%)	8.7	6.8	84.5
Fine liquor (%)	8.5		91.5
White sugar (%)	55.1	3.6	41.3
White sugar (washed, %)	70.4	2.2	27.4
Molasses (%)	0.8	39.5	59.7

3.5. White sugar wash

White sugar, 50 g, calibrated between sieves 0.300–0.425 mm, was mixed with 50 g of methanol saturated with sugar. This mixture was introduced into a flask and placed in a water bath with a rotating system for a period of 30 min at room temperature. The mixture was then centrifuged at 4000 rpm for a period of 5 min to obtain the washed sugar.

4. Results and discussion

The tests described in this article used a Superose 12 column as described in Section 3.4. In order to protect the column, samples were filtered through a 0.20 μm membrane. With this filtration, compounds of very high molecular weight will be removed and are not considered in this study.

During gel chromatography, compounds of very high molecular weight (higher than 2000 kDa with the column used) will pass the column, with the velocity of the eluent forming the first group to be detected. Afterwards, colourants

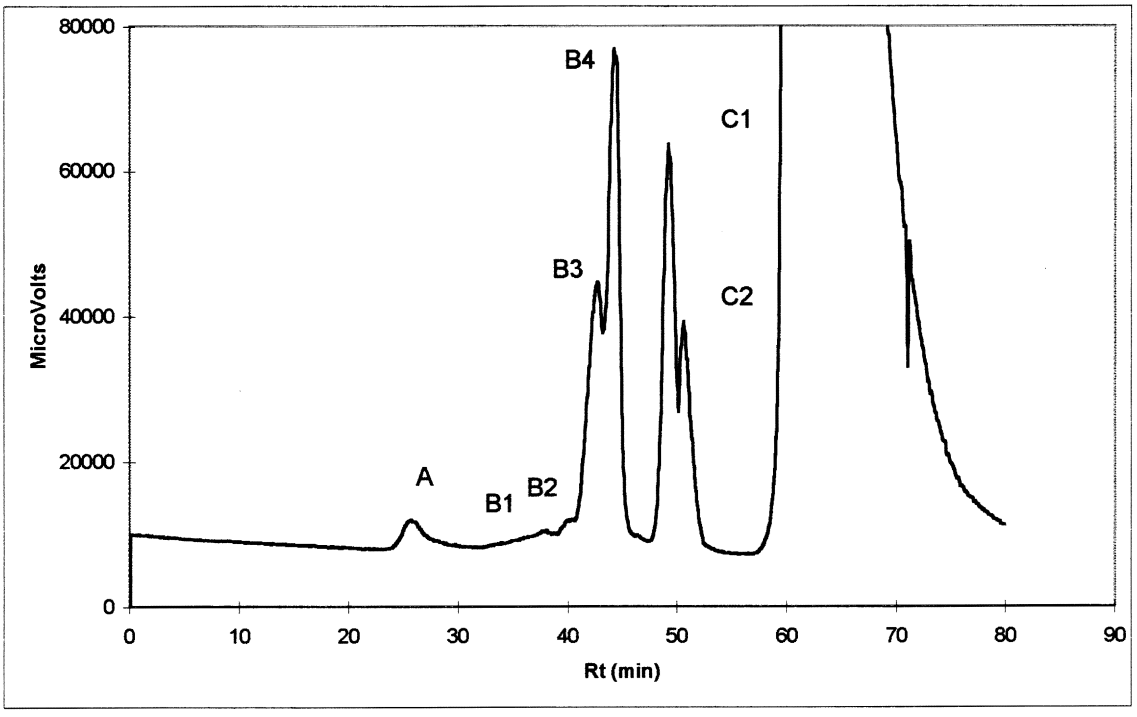

Fig. 3. Cane raw sugar (ELS).

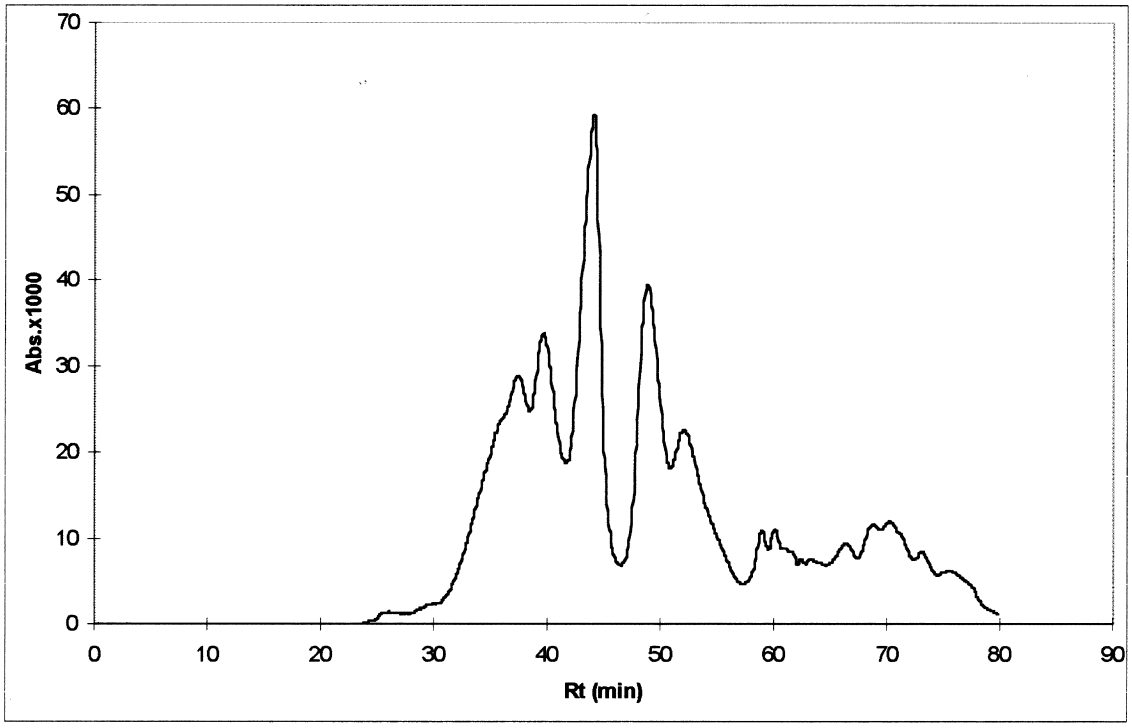

Fig. 4. Cane raw sugar (DAD).

are separated by decreasing molecular weights. Compounds with molecular weights lower than the inferior separation limit of the column will be eluted in one final group: with Superose 12, approx. 1 kDa. Sucrose, simple sugars and other small molecules will be eluted together at the end of the run. As we do not separate sucrose in the samples we can not detect in the ELS detector colourants of low MW as they are eluted mixed with sugars. Therefore, in this study, we are limited to compounds with MW higher than 1 kDa, which will be referred to as HMW material.

As a result of the physical arrangement of detectors there is a time difference of 0.39 min between the two detections. In the following text, when not specified, retention times are referred to the ELS detector. In the figures, R_t is referred to the respective detector. The chromatographic column was calibrated in a previous work (Bento et al., 1997). DAD results are presented at 330 nm. For simplicity, the expression 'colour' means absorbance at this wavelength.

In this study we tested one cane raw sugar from Cuba. Observing the results of can raw sugar with an ELS detector (Fig. 3), we can consider three groups of compounds. The first group (A) corresponds to retention times (R_t) between 20 and 30 min. This group comprises compounds of very high molecular weight (more than 250 kDa). As observed in Fig. 4, this group of colourants has low colour intensity. These compounds must correspond to colourants associated to polysaccharides, as described by Godshall et al. (1992).

In a previous study with a cane raw sugar, Bento and Sá (1997) observed that these compounds remain preferentially in sugar crystals after affination.

Table 2 presents the percentages of peak areas, calculated on ELS detector values, of the three groups considered.

As observed, Group A represents 2.8% of HMW compounds from cane raw sugar sample.

The second group (B), with R_t between 30 and 48 min, comprises compounds with high molecular weight and a high colour intensity. Molecular weight ranged from 12 to 250 kDa. In this group we can consider two peaks with 38 and 40 min of R_t (B1 and B2) that present high colour intensity (Fig. 4). In group B, another peak (B3) is observed with a R_t of 43 min. This peak must comprise compounds

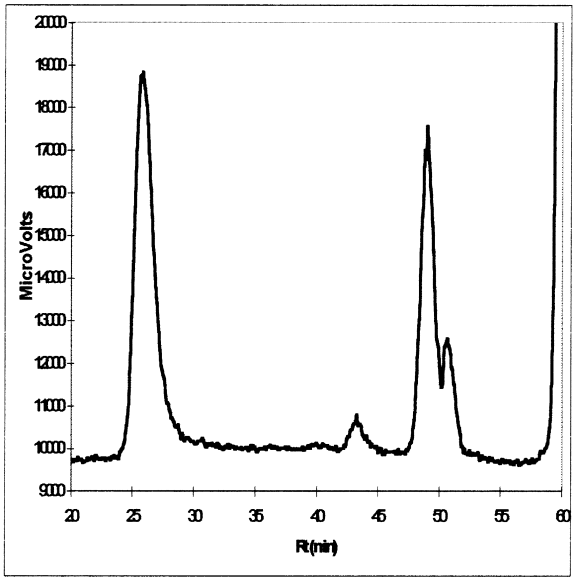

Fig. 5. Cane white sugar (ELS).

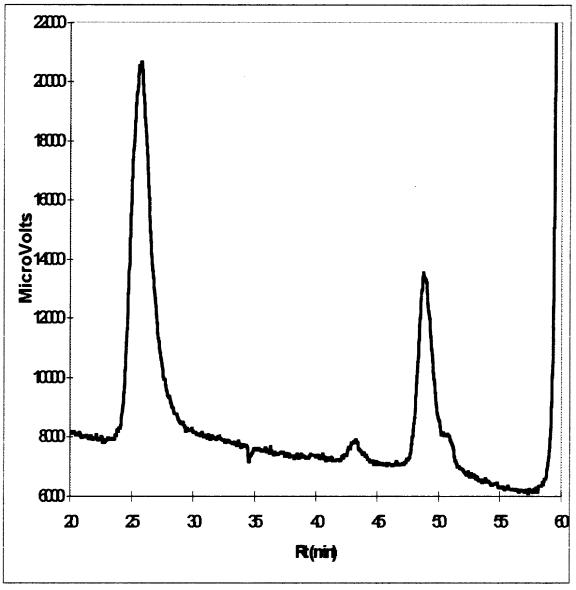

Fig. 6. Washed cane white sugar (ELS).

with low or no colour, as an equivalent peak in the DAD chromatogram is not observed.

Group B represents the majority of HMW compounds from the cane raw sugar sample, that is 56.7% of these compounds.

The next group (C) comprises compounds with lower molecular weight, between 2.5 and 12 kDa, with R_t from 48 to 57 min. In this group we observe two peaks: C1 at 49 min and C2 at 51 min of R_t. This group represents 40.5% of HMW compounds in the raw cane sugar sample. Group C must contain a small quantity of low-MW compounds with high colour intensity as can be observed comparing DAD and ELS results.

Other tests were made using samples of cane white sugar with a colour of 57 IU. Sugar was washed with methanol as described earlier. With this wash, colourants in the outside layer of the crystal were removed. Tests were made with original sugar and washed sugar. Results with ELS detector are presented in Figs. 5 and 6 and in Table 2.

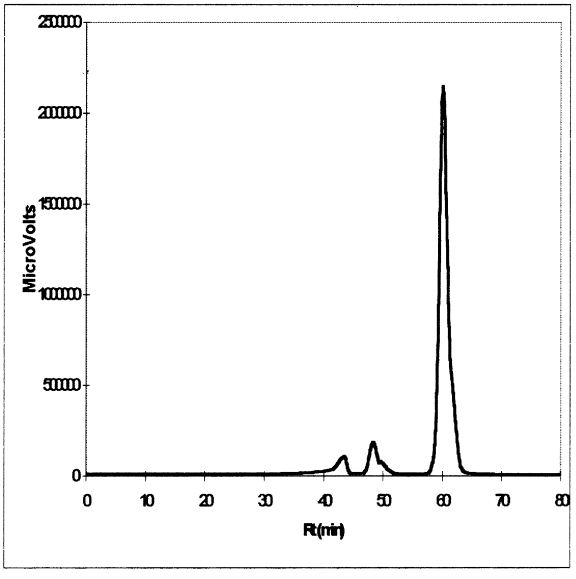

Fig. 7. Molasses (ELS).

It is observed that two main groups of colourants remain in cane white sugar: Group A and Group C1. A small presence of Group B and C2 is observed. After methanol wash, Group C was reduced, from 41.3% to 27.4%, indicating that these compounds are preferentially in crystals syrup layer. Group A compounds of very high molecular weight remained inside the sugar crystals. These compounds remain through the refining process (carbonation + resins) from raw to white sugar.

Fig. 7 and Table 2 present the results, in the ELS detector, of molasses. It is observed that this product presents a higher percentage of group C compounds as compared with the raw sugar.

5. Conclusion

This technique uses medium pressure pumps, a GPC chromatographic column, a UV spectrophotometric detector and an evaporative light-scattering (ELS) detector. The main advantage of this technique is to be able to analyse materials that are not detected by UV spectrophotometers. This technique can be important for studying how decolourizers or separation systems, as membranes, remove or separate different colourant groups. We can also identify colourants more harmful to the refining process, that is, those that have a higher tendency to be included in white sugar crystals.

The ELS detector can be associated with a more accurate chromatographic technique, such as HPLC or IC. By the results obtained here we can preview that the ELS detector can give useful information on high molecular weight materials and sugar colourants in factories and refineries.

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References

Bento L. S. M., Pereira, M. E., Sá, S. (1997). Study of sugar colourants through Gel Permeation Chromatography using UV and Evaporative Light Scattering detectors, *Proceedings of SIT*, Montreal 1997, pp. 383–392.

Bento L. S. M., Sá, S. (1997). Applications of Evaporative Light Scattering Detector to study Beet and Cane sugar colourants separated by Gel Permeation Chromatography, *EuroTechLink 97*, York.

Godshall M. A., Clark M. A., (1988). High molecular weight (HMW) color in raw and defined sugars, *Proc. of S.I.T. Conf.*, pp. 180–193.

Godshall, M. A., Clark, M. A., Miranda, X. A., Blanco, R. S. (1992). Comparison of refinery decolourization systems, *Proc. of the Sugar Proc Res. Conf.*, pp. 281–305.

PL-EMD960 Operator's Manual for Light Scattering Detector, Polymer Laboratories (UK).