CHEMICAL AND PHYSICAL PROPERTIES OF COLOURED DEGRADATION PRODUCTS OF SUGARS

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ABSTRACT

The molecular size distribution of sugar degradation colorants may be determined by Sephadex gel chromatography and molecular weights derived from these data by assuming a suitable molecular shape. In solutions of higher ionic strength colorants take up a random coil configuration similar to that of dextrans. Molecular weights calculated on this basis agree with those determined by vapour pressure osmometry. The molecular weight of colorants tends to increase with increasing net molecular charge. There is, however, a spread of molecular weights within each group of colorants of the same net charge. The significance of these properties to sugar refinery processes is discussed.

INTRODUCTION

As long as white sugar is required by consumers (industrial as well as domestic) the removal of coloured impurities will be a very important stage in the refining process, even though these impurities represent but a small percentage of the total non-sugars. Decolorising processes of any sort will be most effective when a knowledge of the chemical and physical properties of the colorants can be applied to their design and operation. It has been the aim of our investigations to obtain such basic information and apply it to refining process.

A part of the coloring matter in raw sugars and refinery liquors comprises natural pigments originating in the cane or beet. The remainder is produced by the degradation of sugars during the extraction and refining processes. The relative amounts of each type of colorant will depend on the nature of the processes to which the sugar has been subjected. The degradation colorants are most readily formed at high temperatures and pH and especially in the presence of amino acids.

In our laboratories a range of reproducible standard synthetic colorants has been developed for use in the routine assessment of the decolorising performance of ion exchange resins. These colorants closely resemble the degradation colorants isolated from sugar liquors in all properties so far examined (ultraviolet, visible and infra-red spectra; molecular weight and charge distributions; cation/anion balance and precipitation by surfactants). Consequently they have been used as models in our studies of the properties of sugar degradation colorants. In particular the Maillard colorant obtained by degrading glucose in the presence of glycine at 100 C and pH 9.5 represents about 90% of the degradation colorants found in refinery liquors. The remainder is zwitterionic in nature and is closely approximated by material produced by degrading glucose in the presence of lysine.
This paper describes a detailed study of the molecular weight and molecular size distribution of the complex mixture of colored species which comprises the Maillard colorant derived from glucose and glycine. Extensive use is made of the technique of Sephadex gel chromatography.

**EXPERIMENTAL**

**Preparation and Isolation of Colorant**

A model Maillard colorant solution was prepared as described by Cookson et al. Fractions essentially homogeneous with respect to net anionic charge were isolated by chromatography on a column of anion exchange cellulose (Whatman DE32) in 8 Molar urea. Elution with a linear gradient of sodium chloride gave a series of peaks (Fig. 1) corresponding each to colorant molecules of integral net charge. Fractions corresponding either to these peaks or their calculated positions were pooled giving a series of colorant fractions of net charges 2 to 7.

![Figure 1. Net charge profile of a Maillard colorant.](image)

Further colorant fractions were obtained by successively passing the colorant solution over a column (0.5 cm³) of Amberlite XE 279, alternating with 2 Molar sodium chloride to elute the adsorbed colorants. These fractions were not homogeneous in net charge but represented material of successively lower average charge.

Colorant fractions were freed from urea and salts by a modification of the technique of Fleming et al. Adsorption from a solution of pH 3 onto a column (10 cm³) of Phasepak Q (a gas chromatographic packing related to Amberlite XAD-2) was followed by washing with N/1000 hydrochloric acid. Elution with N/1000 methanolic hydrochloric acid gave a concentrated colorant solution of which a portion was esterified with diazomethane (giving methyl esters) and the remainder neutralised and freed from methanol for gel permeation experiments.
Molecular Weight Determinations by Vapour Pressure Osmometry

The number average molecular weights of the colorant esters were measured in chloroform solution with a Mechrolabs Vapour Pressure Osmometer. In this instrument a drop of solution of known concentration is placed on a thermistor bead surrounded by solvent vapour in an accurately thermostatted chamber. The presence of solute causes solvent to condense on to the bead. The resultant thermal effect is measured as a change in thermistor resistance which is related to the molarity of the solution. The instrument needs to be calibrated with a solute of known molecular weight.

Gel Permeation Chromatography

A column (25 cm x 35 cm) of Sephadex G50 (fine) was used, eluted at 0.5 cm³/min with 8 Molar urea (or 8 Molar urea + 0.5 Molar sodium chloride). Samples were introduced without interrupting the flow by layering a solution slightly denser than the eluate onto the top of the column packing. The column effluent was led through a flow cell in a Perkin Elmer 124 Spectrophotometer, the electrical output of which was amplified and integrated by a Honeywell Precision Integrator set to give a digital print out of cumulative colour (at any chosen wave length) at 5 minute intervals. Sensitivity is 50 000 counts per adsorbance unit and baseline stability can be ± 5 counts. This enhanced sensitivity permits the examination by gel permeation of solutions of low optical density (e.g. 0.1 at the wavelength chosen). Accuracy of elution volume measurement was ensured by running at the same time a sample of Blue Dextran (molecular weight 2 000 000). This is totally excluded from the Sephadex gel and acts as a void volume marker.

Ion Exchange Cellulose Chromatography

The colorants, in a solution 8 Molar in urea and dilute enough to avoid premature elution by salts, were adsorbed onto a column (5 cm³) of DE32 (diethyl amino ethyl) cellulose in the chloride form. The flow rate was 0.5 cm³/min. Elution was by a linear gradient (0-0.5 Molar) of sodium chloride in 8 Molar urea. The column effluent was monitored in the same way as the Sephadex column effluents.

RESULTS AND DISCUSSION

Molecular Weights

Gel permeation chromatography separates materials according to their molecular size. The separation depends on the extent to which molecules of different sizes are able to diffuse into the pores of the separation medium. The largest molecules pass most rapidly through the column. Relationships have been derived between molecular (Stokes) radius (r) and the partition coefficient on gel permeation (Kav).

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Kav = \frac{Ve - Vo}{Vo} \quad Ve = \text{elution volume of substance} \\
Vo = \text{void volume of column} \\
Kav = \frac{Vt - Vo}{Vo} \quad Vt = \text{total volume of gel bed}
\]

\[
r = \sqrt{-\ln(Kav)/\pi L - R}
\]

L and R are constants relating to the gel structure
The relationship between molecular radius and molecular weight, however, depends on the molecular configuration in solution. For instance proteins are regarded as being globular, dextran are random coils and acidic polysaccharides are extended random coils on account of the mutual repulsion of charged groups. Acidic polysaccharides change their shape and molecular size with changes in solution ionic strength. At high ionic strengths the repulsive forces are screened, allowing the molecule to contract. We have observed this phenomenon in the case of sugar degradation colorants. This is shown in Fig. 2 which compares the size distribution of a colorant fraction in 8 Molar urea and in 8 Molar urea + 0,5 Molar sodium chloride (no further effect is observed at higher ionic strengths). Molecular radii were obtained by calibrating the Sephadex G50 with molecules of known size.

Eight Molar urea was used as the eluent in these studies in order to minimise adsorptive effects due to hydrogen bonding between solutes and the Sephadex gel. Sephadex G50 was chosen as the medium with the best combination of low adsorption and good separation in the molecular size range being examined. The low adsorption is indicated by the elution of all colorants before the "included volume" of the gel (taken as the elution volume of potassium chromate).

In view of the contraction of colorant molecules under conditions of high ionic strength it seemed reasonable to assume that they took up a random coil-like configuration similar to that of dextran and oligosaccharides. Fig. 3 shows the relationship between the number average molecular weights calculated on this basis from the gel permeation chromatograms of colorant fractions in 8 Molar urea/0,5 molar sodium chloride, and the molecular weights determined by vapour pressure osmometry on the colorant methyl esters. (A calibration curve relating the molecular weights of dextran and oligosaccharides with Kav on G50 Sephadex was drawn up from our own observations on oligosaccharides and the data of Laurent and Killander for dextran fractions).
The colorant fractions examined are of course not single molecular species but mixtures of colorants with a range of molecular weights. Hence the need for expressing molecular weights as a number average ($M_n$) which is calculated from gel permeation data by the formula

$$M_n = \frac{\sum C_i}{\sum \frac{C_i}{M_i}}$$

$C_i =$ amount of material in each fraction

$M_i =$ molecular weight corresponding to that fraction

The errors in the determination of $M_n$ by gel permeation are estimated as less than 10%. The straight line in Fig. 3 shows that, within these limits and under the conditions of ionic strength specified the dextran model is quite a valid one for the molecular shape of the colorants. The colorant molecules are slightly larger than a dextran of the same molecular weight. The relationship is less accurate at lower molecular weights. This may be due to differences in detailed molecular structure between colorants and dextrans becoming more important when only a small number of polymer repeating units are involved.

As the column of G50 Sephadex had also been calibrated for polyethylene glycols the number average molecular weights of the expanded forms of the colorants were calculated on this basis from the gel permeation chromatograms of the colorant fractions run in 8 Molar urea alone. The relationship is similar to that in Fig. 3. Thus polyethylene glycols of a given molecular weight have the same size as colorant molecules in their extended form in the absence of salts.

Charge Densities

Colorants obtained from the degradation of reducing sugars are carboxylic acids and may be fractionated by ion exchange chromatography. The best medium for this has been found to be diethyl-aminoethyl cellulose. If the fractionation is conducted in 8 Molar urea interactions between adsorbent and substrate, other than ion exchange, are reduced to a minimum. Elution of
adsorbed colorants with a linear salt gradient separates colorants according to their net molecular charge.6,7

Our standard Maillard colorant contains small amounts of material with no net charge; while colorant prepared by degrading glucose in the presence of lysine contains up to 40% of material with no net charge. These colorants are naturally not adsorbed by the ion exchanger. A net charge profile of the former colorant (which was fractionated for molecular weight measurements) is shown in Fig. 1. Each peak of the chromatogram is labelled with its net charge (n) assigned with the aid of the linear relationship between log n and the elution position, and also with the molecular weights of the material in the peaks.

The almost linear relationship between molecular weight and net charge for the first few peaks indicates an apparent molecular weight for the polymer repeating unit of approximately 150. It is however dangerous to draw any structural conclusions from these figures as each fraction is a mixture of many components.

A closer examination of each peak in such a charge profile reveals more of this complex situation. The "N value" of colorants has long been used in these laboratories as an indication of molecular weight.

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N = 100 \frac{(\text{Absorbence at 520 nm})^2}{(\text{Adsorbence at 455 nm})}
\]

This property changes across the peaks in the profiles. The leading edge of each peak has the highest N value and thus the highest molecular weight. This is confirmed by gel permeation chromatography of fractions from each peak. The molecular weights corresponding to the maximum in the gel permeation chromatogram of each fraction examined are recorded in Fig. 4.

![Figure 4](image-url)
The mechanism behind this phenomenon has yet to be fully established. The pores of cellulose are too large to cause separations by gel permeation. The variation in properties across the peaks is in fact a change in the charge density on the colorant molecule, (charge divided by molecular weight); molecules with a lower charge density being more readily eluted than those which have a higher, even though they may bear the same net charge.

Sugar Refining Processes — Ion Exchange Decolorisation

The significance of the analytical techniques described lies in the application of the knowledge of colorant properties to refining processes. Sugar liquor decolorisation by ion exchange resins is governed by the two properties described in this paper. Molecular size controls the accessibility of colorant molecules to adsorptive sites, while net charge dictates how firmly the colorant will be held by the ion exchanger and how likely it is to be displaced by molecules of a higher net charge (self-elution).

Accessibility is controlled by the porosity of the resin. A highly cross-linked resin such as Dowex 1 × 10, which has small pores, is found to reject the colorants with high molecular weight as they are too large to diffuse as far as the adsorptive sites.

At the other end of the scale, Dowex 1 × 2, which has a low degree of cross-linking and thus larger pores, fails to adsorb material with low molecular weight and low charge. These colorants have been displaced from the adsorptive sites by more highly charged material which is larger but still has free access.

IRA 401S, which is of medium porosity shows a hybrid behaviour (Figs 5 and 6). Here the molecular size profile of the effluent colour shows two peaks. These are the rejected high molecular weight material and the eluted low

![FIGURE 5. Molecular size distributions of colorant treated with IRA 401S.](image-url)
molecular weight colorants. The charge profile similarly shows material at the high and low ends. Colorants with medium size and charge have gained access to the adsorptive sites and eluted smaller colorants of lower charge.

If this size and charge information can be combined with a detailed knowledge of the porosity and charge structure of ion exchange resins their potential as sugar liquor decolorising agents would be greatly enhanced.

The "Talofloc" Process

This process, which has been described in detail by Bennett, involves the precipitation of colorants together with colloidal matter by phosphatation in the presence of dioctadecylidimethyl-ammonium chloride. The colorants precipitated by this process, and also those remaining in solution, have been examined by ion exchange chromatography (Fig. 7). It is apparent that the more highly charged colorants are preferentially removed. More surfactant molecules are able to combine with them to produce an insoluble compound. The presence of small amounts of material with two and three charges in the precipitated material where the majority is not precipitated is attributable to the charge density differences noted above.

Membrane Processes

There is increasing interest in the use of membrane processes in sugar refining. In such processes it is possible to exploit the molecular size differences between water, salts, sucrose, colorants, etc., to achieve some purification of sugar liquors, as the separation process primarily depends on the molecular sizes of solutes relative to membrane pore sizes.
Re Reverse osmosis uses membranes with very small pores and has potential for desalting and concentrating sugar solutions in that sucrose is retained whereas smaller molecules pass through. Ultrafiltration can be used to separate sugar from the colorants in liquors by employing more open membranes.

Given a detailed knowledge of colorant molecular sizes and their distribution the development and choice of membranes for these processes could be greatly facilitated.

**CONCLUSIONS**

The work described emphasises once again the difficulties involved in examining colorants by revealing the complexity of the mixtures of substances that make up sugar degradation colorants. There is a wide range of net molecular charge, and molecular weights extend from the low hundreds upwards. Molecular radii extend from 0.4-0.5 nm upwards and vary with the ionic strength of the environment between a random coil configuration similar to that of dextrans to a more extended structure similar to polyethylene glycols. The combination of charge and molecular size results in a wide variation of molecular charge densities.

**FIGURE 7.** Net charge profiles of Talofloc treated colorants (40% precipitation).
It is only by detailed examinations of such properties that a true understanding of the working of refinery processes can be achieved, and in gel permeation and cellulose ion exchange chromatography we have two powerful tools for such work. The techniques are mild and may be used to examine colorants with little or no pretreatment. As yet we have applied these techniques chiefly to model colorants but preliminary application to liquor colorants indicates that their full potential for analytical work has yet to be realised.

REFERENCES

PROPIDADES QUIMICAS Y FISICAS DE LOS PRODUCTOS COLORANTES DE DEGRADACION DE AZUCARES

RESUMEN
La distribución del tamaño molecular de los colorantes resultantes de la degradación de azúcares puede ser determinada por cromatografía con gel Sephadex y los pesos moleculares pueden ser derivados de los datos suponiendo una forma molecular adecuada. En soluciones de alta concentración iónica, los colorantes toman una forma helicoidal irregular parecida a la de las dextranas. Los pesos moleculares calculados sobre esta base concuerdan con los determinados por osmometría de tensión de vapor. El peso molecular de los colorantes tiende a aumentar a medida que aumenta la carga molecular neta. Hay, sin embargo, una gama de pesos moleculares dentro de cada grupo de colorantes de la misma carga neta. Se discute el significado de estas propiedades en el proceso de refinación del azúcar.