Journal of Chromatography, 84 (1973) 129–135

CHROM. 6815

EFFECT OF POLYETHYLENE GLYCOLS ON THE MOBILITY OF THE POLYPHENOLIC CONSTITUENTS OF WATTLE EXTRACT ON CELLULOSE

A. E. RUSSELL, M. CORCOY* and D. R. COOPER

Leather Industries Research Institute, Rhodes University, Grahamstown (South Africa)

(Received February 28th, 1973)

SUMMARY

In a previous study, the effects of polyethylene glycols of increasing molecular weight in aqueous solution on the affinity of catechin on cellulose have been investigated by paper chromatography. In this paper, similar effects of the polyethylene glycol series on the affinity of the polyphenolic constituents of wattle extract on paper have been investigated. As for catechin, the migration of the more mobile constituents increased with concentration and, in particular, with the degree of polymerization of the various glycols, conforming to the empirical expression

$$A_n = A_1 n^b$$

where A_n and A_1 are the activity functions for polymer and monomer, respectively (expressed as the molar change in R_M at infinite dilution), n is the number of repeat structural units in the molecule and b is a characteristic constant for the series. The rapid increase in mobility with polar group content of the polymers implicit in the above relationship is considered to be consistent with a mechanism involving cooperative hydrogen bonding of these groups to complementary sites on cellulose, resulting in competitive displacement of bound tannins.

INTRODUCTION

In previous studies in these laboratories, changes in the mobility of the polyphenolic constituents of wattle extract[1] and of (±)-catechin[2–4] in paper chromatography have been examined as a function of composition in the aqueous developing medium as a means of investigating the non-covalent interactions that govern affinity between polyphenolic compounds and cellulose. Although the relationship between chromatographic mobility and solute structure has been well documented and reviewed[5–10], constitutive effects of structural groups in the developing solvent medium have been less extensively studied.

The chromatographic mobility of wattle constituents and catechin on paper was found to increase with hydrocarbon chain length using aqueous aliphatic alcohols, glycols, ketones and nitriles as developing media[1–4]. Molar effects of the solvents

* Present address: Facultad de Biologica, Universidad de Barcelona, Barcelona, Spain.

at infinite dilution on the R_M values of catechin increased substantially in a linear manner with the number of methylene groups present in the hydrocarbon chain, irrespective of the functional group present, resulting in a series of parallel linear trends with intercepts that varied with the functional group[4]. These structural effects in the developing solvent were therefore analogous to similar trends proposed for solute structures[9], indicating that in the absence of disturbing group interactions, Martin's postulates[5] regarding the additive and constitutive nature of solute group contributions could be extended to the developing solvent medium.

In the case of the polyethylene glycol series, however, molar effects on catechin mobility increased rapidly with the molecular weight of the glycols, resulting in a marked deviation from linearity with respect to structural group addition[4]. This effect is attributed to cooperative interactions of the polar groups resulting in multi-point hydrogen bonding of the glycols to complementary sites on cellulose. On this basis, polymer binding could be expected to increase rapidly with the content of active polar groups, accounting for the concomitant displacement and rapid increase in catechin mobility with the degree of polymerization.

In the present study, examination of the effects of polyethylene glycols on chromatographic behaviour has been extended to the polyphenolic constituents of wattle extract.

EXPERIMENTAL

Materials

Polyethylene glycols were BDH (Poole, Great Britain) or Shell Chemicals Ltd. (London, Great Britain) laboratory-grade polymer fractions. Commercial black wattle (*Acacia mearnsii*) fresh bark powdered extract was used throughout, in which the polyphenols constitute the major fraction, about 75% of the anhydrous extract.

Methods

Ascending chromatography in small-scale systems was carried out as previously described[1–4] on 20 × 180 mm Whatman No. 1 paper strips in the machine direction suspended in 1-litre glass-stoppered reagent jars. Polymer concentrations were varied over a range, the upper limit being governed by practical considerations such as solution viscosity. The room temperature was controlled at 20 ± 2°. The polyphenolic constituents were rendered visible after development using an ammoniacal silver nitrate spray.

RESULTS

Typical mobility profiles obtained by plotting R_F values corresponding to the more mobile constituents in the extract as a function of polyethylene glycol concentration in the aqueous developing solution are shown in Fig. 1a. R_F values obtained by either spot development or the frontal analysis technique in which the wattle extract was dissolved in the polyethylene glycol developing solution in low concentration agreed closely.

As reported for catechin[4], polyphenolic mobilities typically increased with polyethylene glycol concentration, approaching the solvent front assymptotically at

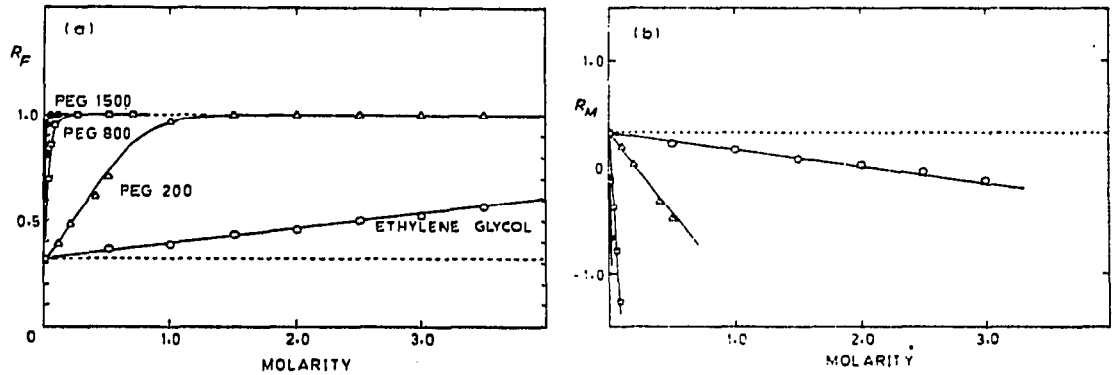

Fig. 1. Typical profiles for wattle polyphenol mobilities in paper chromatography as a function of polyethylene glycol concentration at 20°. (a) R_F variation; (b) R_M variation.

higher concentrations. Similarly, a rapid increase in molar effect on polyphenolic mobility was also apparent as the degree of polymerization increased. Corresponding R_M profiles for the various polyethylene glycols are shown in Fig. 1b.

Quantitative comparison of molar effects was carried out as previously described[4] by determining the initial slopes of the R_F and R_M *vs.* polymer concentration plots from the least-squares parameters obtained by statistical fitting of either a linear or a quadratic equation to the initial data in each case with the aid of a Hewlett-Packard 9100 B programmable calculator. These initial gradients, corresponding to the intrinsic polymer molar activity at infinite dilution in terms of R_F and R_M effects, respectively, are reported in Table I.

TABLE I

MOLAR EFFECTS OF POLYETHYLENE GLYCOLS (PEGs) AT INFINITE DILUTION ON MOBILITY OF WATTLE POLYPHENOLS

Glycol	*Degree of polymerization*	$[\Delta R_F/\Delta M]^0$ *(experimental)* $(l \cdot mole^{-1})$	$[\Delta R_M/\Delta M]^0$ *(experimental)* $(l \cdot mole^{-1})$	$[\Delta R_M/\Delta M]^{0*}$ *(calculated)* $(l \cdot mole^{-1})$	$(\Delta F)(CH_2CH_2O)^{**} = 2.303\ RT\Delta R_M$ (CH_2CH_2O) $(kcal \cdot mole^{-1})$
Ethylene glycol	1	0.08	− 0.15	− 0.14	− 0.34
PEG 200	4.1	0.77	− 1.50	− 1.87	− 1.1
PEG 400	8.7	3.76	− 6.84	− 7.15	− 2.0
PEG 600	13.2	9.27	− 16.8	− 15.3	− 2.8
PEG 800	17.8	11.7	− 23.4	− 26.2	− 3.6
PEG 1000	22.3	28.8	− 52.1	− 39.4	− 4.3
PEG 1500	33.7	48.4	− 95.0	− 83.2	− 6.0
PEG 4000	82.5	191.5	−347	−421	−12.4

* Calculated from least-squares power curve, *i.e.*, $-[\Delta R_M/\Delta M]^0 = 0.143\ n^{1.81}$.

** Calculated from gradients to least-squares power curve at the respective degrees of polymerization, *i.e.*, $\Delta F(CH_2CH_2O) = -2.303\ RT\ d[\Delta R_M/\Delta M]^0/dn = -2.303\ RT\ (0.259)\ n^{0.81}$.

The relationship between intrinsic molar activity and the average degree of polymerization (or molecular size) within the various polyethylene glycol fractions was further examined by plotting initial gradients as a function of the degree of polymerization in Fig. 2a. A similar plot obtained for catechin under the same conditions[4] is reproduced for comparison.

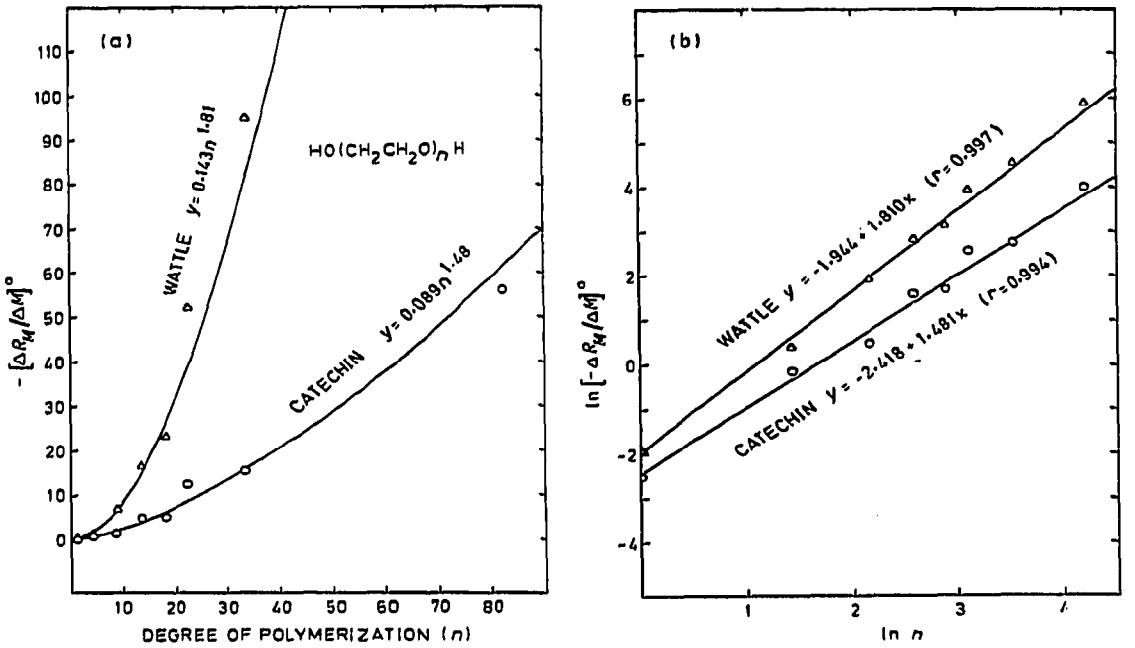

Fig. 2. Relationship between molar effects of polyethylene glycols on R_M at infinite dilution and degree of polymerization. (a) linear plot; (b) double logarithmic plot. Symbols denote experimental points; trends shown are the corresponding least-squares regressions generated by the equations given with associated correlation coefficients, r. Data for catechin taken from ref. 4.

Molar effects on R_M at infinite dilution of the various polyethylene glycols were consistently greater for wattle polyphenolics than for catechin. It was apparent, however, that a qualitatively similar relationship between molar activity and degree of polymerization applied to both, consistent with an empirical expression in which molar activity varied with some power of the degree of polymerization. Accordingly, a double logarithmic plot of the same variables is shown in Fig. 2b, in which both wattle and catechin showed substantially linear trends. A linear least-squares fit to the double logarithmic plot for wattle polyphenols yielded the equation

$$\ln [-\Delta R_M/\Delta M]^{\circ}_{n=r} = \ln [-\Delta R_M/\Delta M]^{\circ}_{n=1} + b \ln n$$
$$= -1.94 + 1.81 \ln n$$

where $\ln [-\Delta R_M/\Delta M]^{\circ}_{n=r}$ is the natural logarithm of the initial gradient for polyethylene glycol of degree of polymerization r, expressed in terms of the number of repeat $-CH_2CH_2O-$ units in the molecule.

The corresponding power transform from the above expression is

$$[-\Delta R_M/\Delta M]_{n=r}^{0} = [-\Delta R_M/\Delta M]_{n=1}^{0} n^b$$

The smooth trend generated by the above expression for wattle polyphenols is shown in Fig. 2a together with the similar trend for catechin. Molar effects calculated at various degrees of polymerization from the above expression are compared with experimental values in Table I and the corresponding free energy changes for structural addition are tabulated.

DISCUSSION

In a previous study of catechin affinity on cellulose in the presence of polyethylene glycols, it was shown that the marked increase in chromatographic mobility with the degree of polyethylene glycol polymerization implied a correspondingly rapid decrease in the transfer free energy (proportional to R_M) governing the binding or partition of catechin between stationary and moving phases[4]. Non-linearity of this free energy change with respect to successive additions of the same structural group in the polyethylene glycol series appeared to contrast with the situation obtaining for polar solvents such as alcohols, diols, ketones and nitriles, where a substantially linear increase in R_M with hydrocarbon chain length was observed.

The additivity of R_M effects with respect to structural addition in the developing solvent medium is analogous with Martin's postulates regarding the solute structural contributions to transfer free energy in chromatography[5]. Hence, in the absence of interfering interactions, Martin's postulates appear to extend with equal validity to structural changes in the developing solution. Deviation of polyethylene glycol effects from linearity using catechin as a reference standard in chromatography was attributed to cooperative interaction between polar groups in the formation of multiple hydrogen bonds between the polyethylene glycols and cellulose. Under these conditions, the stability of the polyethylene glycol–cellulose complex would be substantially enhanced, resulting in increasing displacement of bound catechin with increase in the number of active sites on the polyethylene glycol molecule.

From the close parallel in polyethylene glycol effects on wattle polyphenols in the present study, it would appear that qualitatively the affinity of these condensed tannins closely resembles that of catechin and that a similar mechanism of competitive displacement of bound wattle constituents by polyphenols is operative. Quantitative comparison indicated that the catechin mobility was intermediate within the range of wattle polyphenols, which varied between $R_F=0$ and 0.32 in water. As in the case of catechin, extrapolation of molar effects of the polyethylene glycols to infinite dilution was carried out in order to compare intrinsic activities under the same conditions and eliminate medium effects such as changes in dielectric constant with concentration. The amounts of extract applied to the paper were similarly maintained as low as possible, consistent with a linear adsorption–partition isotherm.

The affinity of various vegetable extract fractions on cellulose has been investigated by previous workers[11,12]. Solvent and chromatographic fractionation of the phenolic constituents of black wattle and quebracho (*Schinopsis* spp.) extracts and subsequent examination of paper chromatographic mobility *versus* molecular-weight relationships have shown that in general, the affinity of the condensed tannins increases

with molecular size. Fig. 3 shows least-squares lines fitted to previous data[11] for R_M as a function of the average molecular weight in various wattle and quebracho fractions. The increase in R_M with molecular weight, implying an increase in the free energy of solute transfer from the paper to the mobile solvent phase, indicates that the reverse process or solute binding is favoured. The range of molecular-weight variation did not appear to be sufficiently wide to determine whether polyfunctionality gave rise to deviations from linearity at higher molecular weights, as in the case of the polyethylene glycols. On the other hand, steric factors restricting multiple interaction would be expected to be operative in the more rigid polyphenolic system.

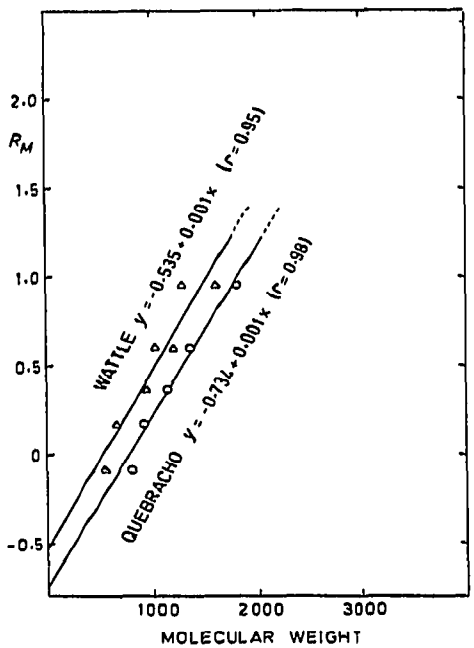

Fig. 3. Relationship between R_M on cellulose and average molecular weight in fractionated polyphenolic constituents of wattle and quebracho condensed tannins chromatographed in water containing 2% (w/v) acetic acid at 20°. Symbols denote experimental values taken from ref. 11. Linear trends are least-squares regressions corresponding to the equations given with associated correlation coefficients, r.

Chromatographic behaviour and the effects of molecular size and polyfunctionality in the polyethylene glycols and polyphenolic constituents of the condensed tannins suggest the operation of reversible physical interactions with relative strengths depending on the degree of surface contact. Non-specificity of the effects is emphasized by the fact that a close parallel has been demonstrated between relative affinities in aqueous medium of condensed tannin constituents for cellulose, collagen and modified collagens[12–14] and a similar parallel can be demonstrated for polyethylene glycol effects on wattle extract mobility using both cellulose and collagen as chromatographic substrates[15]. The observed effects are consistent with a mechanism involving multi-point competitive hydrogen bonding between tannins, glycols and complementary polar sites on cellulose and collagen.

ACKNOWLEDGEMENTS

This work was supported by the annual grants from the South African Livestock and Meat Industries Control Board and the Council for Scientific and Industrial Research.

REFERENCES

1 A. E. Russell, S. G. Shuttleworth and D. A. Williams-Wynn, *J. Soc. Leather Trades Chem.*, 52 (1968) 459.
2 A. E. Russell and D. R. Cooper, *Biochemistry*, 10 (1971) 3890.
3 G. J. Hart, A. E. Russell and D. R. Cooper, *J. Chromatogr.*, 67 (1972) 121.
4 A. E. Russell, M. Corcoy and D. R. Cooper, *J. Chromatogr.*, 78 (1973) 401.
5 A. J. P. Martin, *Biochem. Soc. Symp., Cambridge, England*, 3 (1949) 4.
6 R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, 38 (1944) 224.
7 E. C. Bate-Smith and R. G. Westall, *Biochem. Biophys. Acta*, 4 (1950) 427.
8 J. Green, S. Marcinkiewicz and D. McHale, *J. Chromatogr.*, 10 (1963) 158.
9 J. Green and D. McHale, *Advances in Chromatography*, Edward Arnold, London, 1966, p. 99.
10 G. Zweig and J. R. Whitaker, *Paper Chromatography and Electrophoresis*, Vol. II, *Paper Chromatography*, Academic Press, New York, London, 1971, p. 6.
11 S. R. Evelyn, *Ph. D. Thesis*, Rhodes University, Grahamstown, 1957.
12 D. G. Roux, *Nature (London)*, 181 (1958) 1793.
13 D. G. Roux, *J. Soc. Leather Trades Chem.*, 39 (1955) 80, 153, 321.
14 R. L. Sykes and D. G. Roux, *J. Soc. Leather Trades Chem.*, 41 (1957) 14.
15 A. E. Russell, M. Corcoy and D. R. Cooper, unpublished results.