Journal of Chromatography, 260 (1983) 13–21
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,604

INFLUENCE OF THE PARTICLE SIZE DISTRIBUTION OF THE PACKING MATERIAL IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

C. DEWAELE and M. VERZELE*

Laboratory of Organic Chemistry, State University of Ghent, Krijgslaan, 281 (S.4), B-9000 Ghent (Belgium)

(First received October 14th, 1982; revised manuscript received December 10th, 1982)

SUMMARY

The influence of a large particle size distribution of reversed-phase silica gel packing materials in high-performance liquid chromatography (HPLC) was studied. Mixtures were prepared with ROSiL-C_{18}-D materials with mean particle sizes of 3, 4, 5, 6, 8 and 10 μm. The efficiency of columns packed with mixtures of different compositions was measured and the usual chromatographic parameters were deduced. The conclusion is that a large particle size distribution has no influence on column efficiency if the eluting speed is kept around the optimum value. At higher solvent flow-rates there is a small negative effect. At all eluting speeds a larger particle size distribution has a negative effect on back-pressure and on separation impedance. A good practical ratio for the diameters of larger over smaller particles in a chromatographic material is about 1.5 to 2 for the ratio d_p 90/d_p 10. The negative effect of fines or dust in column packing materials is stressed.

INTRODUCTION

The influence of the particle size of chromatographic packing materials in high-performance liquid chromatography (HPLC) has often been discussed[1–8] and empirical relationships have been derived[3,4,6]. On the particular influence of particle size distribution on column efficiency, little is known, however, and a quantitative correlation certainly does not exist. Intuitively it seems desirable to use narrow sized cuts of the HPLC packing materials[4,9]. Commercial literature emphasizes this, but most journal literature contradicts this point of view. Halász[10] even mentions that painstaking efforts to produce uniform particles led to difficulties in packing columns. Most published studies concern larger particle sizes (d_p = 20 μm) and the conclusions are that a narrowly distributed particle size is not essential for good chromatographic performance[1,6,7]. More recently, Gazda *et al.*[11] concluded that a distribution as large as ±80 μm for a mean particle size of 110 μm has almost no influence on the column efficiency. Bristow[12], however, found for the small particles used in modern HPLC that the range of the particle size should not be that large.

0021-9673/83/$03.00

Halász and Naefe[6] found that permeability and the H/u relationship are not influenced provided that the particle size distribution does not exceed 40%. Done and Knox[7] and Halász[13] state that the smallest particles determine the permeability (K) and that the larger particles are mainly responsible for the other chromatographic properties. Almost all of the literature data cited above are for plain silica gel, and therefore in the normal-phase adsorption mode.

It is mostly in the commercial literature that material is presented as if a narrow particle size distribution were a worthwhile quality factor. Because of this and the not so clear conclusions from the literature, we decided to study this effect again for modern HPLC reversed-phase ultra-fine particle sizes (3–10 μm).

THEORY

The symbols and parameters used are as follows:

(1) Plates per metre: $\frac{100}{L} \cdot N$ with $N = 5.54 \left(\frac{T_R}{W_{\frac{1}{2}}} \right)^2$;
N: theoretical plates of the column;
L: column length (cm).

(2) HETP: $H = L/N$.

(3) Reduced HETP: $h = H/d_p$;
d_p: particle diameter.

(4) Chromatographic efficiency[14]: $CE = 100/h\%$ for h at optimal flow-rate.

(5) Chromatographic permeability:
$$K = \frac{u\eta\ L}{\Delta p};$$
u: linear mobile phase flow-rate;
η: viscosity;
Δp: column pressure.

(6) Resistance factor:
$$\varphi = \frac{d_p^2}{K} \text{ or } \frac{\Delta p t_0 d_p^2}{\eta L^2}$$
t_0: dead time of column

(7) Separation impedance:
E: $h^2\varphi$.

Several methods have been proposed for comparing the chromatographic properties of columns. Bristow and Knox[15] recommend the use of reduced parameters to compare different particles sizes. This is now the accepted method of comparing directly different materials. We have suggested the introduction of a chromatographic efficiency (CE) value, which is $100/h$ expressed as a percentage of the ultimate attainable, which would then be the mean particle size of the packing material[14]. This is also, of course, a reduced parameter but presented as a percentage of the attainable value. Reduced parameters tell us how well we have used the chromatographic materials and instrumentation and how well these are adapted to chromatography. They do not tell us, however, what a particular column can do. The CE

value may be as high as 100%, but if it is for a 20-μm material the column (for equal lengths) will not be as efficient as one filled with a 3-μm packing material even at a *CE* of only 50%. The commercial habit of expressing column quality in number of plates per metre therefore has merit.

The most critical parameter for deducing reduced parameters is d_p. With a narrow particle size distributrion, d_p can be measured with more confidence than when the former is large. With asymmetric particle size distributions the situation becomes very difficult. It is therefore interesting to work with an effective chromatographic diameter deduced from pressure measurements as Endele *et al.*[8] have shown. This approach requires that the resistance factor φ remains constant. For a range of particles from the same origin and for their mixtures, Bristow[12] states that φ is similar or identical. In our study described here this situation prevails. The effective particle diameter is then deduced chromatographically using the equation

$$d_{p_{\text{eff}}} = \left(\frac{\varphi \eta L^2}{t_0 \Delta p} \right)^{\frac{1}{2}}$$

EXPERIMENTAL

Instruments

All chromatography was carried out on a Varian 5020 LC instrument with a Varichrom UV-50 detector and a 10-μl Valco 7000 p.s.i. injector. The detector cell volume was modified to 1.7 μl and the detector time constant reduced to 0.25 sec. The detection wavelength was 254 nm.

Columns

The columns (10 × 0.46 cm I.D.) were made from a single Lichroma tube, and were equipped with Valco fittings and stainless-steel frits of 0.5-μm pore size. The packing materials were obtained from Alltech (Arlington Heights, IL, U.S.A.) and were batches with the same production data of octadecylated deactivated spherical silica gel (ROSiL-C_{18}-D) with mean particle sizes of 3, 4, 5, 6, 8 and 10 μm. The percentage of bonded organic material was 13% in all instances, which is about 2 μequiv./m^2. Residual accessible silanol groups were trimethylsilylated by a deactivation procedure.

Particle size distributions in HPLC

Measurement of the mean particle size value of a silica gel phase is not as easy as it may seem, especially for irregularly shaped particles. Estimating the distribution in particle size is equally difficult, but again easier for spherical particles. The most commonly used techniques are microscopic viewing and Coulter counting. Microscopic viewing is direct but the result is obviously subjective. Evaluating, by viewing, the ratio of larger over smaller particle diameters easily leads to very high figures, because the result is based on selected single particle dimensions standing out clearly in the viewed sample. Coulter counting looks more objective but also presents difficulties. Coulter counting is based on comparison and the question of the trustworthiness of the reference can be introduced. The reference should be as similar to the

measured sample as possible. The solvent must be a deflocculating one for both the reference and the sample material. These conditions cannot always be satisfied completely. Coulter counting results can be presented easily in such a way that the particle sizes can be estimated by limiting the 10% of volume or weight below 10% and above 90% of total volume or mass. The ratio of these limiting particle sizes, d_p 90/d_p 10, can be used as a simple measure of the distribution of HPLC particles. For samples of the commercial materials μBondapak 10 μm, LiChrosorb 10 μm, RSiL 10 μm and Zorbax 7 μm this ratio was 1.7, 1.6, 1.5 and 1.3, respectively[16]. Under a microscope it is easy, however, to find particles with a diameter ratio of 4 or larger for all of these materials. This shows the difficulty of expressing the distribution in particle sizes and the need for a conventional approach.

All the ROSiL materials used in this study had a d_p 90/d_p 10 ratio around 1.5, the value for the larger particle sizes being lower than for the finer particle materials.

Packing procedure

All columns were packed with a 10% (w/v) slurry in pentane. The upward mode and a Haskel pump at 600 kg/cm^2 were used. The slurries were shaken vigorously and subjected to ultrasonic treatment just before use.

Column testing

All columns were tested under the following conditions unless stated otherwise: eluent, acetonitrile–water (75:25); eluent flow-rate, 1 ml/min; sample, naphthalene–anthracene–pyrene mixture (the k value for pyrene was *ca.* 6 in all instances).

RESULTS

Influence of particle size distribution on N, Δp, h, CE and E

The above parameters are presented in Table I. $d_{p_{eff}}$ was calculated with a constant resistance factor and with the 5-μm material as reference ($\varphi\eta L^2/t_0 \approx$ 1400 and $\varphi \approx 960$).

Table I shows that it is relatively easier to pack the coarser materials. The value of *CE* increases with increasing particle size. The values in Table I are not as high as those previously published for ROSiL-C_{18}-D[14] but batches indeed do differ and, even more important, there are variations in the quality of the column tubing that influence the chromatographic performance. The calculated $d_{p_{eff}}$ values correspond reasonably well with the nominal values.

TABLE I

CHROMATOGRAPHIC PARAMETERS FOR ROSiL-C_{18}-D

d_p (μm)	d_{peff}	N/m	h	$CE(\%)$	E
3	2.89	115,000	2.89	34.5	8018
4	3.50	106,000	2.68	37.3	6895
5	5.00	81,710	2.44	41.0	5715
6	5.77	67,000	2.58	38.7	6390
8	7.63	62,710	2.11	47.4	3173
10	10.80	52,900	2.04	49.0	3995

To ascertain the influence of the particle size distribution on N and the back-pressure Δp, equal weight mixtures of the above particle sizes were tested as shown in Table II. On mixing equal weights of 4 and 6 μm, 3 and 8 μm and 3 and 10 μm materials, the first impulse is to make a comparison with the results for the 5-μm material in Table I. There is no difference in efficiency, at least not within the limits that it is measurable, although the ratio $d_p 90/d_p 10$ for the 3 + 10-μm mixtures is at least 5.

The apparent mean d_p, as would be evaluated by Coulter counting, is approximately as given under $d_{p_{app}}$. The chromatographic $d_{p_{eff}}$ is much smaller, however. The large (50%) amounts of 8 and 10 μm material in mixtures b and c have only a small influence on the measured $d_{p_{eff}}$. Comparison of materials thus becomes very difficult as it is not evident what d_p should be taken to calculate the chromatographic parameters. With $d_{p_{app}}$, the mixtures are better than narrowly sized material, but with $d_{p_{eff}}$ this is not so as the higher pressure drop becomes a very negative factor in the calculations. If the N/m value is considered to be the most important, then the column quality is not affected by a wider distribution of particle sizes. On the contrary, the efficiency nearly doubles when 50% of 3-μm material is added to the 10-μm material. This mixture c with $d_{p_{app}} \approx 6.5$ gives even greater efficiency than the regular 5-μm material. The back-pressure, however, is very negatively affected in this instance and with longer columns this effect may become prohibitive. The E and CE values and, of course, also h_{eff} in Table II were calculated with $d_{p_{eff}}$.

TABLE II

CHROMATOGRAPHIC PROPERTIES OF EQUAL WEIGHT MIXTURES OF ROSiL-C_{18}-D PACKING MATERIALS

Mixture	d_p (μm)	Δp	d_{papp}	d_{peff}	N/m	h_{app}	h_{eff}	$CE(\%)$	E
a	4 + 6	74	5	4.35	83,000	2.40	2.77	36.1	7365
b	3 + 8	130	5.5	3.28	81,950	2.21	3.42	29.2	8889
c	3 + 10	128	6.5	3.31	89,000	1.72	3.68	27.1	13,000

In a further step, the 3- and 8-μm materials were mixed in different proportions. In these mixtures the distribution of particle size occurs in an asymmetric way, but then this is often the case for silica gel HPLC materials[10]. These two materials were chosen so as to differentiate the mixtures strongly enough to affect the parameters to be measured. Fig. 1 shows the results for ten such mixtures.

The linear increase in the plate number shows that a wider particle size distribution has no influence on the efficiency. This is so for symmetrical and asymmetric distributions of the particle sizes up to $d_p 90/d_p 10 \approx 4$–5). This conclusion is similar to those in refs. 1, 6 and 11. Fig. 1b also shows the increase in the column back-pressure. Smaller particles strongly influence the back-pressure[7,13] and therefore the $d_{p_{eff}}$ and E values. In this context, we mixed only 2% of 1–2-μm ROSiL-C_{18}-D (experimental batch) with 98% of 10-μm ROSiL-C_{18}-D. A special small-pore frit was provided at the column bottom. The pressure, which was only 12 atm for 100% 10-μm material, increased to 40 atm. This large increase shows the importance

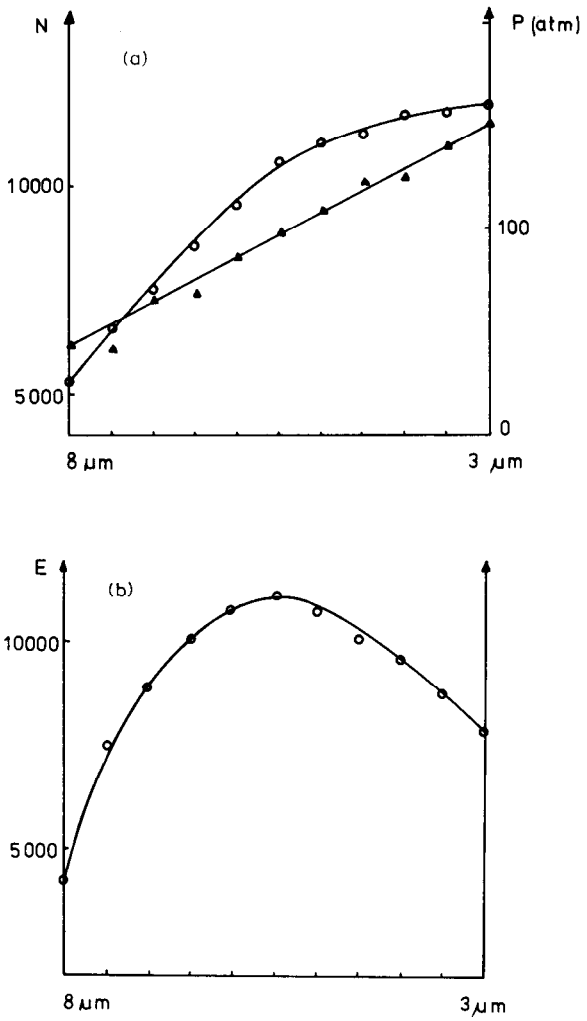


Fig. 1. (a) Plate numbers (▲, N) and back pressures (○, p atm) of 10 × 0.46 cm I.D. columns packed with 8- and 3-µm ROSiL-C_{18}-D packing materials mixed in ten proportions as shown. (b) Separation impedance (E) for the same columns.

of avoiding the presence of dust in HPLC packing materials. Similar results were reported by Bristow[12].

Fig. 1b shows the variation of E, which reaches a maximum for the widest distribution at the 50:50 mixture point.

Particle size distribution and H/u relationship

Halász and Naefe[6] mentioned that a wide distribution of particle size does not affect the H/u relationship. Such curves for some of the mixtures studied here are shown in Fig. 2.

At low solvent flow-rates ($u < 1$ mm/sec) all curves coincide. This is expected as the B/u term of the Van Deemter equation[17] is independent of particle size.

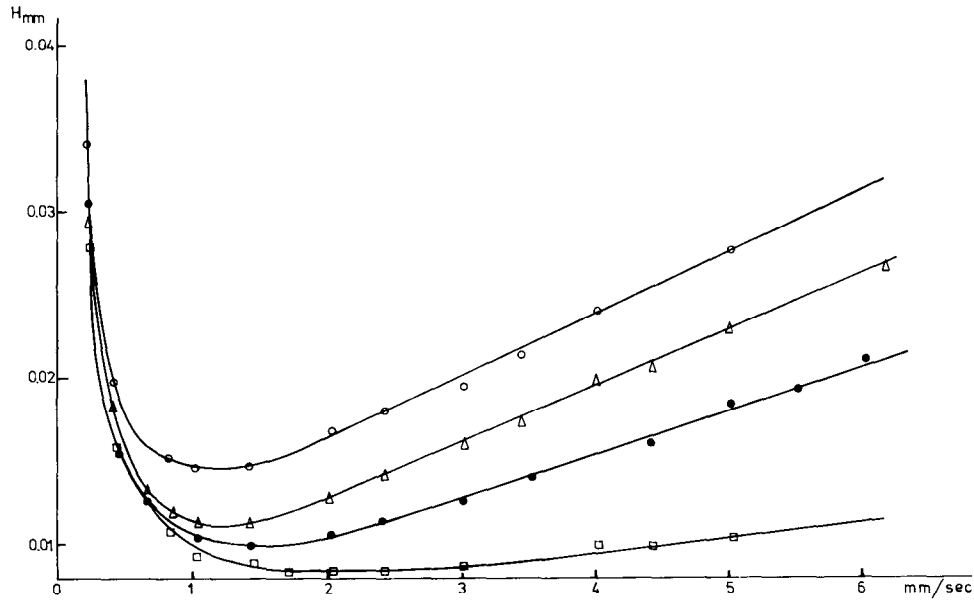
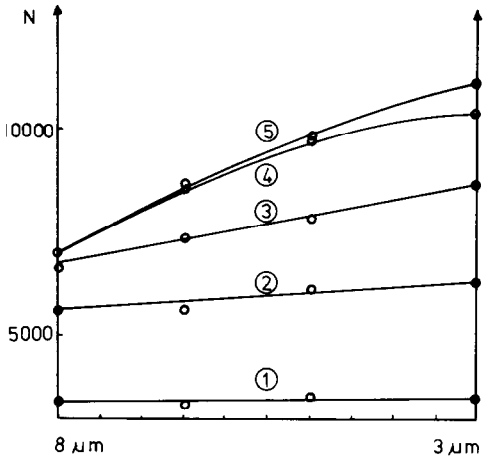
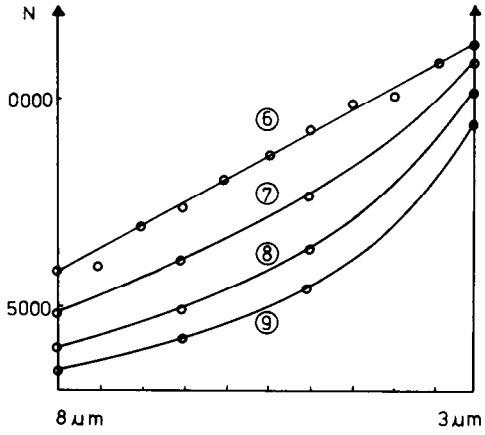


Fig. 2. H/u curves for 8- and 3-μm ROSiL-C_{18}-D packing materials and mixtures of both: ○, 8 μm; △, 3 + 8 μm (3:7); ●, 3 + 8 μm (6:4); □, 3 μm. H (mm) = $A + B/u + Cu$.

The A terms, as determined graphically for the 3, 8, 3 + 8 (3:7) and 3 + 8 μm (6:4) were 5.4, 9.3, 6.4 and 5.8 μm, respectively. Greater particle size distributions therefore do not lead to greater A-term contributions. Still, the A term is the largest single contributor to the H value. Earlier in chromatography the A term was not so important, but with the exceptional efficiencies of the modern very small spherical particles it has become the major contributor to the H value. The above A values suggest that further improvements in chromatographic results cannot be expected from smaller particle size distributions. The C terms for mixtures have intermediate values, as the curves show. With the data for the H/u curves, graphs such as those in Fig. 1 were produced to ascertain whether the linear relationship between N and mixture ratios is also valid at other elution speeds. The results are presented in Figs. 3 and 4.

Fig. 3 shows that at higher elution speeds (>3 mm/sec) a larger particle size distribution has a negative influence on efficiency. At lower solvent rates, where diffusion in the mobile phase is important, there is no such influence. The slight deviation from linearity at 0.5 ml/min is probably due to experimental variability. This change in the influence of particle size distribution with the solvent flow-rate is probably the reason for the contradictory statements in the literature. Intuitively it is not unexpected that higher solvent flow-rates result in a stronger contribution from the irregular packings which must occur with wider particle size distributions. Fig. 4 shows that the decrease in separation impedance with a wider particle size distribution is also more important with higher elution speeds.





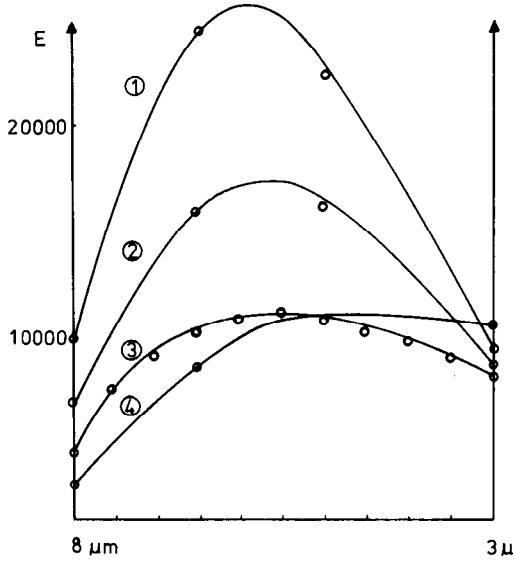


Fig. 3. Plate numbers (N) as in Fig. 1 for the same packing materials at various solvent flow-rates: (1) 0.1; (2) 0.2; (3) 0.3; (4) 0.5; (5) 0.7; (6) 1; (7) 1.5; (8) 2; (9) 2.5 ml/min.

Fig. 4. Separation impedance (E) for mixtures as in Figs. 1, 2 and 3 at various solvent flow-rates: (1) 2; (2) 1.5; (3) 1; (4) 0.5 ml/min.

CONCLUSION

The column efficiency is not affected by a wide particle size distribution when the solvent flow-rate is close to its optimum value. A negative effect becomes apparent only at higher elution speeds. These facts explain the contradictory opinions found in the literature on the influence of particle size distribution. The back-pressure and separation impedance are negatively affected by a larger distribution of the particle dimensions even at lower elution speeds. The ratio of larger over smaller particle

diameters or the distribution of the particle sizes is therefore an important characteristic of chromatographic materials. A simple way of expressing this numerically is with the ratio d_p 90/d_p 10 (d_p 90 and d_p 10 are the particle diameter values above and below which 10% of the total weight is found). This value is around 1.5 for some commercial materials, which seems more than adequate.

The "eddy diffusion" or A term in the Van Deemter equation is less influenced by a wider particle size distribution than would be expected. This A term is the most important contributor to the H value.

ACKNOWLEDGEMENTS

We thank the Ministerie voor Wetenschapsbeleid, the Nationaal Fonds voor Wetenschappelijk Onderzoek —NFWO and the Instituut voor Wetenschappelijk Onderzoek in Nijverheid en Landbouw —IWONL for financial assistance to the laboratory. Thanks are due to Dr. B. Halkes for providing Coulter Counter data.

REFERENCES

1 L. R. Snyder, *Anal. Chem.*, 39 (1967) 698.
2 H. N. M. Stewart, R. Amos and S. G. Perry, *J. Chromatogr.*, 38 (1968) 209.
3 L. R. Snyder, *J. Chromatogr. Sci.*, 7 (1969) 352.
4 R. E. Majors, *J. Chromatogr. Sci.*, 11 (1973) 88.
5 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 129.
6 I. Halász and M. Naefe, *Anal. Chem.*, 44 (1972) 76.
7 J. N. Done and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 606.
8 R. Endele, I. Halász and K. Unger, *J. Chromatogr.*, 99 (1974) 377.
9 R. E. Majors, H. G. Barth and C. H. Lochmuller, *Anal. Chem.*, 54 (1982) 323.
10 R. Ohmacht and I. Halász, *Chromatographia*, 14 (1981) 155.
11 K. Gazda, M. Kamiński, J. Klawiter, J. S. Kowalczyk, B. Makuch, K. Prusiewicz and B. Śledzińska, *J. Chromatogr.*, 191 (1980) 9.
12 P. A. Bristow, *J. Chromatogr.*, 149 (1978) 13.
13 I. Halász, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, p. 330.
14 M. Verzele, J. Vandyck, P. Mussche and C. Dewaele, *J. Liquid Chromatogr.*, 5 (1982) 1431.
15 P. Bristow and J. H. Knox, *Chromatographia*, 10 (1977) 279.
16 B. Halkes, Duphar, Weesp, The Netherlands, personal communication.
17 J. van Deemter, F. Zuiderweg and A. Klinkenberg, *Chem. Eng. Sci.*, 5 (1956) 271.