

AFFECTING OF EFFICIENCY IN ADSORPTION ELUTION CHROMATOGRAPHY

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The factors affecting the efficiency of separation columns with commercial adsorbents commonly used in laboratories are considered. The results of measurements performed with four adsorbents indicate, how relatively high efficiencies ($H \sim 1$ mm) can be achieved with adsorbents of particle size 30—200 μm . The effects of the particle size, linear velocity of the eluent, diameter of the column, temperature, injection volume, and capacity factor of the substance examined were studied. The linear velocity of the eluent and the diameter of the adsorbent particle proved to be the most significant parameters affecting the efficiency, the other parameters are of minor importance. An essential improvement of the efficiency was achieved by fractionation of commercial adsorbents to portions with a narrow mesh cut.

The development of adsorption chromatography is now directed towards increasing the efficiency of chromatographic systems by using adsorbents with the diameter of the particles lower than 10 μm , in many cases of a defined size and structure of the pores. In addition to such adsorbents, the usual adsorbents of grain size 30—200 μm are used frequently as well, particularly for preparative separation and also if the highly efficient columns could be deteriorated by strongly adsorbed components. In addition, a wider use of the high efficient columns is hindered by the difficulties encountered in instrumentation (requirement of high-pressure pulseless pumps, appropriate columns).

This work aimed to find the most significant factors, which affect the efficiency of columns with commercial adsorbents. Adsorbents for column chromatography, fractionated to narrower portions, were used. The application of adsorbents of this kind, particle size about 100 μm or higher, does not yield as good results for analytical purposes as particles about 10 μm , but for separation purposes these adsorbents represent an appropriate and inexpensive material. In addition, a reproducible dry filling of the column is relatively quick and simple.

The efficiency of chromatographic columns is expressed through several parameters, which can be determined experimentally. Their interrelation is clear from the following equations^{1,2}.

The sample is characterized by the capacity factor k' ,

$$k' = (t_R - t_m)/t_m, \quad (1)$$

$$k' = 1 + (\sqrt{N})/4 \ln(t_R/t_m), \quad (2)$$

where t_R and t_m are the retention times of the measured component and of the inert, respectively, and N is the number of theoretical plates of the column.

$$N = 16(t_R/w)^2, \quad (3)$$

where w is the width of the elution band at the base. The height equivalent to a theoretical plate is

$$H = L/N, \quad (4)$$

where L is the column length.

The values of these parameters depend on the experimental conditions, on the adsorbent particle size, linear velocity of the eluent, diameter of the column *etc.* In addition to these factors, which can be affected directly by an appropriate choice, several other factors occur, which cannot be influenced directly, such as the diffusion coefficient, rate of desorption *etc.* It is obvious that for a rapid adjustment of the optimum parameters of the chromatographic system applied, potentialities are to be sought in the former group of factors.

A decisive factor is the diameter of the column, d_c , in relation to that of the adsorbent particle, d_p . Usually the difference of at least one order is supposed to be sufficient. It proved, however, that d_c and d_p cannot be considered quite independent of the column length, as the number of plates of the column increases with the length linearly only up to a certain limit²⁻⁴. DeStefano and Beachell⁴ give therefore the relation

$$d_c = (2.4d_pL)^{1/2} \quad (5)$$

including the interdependence of these parameters.

Regarding the fact that the efficiency of the column increases with decreasing diameter of the adsorbent particles, most of the authors concerned with this problem focus now attention on particles of diameter lower than $100 \mu\text{m}^{5-12}$. Simultaneously with the effect of the particle size, the effect of linear velocity of the mobile phase has been studied as well in many cases^{3,7,8,13}. Many authors attempted to express the relation between H and d_p by an empirical expression. Jardy and Rosset⁷ suggest

$$H = kd_p^\beta, \quad (6)$$

where β is an empirical exponent depending on the linear velocity of the eluent, and k is a constant, represented by the slope of the plot of $\log H$ vs $\log u$. Similar expressions for H were suggested by Martin and coworkers³ and Beachell and DeStefano¹⁰.

The increasing of the column efficiency *via* lowering of the linear velocity of the eluent is not unlimited. At extremely low velocities the diffusion along the column axis plays a part, which is reflected by a worse efficiency. Snyder⁵ described the dependence of H on the linear velocity of the eluent by the expression

$$H = Du^{0.4} \quad (7)$$

where D is a constant for the given system. Similar formulations of conclusions appeared in the papers by Martin and coworkers³ and Jardy and Rosset⁷. Many authors^{1-3,6} agree with the validity of the equation (7) in the range of linear velocities 0.1—10 cm/s. Treating the dependence of H on u , Halász and Walkling¹⁴ included the laminar and turbulent flow of the eluent, based on the Reynolds' criterion.

Data describing the effect of temperature on the adsorption-chromatographic process are relatively less frequent. Among the latest papers, the works of Grushka^{1,2,15} deal with that problem. The opinions about the influence of temperature on the efficiency are vague and even in contradiction to one another. There are data available evidencing a decrease of H with increasing temperature¹⁶, other experiments indicate no or only a negligible effect of temperature¹⁷, some papers report even a negative effect of temperature^{1,2}. The results of the work of Krejčí and Kouřilová¹⁸, where the dynamical temperature gradient has been employed, show that the effects of temperature probably cannot be generalized for substances of different nature.

Among further factors, which can under some circumstances affect the efficiency of a chromatographic column, diffusion should be mentioned. Molecular diffusion in the mobile phase of the system is, however, usually insignificant. The efficiency of the columns is dependent also on the value of the diffusion coefficient in the mobile and stationary phases, on the kinetics of desorption, porosity, pore structure, *etc.*³.

Kiselev and coworkers¹⁹ measured the dependence of H on u on silica gel with different pore diameters and found no significant effect of the pore size on the H value for the velocities used (0.5–4 cm/s).

For an increase of the number of theoretical plates of a chromatographic system, a series connection of two or more columns proves to give good results in some cases. Kwok and Snyder²⁰ inspected this problem thoroughly and came to the conclusion that columns with a high difference in efficiency, size, and particle size should not be combined.

Efficiency can be increased by gradient elution²¹. In the optimum case all the peaks have the same width w , which brings about a decrease of the number of theoretical plates required for a certain resolution power.

EXPERIMENTAL

Adsorbents. Silica gel Woelm (Eschwege, FRG) and Silpearl (Kavalier, Votice), alumina Woelm Neutral (Eschwege, FRG) and Reanal (Budapest, Hungary). The number of fractions used and the particle size are given in Table I. The adsorbents were dried (silica gel 180°C/4 h, alumina 400°C/6 h) and deactivated by an addition of 3 and 3.5% of water, respectively.

Columns. Glass columns 2 mm i.d. × 50 cm, 3 mm i.d. × 50 cm (Nester-Faust, USA) and 4 mm i.d. × 30 cm (Pye Unicam, GB) were used.

Pump. Pressure gradient pump ISCO Dialagrad Mod. 384 with a pulseless injection of the eluent up to the pressure of max. 200 atm. (Instrumentation Specialties Co., USA).

Detector. UV Monitor Waters Assoc. (USA) cell dead volume 8 μ l, path length 1 cm, working at the wavelength 254 nm.

Eluent. n-Pentane, purity tested by UV spectrophotometry (instrument SP 800 B, Pye Unicam).

RESULTS

The dependences of H on the linear velocity of the eluent in the range of 1–12 mm/s were measured on the column 3 mm i.d. × 50 cm for all adsorbent fractions (Table I, Fig. 1, 2). From these plots of H vs u , the H values were read for $u = 2$ mm/s and the dependence of H on d_p was plotted for a constant linear velocity of the eluent (Fig. 3). A solution of benzene, naphthalene, and phenanthrene or pyrene in iso-octane was used as the testing mixture, injection 4 μ l of 0.3% solutions of the stant

TABLE I
Fractional Composition of the Adsorbents Used in % wt

Particle diameter μm	Silical gel		Alumina	
	Woelm	Kavalier	Woelm	Reanal
220	43	—	3	0.5
150	51	0.5	24	7
100	4	8.5	63	38
62	2	55	8	15
48	—	22	0.5	5.5
30	—	14	1.5	33

TABLE II
Mean Values of the Capacity Factors k' of the Components of the Testing Mixture of Standards

Adsorbent	Benzene	Naphthalene	Phenanthrene	Pyrene
Silica gel Woelm	2.15	5.15	14.40	—
Silpearl Kavalier	2.24	4.45	11.45	—
Alumina Woelm	0.52	1.22	4.75	—
Alumina Reanal	0.54	0.96	3.65	6.20

TABLE III
Values of the Constants k and β of Equation (6) (Fig. 3)

Adsorbent	k	β
Silica gel Woelm	21.62	1.024
Silpearl Kavalier	5.425	0.537
Alumina Woelm	39.36	1.406
Alumina Reanal	7.93	0.514

dards. Working pressures as high as 15 atm were used for the lowest particle size and the highest linear velocity of the eluent. The mean values of the capacity factors k' for the various compounds are given in Table II.

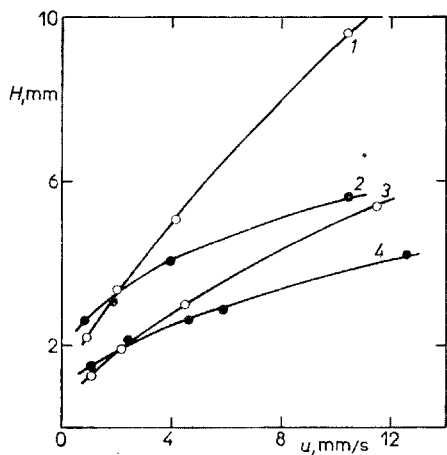


FIG. 1

Dependence of H on Linear Velocity of the Eluent u

Testing substance phenanthrene, column 3 mm i.d. \times 50 cm. Original adsorbents: 1 Silica gel Woelm, 2 Silpearl Kavalier, 3 alumina Woelm, 4 alumina Reanal.

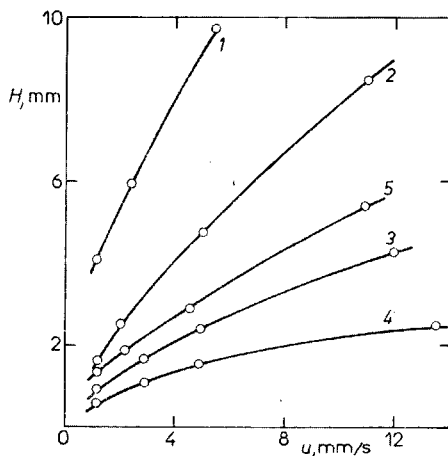


FIG. 2

Dependence of H on Linear Velocity of the Eluent u and Particle Size d_p

Alumina Woelm, testing substance phenanthrene, column 3 mm i.d. \times 50 cm. Value d_p : 1 220 μm , 2 150 μm , 3 100 μm , 4 62 μm , 5 original adsorbent.

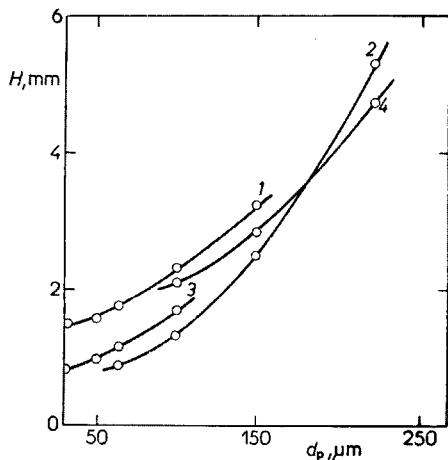


FIG. 3

Dependence of H on the Particle Size d_p

Linear velocity of the eluent $u = 2$ mm/s, testing substance phenanthrene, column 3 mm i.d. \times 50 cm. Adsorbents: 1 alumina Reanal, 2 alumina Woelm, 3 Silpearl Kavalier, 4 silica gel Woelm.

The effect of the column diameter on the H values was shown on the case of a fraction of alumina Reanal ($d_p = 62 \mu\text{m}$) and Silpearl ($d_p = 100 \mu\text{m}$); the results were plotted (Fig. 4). In addition to these parameters affecting the efficiency, as mentioned, the effect of temperature (Fig. 5) and the effect of the injection volume (Fig. 6) were examined on a selected adsorbent. All experiments (except the investigation of the dependence of H on temperature) were carried out at the ambient temperature (approx. $20-22^\circ\text{C}$). For repeated packing of a column with the same adsorbent, the differences of the H values did not exceed $\pm 15\%$. The data given resulted from at least two measurements on a column packed two times.

In all cases the columns were packed on a dry way by employing vibrations along the longitudinal axis of the column. The adsorbent was added gradually in small portions (15–20 mm of the column) and pressed down by means of a vibrator.

DISCUSSION

Dependence of H on eluent linear velocity u and the particle size d_p . Fig. 1 shows the effect of linear velocity of the eluent u , obtained by measuring on original non-fractionated adsorbents. An enhanced sensitivity of the adsorbents Woelm to a change of u , particularly for $u \geq 4 \text{ mm/s}$, is apparent. It is caused probably by the different fractional composition (Table I), where the presence of fractions with a higher diameter brings about an unfavourable effect (Fig. 2).

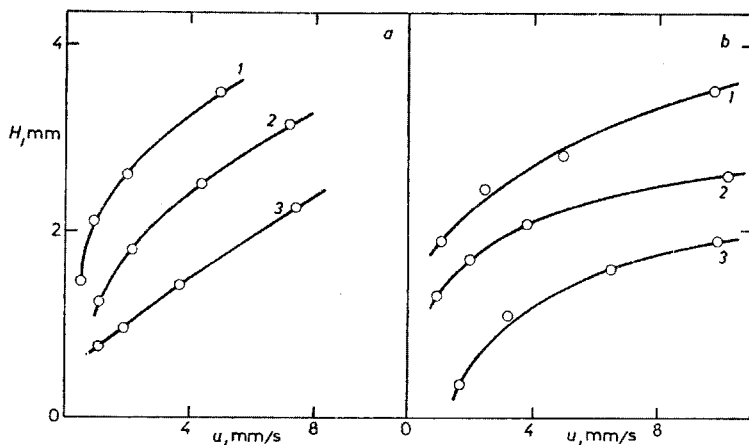


FIG. 4

Dependence of H on the Column Diameter d_c for Various Linear Velocities of the Eluent u

Particle size $62 \mu\text{m}$ (alumina) or $100 \mu\text{m}$ (silica gel), testing substance phenanthrene. *a* Alumina, *b* silica gel. Values d_c : 1 mm, 2 mm, 3 mm.

A typical case of results obtained by measuring on a series of fractions from alumina Woelm is presented in Fig. 2; analogous results were obtained also from measurements with Silpearl and silica gel Woelm as well as on alumina Reanal. Fig. 2 shows that great differences in the efficiency of the various fractions occur and that the efficiency of the adsorbent improves considerably by narrowing the mesh cut and decreasing the particle size. From the figure it is also apparent that for narrow fractions, values of $H \sim 1$ mm and lower can be attained under suitable conditions.

Fig. 3 shows the effect of the particle size, as observed during measurements at a constant linear velocity, $u = 2$ mm/s. All the curves of the figure agree well with the exponential dependence (6). The values of the constant k and the exponent β calculated by correlation of $\log H$ with $\log d_p$ (dimensions in mm) are given in Table III. The constant k is a complex characteristics of the whole chromatographic system and involves all the side factors except the particle size. Physically the constant k represents the height H for the particle diameter $d_p = 1$ mm. The exponent β characterizes the sensitivity of the column efficiency to the change of the particle size for the various kinds of adsorbents. The values given in Table III show that in the cases of alumina Reanal and Silpearl the column efficiency is approximately proportional to the square root of the particle diameter, while the characteristics for the adsorbents Woelm is less favourable.

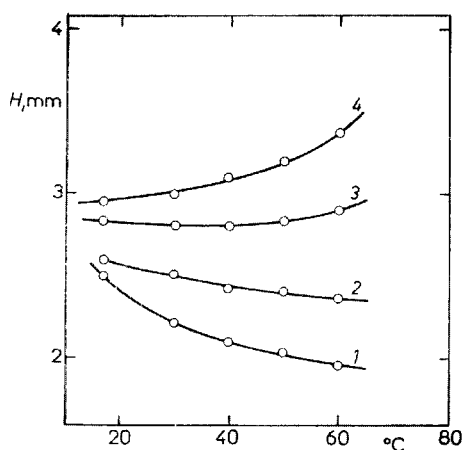


FIG. 5

Dependence of H on Temperature

Alumina Reanal original, column 3 mm i.d. \times 50 cm, linear velocity of the eluent $u = 5$ mm/s. Testing substance: 1 benzene, 2 naphthalene, 3 phenanthrene, 4 pyrene.

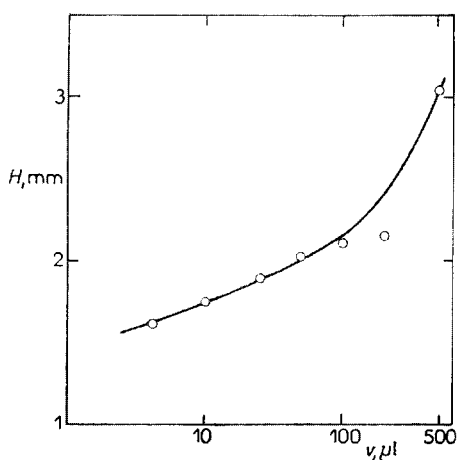


FIG. 6

Dependence of H on the Injection Volume v

Alumina Reanal ($d_p = 100 \mu\text{m}$), testing substance phenanthrene, column 3 mm i.d. \times 50 cm (free volume 1.3 ml), linear velocity of the eluent $u = 5$ mm/s.

Dependence of H on the diameter of the column. Fig. 4 presents the results obtained from measurements with column diameters 2, 3, and 4 mm. The effect of the increasing diameter of the column is negative in the case of silica gel as well as of alumina, which is in contrast with the theory of minimum column diameter with respect to the size of the used adsorbent particles⁴ (Equation (5)). A detailed study of this effect, employing the measurements of H on alumina, particles 40, 62, 100, and 150 μm , showed that the dependences described, *e.g.*, by DeStefano and Beachell⁴ hold for the columns used only in the range as high as $d_p = 150 \mu\text{m}$, where the increase of the column diameter was accompanied by a mild decrease of the H value. The differences in the H values measured, however, do not exceed in this case the mean experimental error; it can be therefore stated that for a ratio of $d_c : d_p \gg 20$ the diameter of the column does not belong to factors affecting considerably the efficiency.

Dependence of H on the injection volume. The effect of the injection volume was studied with alumina Reanal, particle size 100 μm , in a column 3 mm i.d. \times 50 cm. From Fig. 5 a marked negative effect of increasing injection volume is apparent. The differences of the H values measured with injections 4 and 50 μl differ mutually by 0.37 mm. This difference represents about 20% of the H value measured with the injection of 25 μl . From these facts it follows that injection volumes up to 50 μl (in this case approx. 4% of the dead volume of the column) can be considered satisfactory with respect to experimental accuracy. On the other hand, injection volumes higher than 200 μl (approx. 15% of the dead volume of the column) exert a strongly negative effect on the measured H values. These conclusions hold for work in the range of linear capacity of the column.

Dependence of H on temperature. This dependence was measured in the range

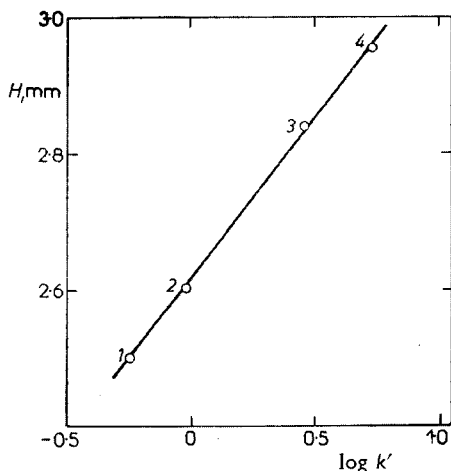


FIG. 7

Dependence of H on the Capacity Factor k'

Alumina Reanal (original), column 3 mm i.d. \times 50 cm, linear velocity of the eluent $u = 5 \text{ mm/s}$. Testing substance: 1 benzene, 2 naphthalene, 3 phenanthrene, 4 pyrene.

of 17–60°C in a column 3 mm i.d. × 50 cm (alumina Reanal original). For this case a higher linear velocity of the eluent, $u = 5$ mm/s, was chosen, with regard to higher differences of the H values for different capacity factors k' . The results obtained are shown in Fig. 6. A high dependence appeared on the change of k' of the substance whose chromatographic band served for the calculation of the H value. With increasing k' the temperature effect converts from a positive to a negative one. In the case of phenanthrene under given experimental conditions the temperature effect can be considered to be zero, for pyrene a negative temperature effect can be observed.

Snyder and Kirkland⁵ suggest a relation between the diffusion coefficient D_m and the other parameters, from which it follows that the D_m value increases with absolute temperature. As for the contribution of longitudinal diffusion the authors⁵ give the expression

$$H_d = C_d D_m / u, \quad (8)$$

where C_d is a constant for the adsorbent used, it is clear that the increasing temperature and decreasing linear velocity of the eluent is accompanied by an increase of the value H_d representing the effect of molecular diffusion on the total H value.

Dependence of H on the capacity factor k' . The experimental column efficiency depends to an extent also on the capacity factor k' of the substance used for testing. Generally the measured H values are higher with increasing k' . This is quite clear from Fig. 7, which shows that during the testing of the same column with benzene and pyrene, respectively, the H value is 15% lower in the former case.

CONCLUSION

Separation efficiency of commercial adsorbents can be markedly improved by removing fractions of particles larger than approx. 150 μm , because the increase of the particle size, particularly in this range, brings about an essential increase of the H values. An appropriate particle size of the adsorbent along with the linear velocity of the mobile phase make it possible to attain the plate height of about 1 mm, which corresponds to the high-efficiency fine-mesh adsorbents. The study of the effect of the diameter of the column and size of the adsorbent particles showed that the efficiency of the column is very good for particles smaller than 150 μm , if the inner diameter of the column is at least 2 mm. The injection volume decreases the column efficiency – or increases H – only if it is higher than approx. 5% of the dead volume of the column.

The effect of temperature cannot be generalized uniquely, because it depends upon the capacity factor as well as the character of the solute measured. Compounds

with a low capacity factor indicate a favourable temperature effect; this positive effect converts gradually to a negative one with increasing k' .

The practical effect of the measurements performed can be summarized as follows. From a common adsorbent a fraction below 100 μm can be easily prepared, and if used in a suitable column, in connection with the use of the linear velocity of the eluent approx. 1–2 mm/s, the efficiency of 500–1000 theoretical plates can be attained with a column 1 m long. Regarding the error in the reproduced measurements, the variations of the ambient temperature and injection volume up to 5% of the dead volume of the column can be considered to be insignificant factors, which do not exert a decisive effect on the separation properties of the system.

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