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## STUDY OF THE PERTINENCY OF PRESSURE IN LIQUID CHROMATOGRAPHY

### I. THEORETICAL ANALYSIS

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#### SUMMARY

Probable future liquid chromatographs are discussed, with special attention to the question whether there is any other limitation than today's technology to the use of finer particles and higher pressures. This discussion is based on new equations, relating pressure and column length at constant analysis performance parameters (*i.e.*, constant retention time and resolution).

The main conclusions seem to be that although pressure is always required and a minimum pressure is necessary to ensure any given analysis, the necessary pressure is much lower than the pressures currently used. This pressure optimization allows either to "save" pressure or to employ it optimally in analysis for which extreme performance is needed.

With respect to the particle diameter there seems to be a technological limit, or at least a barrier, not far below 4–5  $\mu\text{m}$ . Practical implications of the results obtained are discussed, emphasizing detector requirements and column design.

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#### INTRODUCTION

Since the introduction of high-performance liquid chromatography (LC) in the late 1960s, its development has illustrated the efficiency and versatility of this new analytical method<sup>1</sup>. Up to now, most advances have been made through the use of increasingly finer particles and higher pressures. High pressures are needed, of course, in order to operate columns packed with very fine particles, but the necessity of operating them at very high flow velocities has also been considered to be essential.

Most commercially available equipment is now designed to operate at pressures up to 250–300 atm. The use of even higher pressures (*ca.* 4000 atm) has been reported<sup>2</sup> or advocated. It might seem, therefore, from a survey of the recent scientific and commercial literature, that the limit to the pressures that can be used is set only by technological and financial considerations, and there seems to be little point in this costly exercise.

In contrast with general trends, we have shown recently that the use of fine particles, resulting in the preparation of very efficient columns, makes it possible to

achieve a given separation with a short column and a low inlet pressure<sup>3</sup>. In other words, a reduction in the particle diameter is not necessarily accompanied by an increase in the inlet pressure, provided that one is interested in separation and analysis time, and not in column length or flow velocities, which are only means of achieving these aims. These theoretical results have recently been confirmed by experimental results, showing that very short columns, packed with fine particles, can be used to achieve difficult separations in a reasonable time using only moderate pressures<sup>4-10</sup>.

This apparent inconsistency raises the question of the pertinency of the pressure in LC and it is the aim of this paper to clarify this aspect. In fact, pressure is required in order to move the carrier liquid and hence to perform LC and, in some respects, it appears to be the price that must be paid in order to make the analysis feasible. Therefore, it is interesting to minimize that price or, in other words, to make the best use of the pressure.

We show later that this optimization can now be performed only as the result of having good, small particles that can be efficiently packed with improved packing techniques; thus our general conclusions would not have been realistic a few years ago. Furthermore, it is worth pointing out that our optimization, as with any other optimization, does not provide a universal solution as its virtue depends only on the ultimate aim. Other treatments of the optimization of LC conditions reported recently<sup>3,11-21</sup> have not usually arrived at the same conclusions for the "directions for use" of LC. The reason for this disagreement is that the aims of these approaches are fundamentally different.

Thus the first and probably most important step in optimization is to define its objective by answering the question: What does the analyst really want? Once the problem has been defined, its solution is straightforward, as illustrated below.

In order to simplify this paper and to help in understanding its main points, we have restricted the mathematics to simple equations or results. The derivation of these theoretical results is given in the Appendix.

## THE GENERAL PROBLEM OF OPTIMIZATION IN LIQUID CHROMATOGRAPHY

The difficulty of optimization in LC is due to the fact that the separation of each pair of compounds depends upon many variables. For the sake of convenience they can be separated into main and secondary parameters.

The nine main parameters are: the HETP ( $H$ ), the column length ( $L$ ), the number of plates ( $N$ ), the velocity of the mobile phase ( $u$ ), the column pressure drop ( $\Delta P$ ), the particle diameter ( $d_p$ ), the retention time of the second solute of the pair of interest ( $t_R$ ), the resolution ( $R_s$ ) and the maximum concentration of the peak ( $C_{\max}$ ).

The secondary parameters, of lesser importance, are: the solvent viscosity ( $\eta$ ), the capacity ratio ( $k'$ ), the specific column permeability ( $k_0$ ), the total and external porosities ( $\varepsilon_m$ ,  $\varepsilon_e$ ), the temperature ( $T$ ), the diffusion coefficient ( $D_m$ ), the sample size ( $m$ ), the selectivity ( $\alpha$ ), the Knox HETP coefficients ( $A$ ,  $C$ ,  $\gamma$ )<sup>22</sup>, the column diameter ( $d_c$ ), etc.

The basis for this classification is that the secondary variables are supposed to be virtually constant for a given system and a given packing method at a given temperature. Obviously, some secondary parameters have to be changed into primary parameters in some instances (e.g.,  $d_c$  and  $m$  on the preparative scale).

Not all of the main variables are independent but are related via the following classical equations<sup>22-24</sup>:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{1 + k'} \quad (1)$$

$$N = \frac{L}{H} \quad (2)$$

$$H = \frac{2 \gamma D_m}{u} + \frac{A}{D_m^{1/3}} \cdot u^{1/3} d_p^{4/3} + \frac{C d_p^2 u}{D_m} \quad (3)$$

or any other HETP equation,

$$t_R = \frac{L}{u} (1 + k') \quad (4)$$

$$u = \frac{k_0 d_p^2}{\eta} \frac{\Delta P}{L} \quad (5)$$

$$C_{\max.} = \frac{4 m \sqrt{N}}{\pi \varepsilon_m (1 + k') d_c^2 L \sqrt{2 \pi}} \quad (6)$$

Eqn. 3 is derived from the Knox equation<sup>22</sup> given in reduced coordinates, with the slight approximation of  $0.33 \approx 1/3$ . With these six equations, it turns out that only three of the nine main parameters are independent and can be chosen at will. Note that  $R_s$  and  $N$  are so closely related that we may prefer not to consider  $R_s$ . Then the number of main parameters is only eight but the number of independent variables remains unchanged.

Any optimization procedure aims to find the optimum value of one of these parameters as a function of the other seven, in fact as a function of any three independent variables chosen arbitrarily among these seven factors. As these variables can be any set of three parameters out of the seven, there are  $C_7^3 = 35$  possible sets of independent variables for each of the eight possible parameters to be optimized. The number of possible optimization theories of LC is thus  $8 \times C_7^3 = 280$ . Not all of these theories are of interest, however, as the analyst is not equally interested in all of them. The column length, plate height and fluid velocity are rather irrelevant parameters to optimize and  $C_{\max.}$  is not a practical variable, so we are left with  $N$  (or  $R_s$ ),  $t_R$ ,  $\Delta P$ ,  $d_p$  and  $C_{\max.}$  as the parameters that are interesting to optimize, and with  $N$ ,  $t_R$ ,  $\Delta P$ ,  $d_p$  and  $L$  as possible independent variables. Then the number of possible theories is reduced to  $4C_4^3 + C_5^3 = 26$ .

This illustrates the many different aspects that must be considered in chromatography, depending upon what is required and indeed we know that an analysis has to be carried out under completely different conditions depending on whether we are interested in speed of analysis ( $t_R$ ) or in trace analysis ( $C_{\max.}$ ):

Usually, however, theoreticians consider that analysts are interested in short analysis times and large resolution ( $R_s$  or  $N$ ), but these two parameters can hardly be

optimized separately. In order to effect a compromise, it is customary to consider the ratio  $N/t_R$ ; therefore, some optimization procedures in LC originate from the idea of generating the maximum number of plates within the shortest possible time<sup>11,17,25</sup>. Undoubtedly this can be achieved only by using very high pressure because, from eqns. 2-4:

$$\frac{t_R}{N} = (1 + k') \cdot \frac{H}{u} = (1 + k') \left( \frac{2 \gamma D_m}{u^2} + \frac{A d_p^{4/3}}{D_m^{1/3} u^{2/3}} + \frac{C d_p^2}{D_m} \right) \quad (7)$$

and  $t_R/N$  decreases monotonously with increasing flow velocity to a minimum value reached when  $u$  is infinite.

Thus, the higher the pressure, the greater is the number of plates per unit time, whatever the column used. Because of equipment limitations, however, this will lead to too short an analysis time and too high an inlet pressure<sup>3</sup>, thus indicating that the problem has been wrongly stated: in effect, this is not what the analyst needs.

Instead, we consider that the analyst needs merely to obtain a given separation, and hence a given resolution, in a reasonably short time, although in practice this analysis time will never be less than one or even several minutes. Thus, for a given chromatographic system, the problem becomes one of finding the experimental conditions that lead to the desired values of  $N$  and  $t_R$ . In this paper, we optimize the column inlet pressure, which is one of the most costly factors in modern LC, and we have chosen the particle diameter as the third parameter.

Obviously, optimization of pressure is the determination of the lowest possible pressure necessary to achieve any set of values of  $t_R$  and  $N$  and the corresponding particle diameter and column length. Note that when formulated in this way, our aim differs from that of classical optimization, as we intend to find the best use of a given system instead of the best use of a given column. Note also that a set of values of  $t_R$  and  $N$  completely defines a chromatogram, and this chromatogram can be achieved with very different columns, pressures, etc.

#### MINIMUM PRESSURE TO ACHIEVE A CHROMATOGRAPHIC SEPARATION

The optimization problem we are now dealing with is the determination of the minimum pressure necessary to achieve a given separation, as a function of three parameters, namely the efficiency ( $N$ ), the analysis time and the column length or the particle diameter.  $N$  is usually determined by the analytical problem, from eqn. 1, and the too often implicit choice of the resolution; within certain practical limits, the analyst can usually select the values of the other parameters that suit his requirements best.

For the sake of simplicity, we shall assume that the number of plates and the retention time are prerequisites and we shall optimize the column length or the particle diameter for minimum inlet pressure. The mathematical discussion is given in the Appendix and only its results are discussed here. Figs. 1 and 2 show the variation of the column pressure as a function of column length and particle diameter, respectively, in two different cases: analysis times are 60 sec and 5 min, respectively; the efficiency is 5000 plates in both cases, and the other parameters are given in the figure captions. The viscosity of the mobile phase, the diffusion coefficient and the specific permeability,

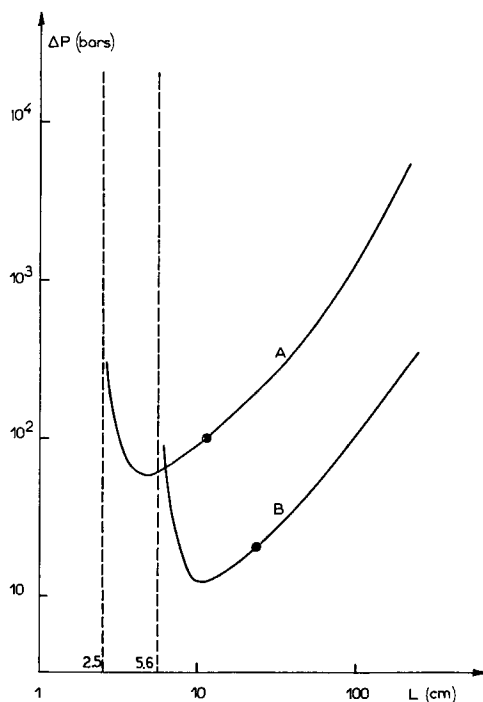


Fig. 1. Column inlet pressure as a function of the column length. The problem is to obtain a peak with 5000 plates in 60 sec (curve A) or 300 sec (curve B). Values of the other parameters:  $k' = 2$ ; mobile phase viscosity = 0.4 cP; diffusion coefficient in the mobile phase =  $3.5 \cdot 10^{-5}$  cm<sup>2</sup>/sec; specific permeability,  $k_0 = 8.46 \cdot 10^{-4}$  ( $\epsilon_m = 0.85$  and  $\epsilon_e = 0.35$ ; *cf.*, ref. 3); Knox coefficients,  $\alpha = 0.9$ ,  $A = 1.7$ ,  $C = 5 \cdot 10^{-2}$ .

$k_0$ , are typical of values encountered in modern LC. The Knox coefficients are typical of those found for good columns packed by advanced methods of slurry packing<sup>26-29</sup>. The value of  $k'$  ( $k' = 2$ ) selected might seem small, but the effect on the optimum pressure of increasing  $k'$  at constant  $t_R$  is the same as that of decreasing  $t_R$  at constant  $k'$ .

Figs. 1 and 2 are not independent as in each case there is a relationship between  $L$  and  $d_p$ , because once  $N$ ,  $t_R$  and  $L$  are determined the other parameters are given by eqns. 1-6.

It is obvious from Fig. 2 that when using the presently available 7- $\mu$ m particles, the analyst can obtain performances of a very high standard (5000 plates in 300 sec with  $k' = 2$ , *i.e.*, 7.4 effective plates per second) with an inlet pressure of less than 12 atm and a short column (11 cm).

From Figs. 1 and 2, or from similar figures drawn for other specific numerical problems, the analyst can select the column design parameters that will allow him the best use of his equipment by working at the lowest pressure. Some of the consequences will be discussed later, but we shall point out here some characteristics of these plots.

In Fig. 1, curves A and B have vertical asymptotes, showing that the performances desired ( $t_R$ ,  $N$ ) cannot be achieved with shorter columns. Mathematical consid-

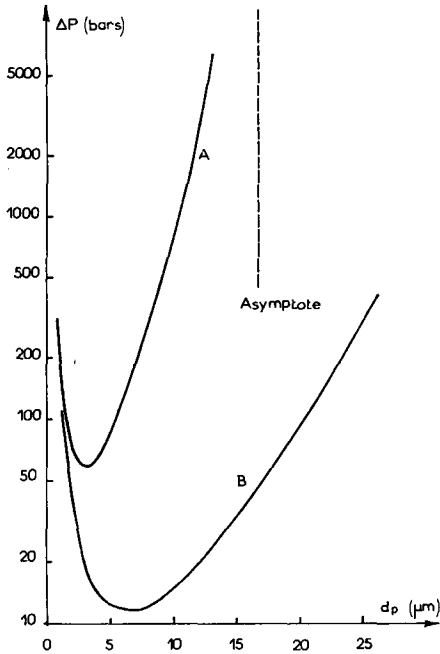


Fig. 2. Column inlet pressure as a function of the particle diameter. Problem and parameters as in Fig. 1. The curves have vertical asymptotes for  $d_p = 16.7 \mu\text{m}$  (curve A) and  $37.3 \mu\text{m}$  (curve B).

erations show that this limit is

$$L \geq L^*: L^* = \sqrt{\frac{2 \gamma D_m t_R N}{1 + k'}} \quad (8)$$

Similarly, Fig. 2 shows that there is an upper limit to the particle diameter above which the analysis cannot be performed:

$$d_p \leq d_p^*: d_p^* = \sqrt{\frac{D_m t_R}{C N (1 + k')}} \quad (9)$$

Although instructive, Figs. 1 and 2 suffer from the disadvantage that the plots are specific to a given problem and depend on the value of the parameters selected ( $N$ ,  $t_R$ ,  $k_0$ ,  $k'$ ,  $\eta$ ,  $D_m$ , Knox coefficients<sup>22</sup>, etc.). In order to obtain a universal plot, we shall define reduced parameters:

$$p = \frac{\Delta P}{\Delta P_0} \quad (10)$$

$$l = \frac{L}{L_0} \quad (11)$$

$$\delta_p = \frac{d_p}{d_{p0}} \quad (12)$$

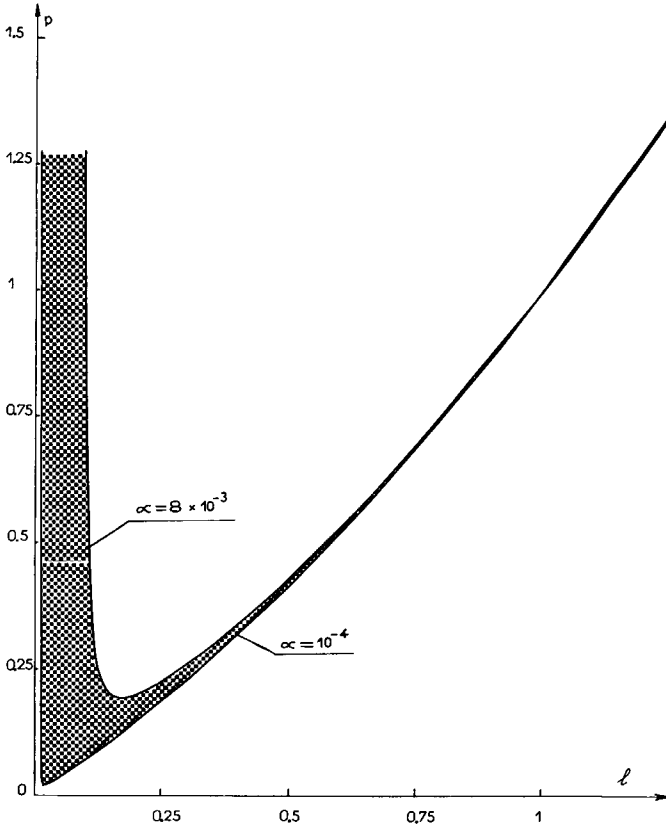


Fig. 3. Variation of the reduced pressure with the reduced length for two extreme values of  $\alpha$ .

where, for the reasons discussed in the Appendix, the standards  $\Delta P_0$ ,  $L_0$  and  $d_{p_0}$  are defined as follows:

$$\Delta P_0 = \frac{27 C^3}{A^3} \left( 2\gamma + \frac{4 A^3}{27 C^2} \right)^2 \cdot \frac{\eta (1 + k') N^2}{t_R k_0} \tag{13}$$

$$L_0 = \sqrt{\frac{D_m t_R N}{1 + k'} \left( 2\gamma + \frac{4 A^3}{27 C^2} \right)} \tag{14}$$

$$d_{p_0} = \sqrt{\frac{A^3 D_m t_R}{27 C^3 \left( 2\gamma + \frac{4 A^3}{27 C^2} \right) (1 + k') N}} \tag{15}$$

The fact that these standards are not clearly defined when  $C$  becomes zero has very little importance in practice as it occurs only for unretained solutes eluted through a bed of non-porous glass beads, which is a case of little importance in analytical chromatography.

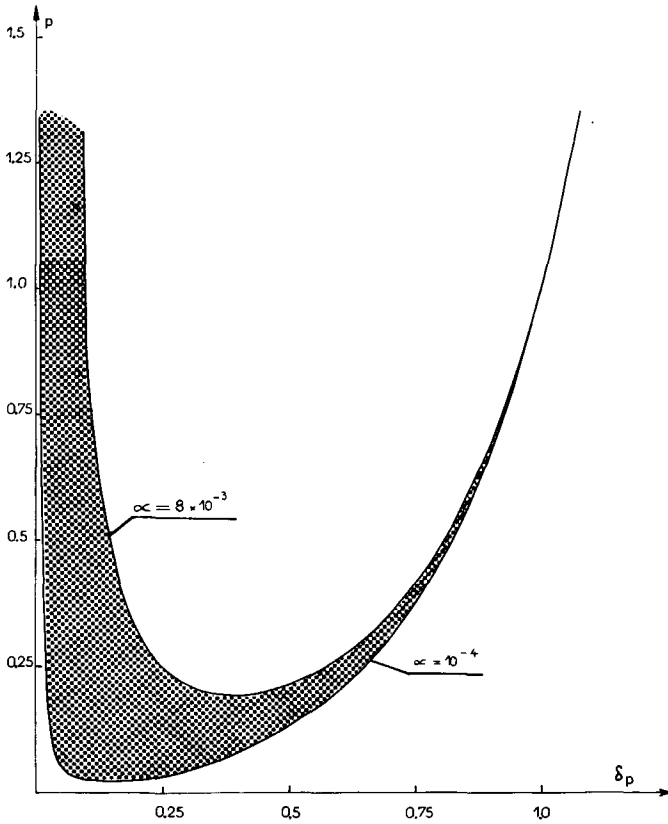


Fig. 4. Variation of the reduced pressure with the reduced particle diameter for two extreme values of  $\alpha$ .

Finally it is useful to consider the number

$$\alpha = \frac{2\gamma}{2\gamma + \left(\frac{4A^3}{27C^2}\right)} \quad (16)$$

which appears in eqns. 13–15. Analysis of the values of the Knox parameters for many columns<sup>22,26,30</sup> shows that  $\alpha$  is usually between  $10^{-4}$  and  $8 \cdot 10^{-3}$ .

Figs. 3 and 4 give the plots of the reduced pressure,  $p$ , versus the reduced length,  $l$ , and the reduced particle diameter,  $\delta_p$ , for the two extreme values of  $\alpha$ . The minimum reduced pressures are the same on the two plots, as they obviously should be. Note that with the reduced parameters, both of the functions  $p = f(l)$  and  $p = f(\delta_p)$  are dependent only on  $\alpha$ .

To this minimum pressure correspond the optimum values of the reduced column length and particle diameter. These optimum values are given in Table I for different values of  $\alpha$ , together with the lower limit of the reduced column length, corresponding to an infinite pressure, and which is given by (*cf.*, Appendix)

$$l^* = \sqrt{\alpha} \quad (17)$$



TABLE I

VALUES OF THE OPTIMUM REDUCED PRESSURE, LENGTH AND PARTICLE DIAMETER FOR DIFFERENT VALUES OF  $\alpha$

$\alpha$	$l^* = \sqrt{\alpha}$	$l_{min.}$	$\delta_{p_{min.}}$	$p_{min.}$
$10^{-4}$	0.01	0.0199	0.140	0.0202
$4 \cdot 10^{-4}$	0.02	0.0396	0.196	0.0408
$10^{-3}$	0.0316	0.0623	0.244	0.0651
$2 \cdot 10^{-3}$	0.0447	0.0877	0.287	0.0931
$5 \cdot 10^{-3}$	0.0707	0.1372	0.354	0.1500
$8 \cdot 10^{-3}$	0.0894	0.1724	0.393	0.1920

whereas the upper limit of the reduced particle diameter is given by

$$\delta_p^* = 2/\sqrt{(1 - \alpha)} \tag{18}$$

It is remarkable that the optimum reduced length is almost equal to  $2l^*$ , the deviation being always less than 4%.

It is interesting to compare the flow velocity corresponding to these optimum conditions with that which corresponds to the minimum value of the classical HETP curve. It can be shown mathematically (*cf.* Appendix) that they are the same. In other words, our optimization procedure is equivalent to selecting the column which, operated at the minimum plate height, gives the number of plates needed in the analysis time desired.

In order to illustrate this point, Fig. 5 shows the variation of the retention time (inversely proportional to the flow velocity) with the plate number of the column corresponding to the minimum pressure for the analytical problem relating to Figs. 1 and 2 (column length, 11 cm; particle diameter,  $7 \mu\text{m}$ ). The maximum efficiency is

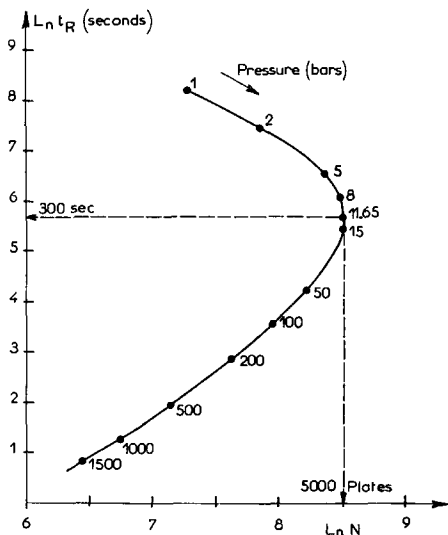


Fig. 5. Variation of the retention time with the number of plates for a hypothetical column of 11-cm length packed with  $7\text{-}\mu\text{m}$  particles. The coefficients used are the same as in Fig. 1.

observed for the velocity that corresponds to the minimum pressure derived in Fig. 1 or 2. In the Appendix, it is shown that this is always the case and is not simply a numerical coincidence.

At this point, we can compare the results of this optimization procedure with the classical procedure, which assumes that large velocities should be used to maximize the number of plates per unit time. Again, we shall use a numerical comparison in order to illustrate better the differences in the results and the opposite viewpoints. From eqns. 1-5 and with the numerical values of the secondary parameters selected for Fig. 1, it is found that a 2-m column packed with 25- $\mu\text{m}$  particles generates 5000 plates in 300 sec, and thus gives exactly the same chromatogram as our optimum column (11 cm long, 7- $\mu\text{m}$  particles). The inlet pressures, however, are 304 and 11.5 atm, respectively. From the point of view of the analyst, this shorter column is certainly more convenient and safer to use. The reduced velocities ( $v = u d_p / D_m$ ) are 142 and 2.15, respectively. The first column is certainly operated at a velocity that is well in the classical optimum range. The second column is operated at its maximum efficiency, at the minimum plate height, which so many chromatographers forgot when they shifted from gas chromatography to LC. It could be argued that the short column is not operated at its optimum and that by using a higher pressure and a higher velocity, a larger number of plates per unit time could be obtained. This is certainly true, but again it will be possible to find an equivalent system, using a shorter column packed with smaller particles. So there appear to be two ways (at least) of improving LC column performances: the use of increasingly higher pressure and the use of shorter columns. In both instances, small particles have to be used. The consequences for the design of equipment are different, but equally serious: extreme pressures in one instance and extremely small volumes of detector and tubing and considerable sampling problems in the other. These problems will be discussed in a subsequent paper<sup>31</sup>.

Anyway, it is now easy to find the experimental conditions corresponding to the minimum pressure. If the Knox coefficients are unknown for the material selected as the stationary phase, they can be derived from the HETP plot for one column. Figs. 3 and 4 and/or Table I can then be used. Knox coefficients, however, can be found for many packings<sup>22,26,30</sup>. It is unlikely, of course, that particles of the correct diameter will be available but, as can be seen from the figures, the pressure does not increase much for relatively large variations in  $d_p$  around the optimum, which is not very critical (*cf.*, Table II).

As an example, the two separations reported in Figs. 6 and 7 have been optimized simply by using the above procedure. The chromatograms are similar and the lengths and particle diameters agree well with predicted values. The slight difference that remains between the two chromatograms can be attributed mainly to a change in the surface activity between the two packing materials<sup>32</sup>, in the pore volume and in the Knox coefficients. Nevertheless, this illustrates the general trend of our procedure.

## PRACTICAL CONSEQUENCES

The first important consequence is that it is possible to obtain excellent analysis with moderate pressures<sup>4-10</sup>. Conversely, using the high pressures that are feasible at present, it should be possible to achieve extremely high efficiencies. The discussion

TABLE II  
 PRESSURE DROP, PARTICLE DIAMETER AND REDUCED VELOCITY NECESSARY TO ENSURE AN EFFICIENCY OF 5000 PLATES\* IN AN ANALYSIS TIME OF 5 min AS FUNCTION OF COLUMN LENGTH

$L$ (cm)	$\Delta P$ (bar)	$d_p$ ( $\mu m$ )	$v = \frac{u d_p}{D_m}$
6	86	1.4	0.24
8	14.5	4.6	1
10	11.8	6.3	1.8
10.9	11.65	6.9	2.15
20	16.3	10.8	6.1
50	44.1	16.4	23
100	110	20.8	59.2
150	196	23.3	99.6
200	304	25.0	142

\* Effective plate number: 2222 (i.e., 7.4 plates/sec).

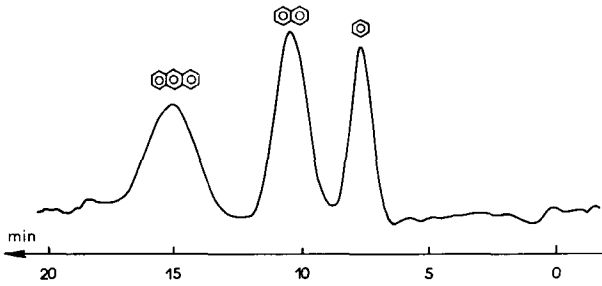


Fig. 6. Analytical separation of benzene, naphthalene and anthracene using a 2-m column packed with 25–31.5- $\mu m$  Spherosil particles operated at a pressure of 305 bar.

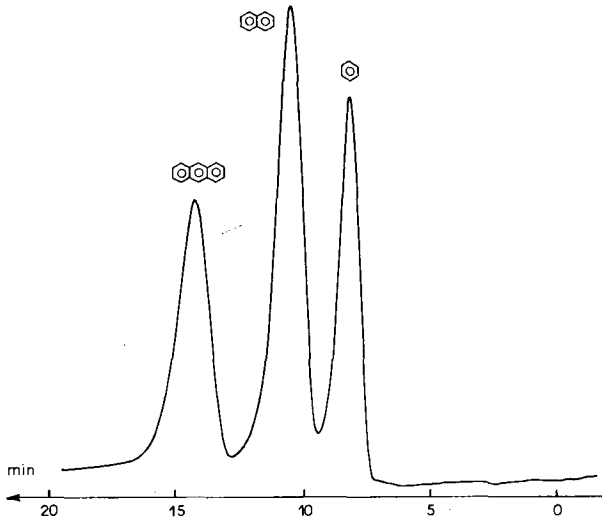


Fig. 7. Analytical separation of benzene, naphthalene and anthracene using a 6-cm column packed with 7- $\mu m$  Spherosil particles operated at a pressure of 1.75 bar.

can be made best by considering eqn. 13. Eqn. 13 shows that  $\Delta P_0$ , and consequently the minimum necessary pressure for a given packing material and a given packing quality (*i.e.*, a given  $\alpha$  value), is proportional to the ratio  $N^2/t_R$ : in other words, we again have to compromise between speed and efficiency. It also seems from eqn. 13 that  $N^2/t_R$  is a more appropriate parameter for expressing this competition than is the empirical ratio  $N/t_R$  commonly used.

Fig. 8 illustrates the relationship between  $N$  and  $t_R$  at constant pressure drop, under optimum conditions (minimum  $p$  from Figs. 3 and 4). Obviously, the column

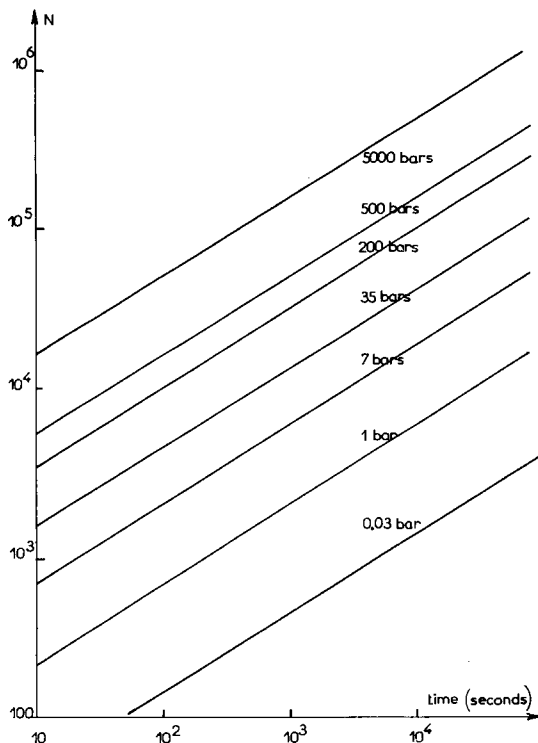


Fig. 8. Relationship between efficiency and retention time for columns operated under optimum conditions. Parameters as in Fig. 1.

length and particle diameter vary along these lines. Although the exact values are valid only within the framework of parameters selected (*cf.*, Fig. 1), it is clear from Fig. 6 that extremely difficult separations can be achieved in a reasonable time with most modern equipment: 20,000 plates can be achieved in 15 min with a moderate inlet pressure of 60 atm and 100,000 plates are not impossible. Table III lists a number of similar possibilities with the corresponding column parameters.

Working with moderate pressures has some important further advantages regarding the heat effect, sensitivity and safety.

It has been shown by Halász *et al.*<sup>33</sup> that the energy used to pump the mobile phase through the column is turned into heat evolved in the column by frictional

TABLE III

COLUMN LENGTH, PARTICLE DIAMETER AND MINIMUM PRESSURE DROP NECESSARY TO ENSURE AN EFFICIENCY OF  $N$  PLATES IN A TIME  $t_R$

The lower left-hand part of the table corresponds to impractical conditions.

$N$	Parameter	$t_R$						
		10 sec	1 min	5 min	15 min	30 min	1 h	5 h
200	$\Delta P$ (mbar)	560	93	19	6.2	3.1	1.6	0.31
	$L$ (cm)	0.4	1	2.2	3.8	5.3	7.5	16.8
	$d_p$ ( $\mu\text{m}$ )	6.3	15.5	34.6	60	85	120	268
1000	$\Delta P$ (bar)	14	2.3	0.47	0.16	0.078	0.039	0.0078
	$L$ (cm)	0.9	2.2	4.9	8.4	11.9	16.8	37.6
	$d_p$ ( $\mu\text{m}$ )	2.8	6.9	15.5	26.8	38	54	120
5000	$\Delta P$ (bar)	350	58	11.6	3.9	1.9	1	0.2
	$L$ (cm)	2	4.9	10.9	18.8	26.6	37.6	84
	$d_p$ ( $\mu\text{m}$ )	1.3	3.1	6.9	12	17	24	54
20,000	$\Delta P$ (bar)	5600	930	186	62	31	15.5	3.1
	$L$ (cm)	4	9.7	21.7	37.6	53	75	168
	$d_p$ ( $\mu\text{m}$ )	0.6	1.6	3.5	6	8.5	12	26.8
100,000	$\Delta P$ (bar)	140,000	23,300	4700	1550	780	390	78
	$L$ (cm)	8.9	21.7	48.6	84	119	168	376
	$d_p$ ( $\mu\text{m}$ )	0.3	0.7	1.5	2.7	3.8	5.4	12

forces. The ensuing temperature increase of the mobile phase along the column is proportional to the pressure drop of the column and becomes

$$\Delta T = \frac{\Delta P}{C_v} \quad (19)$$

in the adiabatic case<sup>33</sup>, where  $C_v$  is the heat capacity at constant volume of the mobile phase ( $C_v \approx 10$  for most liquids except water,  $\Delta P$  is measured in atm and  $\Delta T$  in  $^{\circ}\text{C}$ ). It is still unclear what the effects of this temperature gradient are and to what extent it affects the column performance. Anyway, minimizing the inlet pressure also minimizes the temperature gradient and its adverse consequences. In fact, from eqn. 19, the gradient  $\Delta T/L$  is a minimum for minimum  $\Delta P/L$ , *i.e.*, under the conditions corresponding to the contact between the curve in Fig. 1 and its tangent from the origin, conditions which are not far from the optimum.

Eqn. 6 relates the concentration at peak maximum to the column length and other parameters. As sensitivity is critical in many applications, it would be interesting to minimize the dilution effect associated with all chromatographic separations<sup>34</sup>. Eqn. 6 shows that the shorter the column, the larger is the maximum peak concentration. In order to achieve a given separation or plate number in a given time, the above discussion indicates that the shortest possible column length is  $L^*$ , as given by eqn. 8. Unfortunately, the corresponding pressure is infinite (Fig. 3) but, as shown in the Appendix, the column length corresponding to the minimum pressure is only twice as large; the concentration at the peak maximum is reduced only by a factor of 2, whereas when high pressures and large flow velocities are used, which necessitates the

use of much longer columns, a marked dilution and a larger detection limit result.

Finally, working with low pressures is much safer because of the possible progressive reduction in metal strength under continuous stress.

The use of short columns also has some drawbacks: sampling is much more critical, dead volumes should be drastically reduced, column packing of fine particles is very difficult and, at present, the technique is well controlled in only a few laboratories. If the column is not packed as well as it can be, the Knox coefficients are larger or the Knox equation, although always theoretically sound, may even not be the most appropriate for describing the variation of  $H$  with  $d_p$ . In these cases, however, the minimum pressure will still correspond to the optimum flow velocity, and it is possible by trial and error to find the column length and particle diameter that will enable the desired separation to be performed in a reasonable time under a low pressure. The use of the equations and figures discussed here may help the analyst to find an acceptable compromise between the separations he desires and those which he is able to achieve.

## CONCLUSION

Despite the general belief that analysis in LC must be carried out at very large reduced velocities, we have shown that it is usually better to operate at a velocity corresponding to the minimum HETP when an appropriate column has been found. This arises from the fact that there is always a minimum pressure sufficient to ensure any desired analysis and that this pressure is usually much lower than those used in practice.

Such an optimization procedure offers several advantages compared with the classical procedure: (1) most moderately difficult analyses (up to 5000 plates) can be carried out with inexpensive pumping devices; (2) it is possible to make better use of any pressure device so that currently available equipment appears able to perform very demanding analyses (50,000 plates or more); (3) the use of low pressures reduces the heat effect generated by the friction forces between the liquid and the bed; and (4) it prevents excessive dilution of the sample during the chromatographic process.

However, these advantages are balanced by the necessity for much more carefully designed equipment. A forthcoming paper will discuss these design problems<sup>31</sup>.

## APPENDIX

### *Expression of the function $\Delta P = f(L)$*

As already mentioned, we assume that the system is chosen so that the set of secondary parameters is constant. In order to optimize the inlet pressure as a function of  $L$ , we must eliminate the diameter of the particles ( $d_p$ ), the linear velocity ( $u$ ) and the HETP ( $H$ ) between the four equations 2, 3, 4 and 5. It is then a simple matter to show that

$$\frac{L}{N} = \frac{2 \gamma D_m t_R}{L(1+k')} + \frac{AL^{5/3} \eta^{2/3} (1+k')}{D_m^{1/3} k_0^{2/3} t_R \Delta P^{2/3}} + \frac{C \eta L^3 (1+k')^2}{t_R^2 k_0 D_m \Delta P} \quad (\text{A1})$$

Note that this equation becomes much simpler when  $C = 0$ . However, this can only

happen when  $k' = 0$  and for non-porous supports, which is without interest as no separation can be achieved.

For further reference, it is worth pointing out that eqn. A1 implies that

$$\frac{L}{N} \geq \frac{2 \gamma D_m t_R}{L(1 + k')} \tag{A2}$$

so that a number of plates,  $N$ , can be generated in the time  $t_R$  only if

$$L \geq \sqrt{\frac{2 \gamma D_m t_R N}{1 + k'}} \tag{A3}$$

The problem now is to express  $\Delta P$  as a function of  $L$  from eqn. A1. It is convenient to re-write eqn. A1 as

$$x^3 + ax + b = 0 \tag{A4}$$

where

$$x = (\Delta P)^{1/3} \tag{A5}$$

$$a = -\frac{A \eta^{2/3} (1 + k')^2 L^{8/3} N}{D_m^{1/3} k_0^{2/3} t_R [(1 + k') L^2 - 2 \gamma D_m t_R N]} \tag{A6}$$

$$b = -\frac{C \eta (1 + k')^3 L^4 N}{t_R^2 k_0 D_m [(1 + k') L^2 - 2 \gamma D_m t_R N]} \tag{A7}$$

Now  $\Delta P^{1/3}$  is the solution of eqn. A4 and its expression depends on the sign of

$$\Delta = \frac{b^2}{4} + \frac{a^3}{27} = \frac{C^2 \eta^2 (1 + k')^6 L^8 N^2}{4 t_R^4 k_0^2 D_m^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]^2} \cdot \left[ 1 - \frac{4 A^3 D_m t_R N}{27 C^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]} \right] \tag{A8}$$

It is easily shown that  $\Delta = 0$  for

$$L_0 = \sqrt{\frac{D_m t_R N}{(1 + k')}} \left( 2 \gamma + \frac{4 A^3}{27 C^2} \right) \tag{A9}$$

In this case, the pressure and the diameter of particles are, respectively:

$$\Delta P_0 = \frac{27 C^3}{A^3} \left( 2 \gamma + \frac{4 A^3}{27 C^2} \right)^2 \frac{\eta (1 + k') N^2}{t_R k_0} \tag{A10}$$

and

$$d_{p_0} = \sqrt{\frac{A^3 D_m t_R}{27 C^3 \left( 2 \gamma + \frac{4 A^3}{27 C^2} \right) (1 + k') N}} \tag{A11}$$

Thus  $b^2/4 + a^3/27$  is positive when  $L > L_0$  and negative when  $L < L_0$ . In the first case, there is only one real root, while there are three in the second case.

In the case when  $L > L_0$ , the root is:

$$x = \sqrt[3]{-\frac{b}{2} + \sqrt{\frac{b^2}{4} + \frac{a^3}{27}}} + \sqrt[3]{-\frac{b}{2} - \sqrt{\frac{b^2}{4} + \frac{a^3}{27}}} \quad (\text{A12})$$

and  $\Delta P$  is given by the following equation:

$$\Delta P = \frac{C \eta (1 + k')^3 N L^4}{t_R^2 k_0 D_m [(1 + k') L^2 - 2 \gamma D_m t_R N]} \cdot \left\{ 1 + \left( \frac{4 A^3 D_m t_R N}{8 C^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]} \right)^{1/3} \varphi \right\}$$

where

$$\varphi = \left[ 1 + \left( 1 - \frac{4 A^3 D_m t_R N}{27 C^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]} \right)^{1/2} \right]^{1/3} + \left[ 1 - \left( 1 - \frac{4 A^3 D_m t_R N}{27 C^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]} \right)^{1/2} \right]^{1/3} \quad (\text{A13})$$

In the case when  $L < L_0$ , eqn. A7 has three roots, one of which is positive and acceptable:

$$x = 2 \sqrt[3]{-\frac{a}{3}} \cdot \cos \left[ \frac{1}{3} \cdot \arccos \left( -\frac{b}{2} \sqrt[3]{-\frac{27}{a^3}} \right) \right] \quad (\text{A14})$$

Thus,  $\Delta P$  is given by:

$$\Delta P = \frac{C \eta (1 + k')^3 N L^4}{t_R^2 k_0 D_m [(1 + k') L^2 - 2 \gamma D_m t_R N]} \cdot \left\{ 1 + 3 \left( \frac{4 A^3 D_m t_R N}{27 C^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]} \right)^{1/2} \varphi' \right\}$$

where

$$\varphi' = \cos \left[ \frac{1}{3} \cdot \arccos \left( \frac{27 C^2 [(1 + k') L^2 - 2 \gamma D_m t_R N]}{4 A^3 D_m t_R N} \right)^{1/2} \right] \quad (\text{A15})$$

Although the expression of  $\Delta P$  as a function of  $L$  is given by two different equations, depending upon the conditions  $L < L_0$  or  $L > L_0$ , they are self-consistent as both equations give the same value of  $\Delta P = \Delta P_0$  when  $L = L_0$  and the derivatives  $d\Delta P/dL$  tend to the same value at this point:

$$\left( \frac{d\Delta P}{dL} \right)_{L=L_0} = \frac{972 C^5}{A^3} \cdot \left( \frac{A^3}{27 C^2} - \gamma \right) \left( 2 \gamma + \frac{4 A^3}{27 C^2} \right)^{3/2} \cdot \frac{\eta (1 + k')^{3/2}}{k_0 D_m^{1/2}} \left( \frac{N}{t_R} \right)^{3/2} \quad (\text{A16})$$



The physical meaning of the transition length is not clear at this stage. Note, however, that the mass transfer resistance term ( $Cd_p^2 u/D_m$  in eqn. 3) is equal to one third of the coupling term ( $Au^{1/3} d_p^{4/3}/D_m^{1/3}$ ) for  $L = L_0$  and increases with increasing  $L$ .

#### Reduced variables

Eqns. A13 and A15 can be easily simplified by introducing the reduced parameters

$$p = \Delta P/\Delta P_0 \quad (\text{A17})$$

$$l = L/L_0 \quad (\text{A18})$$

The expression of  $p$  as a function of  $l$  obviously keeps the same form as in eqns. A13 and A15, but now it is only a function of the single parameter  $\alpha$ , which itself is a function of the Knox coefficients:

$$\alpha = \frac{2\gamma}{2\gamma + \left(\frac{4A^3}{27C^2}\right)} \quad (\text{A19})$$

We thus obtain:

for  $l < 1$ :

$$p = \frac{l^4}{4} \cdot \left(\frac{1-\alpha}{l^2-\alpha}\right) \left\{ 1 + 3 \left(\frac{1-\alpha}{l^2-\alpha}\right)^{1/2} \cdot \cos \left[ \frac{1}{3} \cdot \arccos \left(\frac{l^2-\alpha}{1-\alpha}\right)^{1/2} \right] \right\} \quad (\text{A20})$$

for  $l > 1$ :

$$p = \frac{l^4}{4} \cdot \left(\frac{1-\alpha}{l^2-\alpha}\right) \left\{ 1 + \frac{3}{2} \left(\frac{1-\alpha}{l^2-\alpha}\right)^{1/3} \left[ \left(1 + \left(\frac{l^2-1}{l^2-\alpha}\right)^{1/2}\right)^{1/3} + \left(1 - \left(\frac{l^2-1}{l^2-\alpha}\right)^{1/2}\right)^{1/3} \right] \right\} \quad (\text{A21})$$

Of course, it can be seen that these two expressions give  $p = 1$  for  $l = 1$ . The derivatives ( $dp/dl$ ) are not definite at this point but tend to the same value:

$$\left(\frac{dp}{dl}\right)_{l=1} = \frac{4(1-3\alpha)}{3(1-\alpha)} \quad (\text{A22})$$

The limiting value of  $l$  is then

$$l^* = \sqrt{\alpha} \quad (\text{A23})$$

The function  $p$  exhibits a minimum when  $l < 1$  if  $\alpha < 1/3$ , which is always the case as typical values of  $\alpha$  are between  $10^{-4}$  and  $8 \cdot 10^{-3}$ . The value of that minimum is almost impossible to calculate formally; we have shown, however, that  $p$  is a minimum for a value of  $l$  very near to  $2\sqrt{\alpha}$  whenever  $\alpha$  remains small. Actually, this relationship is strictly exact only when  $C = 0$ . Nevertheless, Figs. 1 and 3 show the usefulness of that approximation as the error always remains less than 4% for practical values of  $\alpha$ .

A further feature of the  $p = f(l)$  relationship is that the function becomes like a parabola:

$$p \approx \left( \frac{1 - \alpha}{4} \right) \cdot l^2 \quad (\text{A24})$$

when  $l$  is large. In terms of pressure, the limiting parabola is

$$\Delta P = \left( \frac{C \eta (1 + k')^2 N}{k_0 D_m t_R^2} \right) \cdot L^2 \quad (\text{A25})$$

This shows clearly that the function  $\Delta P = f(L)$  is not an HETP curve although it seems similar.

Next, it is convenient to define a reduced particle diameter,  $\delta_p$ , after eqn. A11:

$$\delta_p = \frac{d_p}{d_{p0}} \quad (\text{A26})$$

Combining eqns. 4, 5, A17 and A18 with A26:

$$\delta_p = \frac{l}{\sqrt{p}} \quad (\text{A27})$$

As a result of eqn. A24,  $\delta_p$  reaches a limit for very large values of  $l$ :

$$\delta_p = \frac{2}{\sqrt{1 - \alpha}} \quad (\text{A28})$$

In term of particle diameters, this means that if  $N$  and  $t_R$  are given, the analysis is feasible only if there are available particles with a diameter smaller than

$$d_p \leq \sqrt{\frac{D_m t_R}{C (1 + k') N}} \quad (\text{A29})$$

*Relationship between the minima of  $H = f(u)$  and  $\Delta P = f(L)$*

We have shown that the curve  $\Delta P = f(L)$  exhibits a minimum. Let  $\Delta P$ ,  $L$ ,  $d_p$  be the corresponding parameters and consider a column of length  $L$  packed with particles of diameter  $d_p$ . We can show that the minimum HETP of this column is obtained when it is operated under a pressure  $\Delta P$ .

By definition,  $p = \Delta P / \Delta P_0$ ; thus  $\Delta P$  from eqn. A10 is

$$\Delta P = p \lambda \cdot \frac{N^2}{t_R} \quad (\text{A30})$$

where

$$\lambda = \frac{27 C^3}{A^3} \cdot \left( 2 \gamma + \frac{4 A^3}{27 C^2} \right)^2 \cdot \frac{\eta (1 + k')}{k_0} \quad (\text{A31})$$

As the system and the method of packing have been chosen,  $\lambda$  can be considered to be constant. Thus

$$\frac{N^2}{t_R} = \frac{\Delta P}{\lambda p} \quad (\text{A32})$$

In the following,  $N_0$  and  $t_{R_0}$  refer to the values of plate number and analysis time desired.  $N$  and  $t_R$  are the values obtained with a given column in conditions other than the optimal ones. Therefore:

$$\frac{N_0^2}{t_{R_0}} = \frac{\Delta P}{\lambda p} \quad (\text{A33})$$

The minimum of the  $H$  vs.  $u$  curve coincides with the maximum of the  $N$  vs.  $\Delta P$  curve for

$$H = \frac{L}{N} \quad (\text{A34})$$

and

$$u = \frac{k_0 d_p^2}{\eta} \cdot \frac{\Delta P}{L} \quad (\text{A35})$$

Combining eqn. A34 with eqn. 4 gives the expression of  $t_R$ :

$$t_R = \frac{\eta L^2 (1 + k')}{k_0 d_p^2} \cdot \frac{1}{\Delta P} \quad (\text{A36})$$

As  $t_R = t_{R_0}$  when  $\Delta P = \Delta P_{\text{opt.}}$ , if the maximum of the function  $N = f(\Delta P)$  does not correspond to  $\Delta P = \Delta P_{\text{opt.}}$ , then there must exist a pressure  $\Delta P_1 \neq \Delta P_{\text{opt.}}$  such that  $N_1 > N_0$  and  $t_{R_1} \neq t_{R_0}$ . In this case:

$$t_{R_1} = \frac{\eta L_{\text{opt.}}^2 (1 + k')}{k_0 d_{p_{\text{opt.}}}^2} \cdot \frac{1}{\Delta P_1} = t_{R_0} \cdot \frac{\Delta P_{\text{opt.}}}{\Delta P_1} \quad (\text{A37})$$

but, from eqn. A31:

$$\frac{N_1^2}{t_{R_1}} = \frac{\Delta P_1}{\lambda p_1} \quad (\text{A38})$$

Comparing with eqn. A36, this would give:

$$\frac{N_1^2}{t_{R_0}} = \frac{\Delta P_{\text{opt.}}}{\lambda p_1} \quad (\text{A39})$$

However, as  $N_1$  is greater than  $N_0$ , we can write:

$$\frac{N_1^2}{t_{R_0}} > \frac{N_0^2}{t_{R_0}} \quad (\text{A40})$$

which, from eqns. A33 and A39, implies that

$$\frac{\Delta P_{\text{opt.}}}{\lambda p_1} > \frac{\Delta P_{\text{opt.}}}{\lambda p_{\text{opt.}}} \quad (\text{A41})$$

and consequently

$$p_1 < p_{\text{opt.}} \quad (\text{A42})$$

which is impossible, for no pressure less than  $p_{\text{opt.}}$  can ensure the analysis.

Consequently, the maximum of the  $N(\Delta P)$  curve is indeed obtained for  $\Delta P = \Delta P_{\text{opt.}}$  as well as for the minimum of the HETP curve.

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