Journal of Chromatography, 367 (1986) 45–58
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 823

STUDIES ON THE OPTIMIZATION OF PARAMETERS OF PREPARATIVE LIQUID CHROMATOGRAPHIC COLUMNS FOR PRODUCTION OF CARDIAC GLYCOSIDES*

M. KAMIŃSKI*, B. ŚLEDZIŃSKA and J. KLAWITER
Institute of Inorganic Chemistry and Technology, Technical University of Gdańsk, 80-952 Gdańsk 6 (Poland)
(First received February 24th, 1986; revised manuscript received May 20th, 1986)

SUMMARY

Investigations aimed at maximization of the output of the production of cardiac glycosides from their mixtures using liquid chromatography are reported. The dependences of the yield of the process on column length, particle size and surface area of the packing and linear velocity of the mobile phase were examined. It has been established that a simultaneous decrease in the particle size and an increase in the column length and mobile phase flow-rate results in an improvement in the yield. However, such a procedure is limited by technological considerations. Optimum production conditions have been characterized.

INTRODUCTION

Among numerous publications concerning the application of liquid chromatography to the separation of pure substances, there are some in which the column dimensions resulted in preparative-scale separations[1,2]. In order to scale up a separation process, the technical and economic parameters must be optimized, *i.e.*, the selectivity, particle size, column length and linear velocity of the mobile phase. Other parameters, *e.g.*, the column diameter or pressure drop, determine only the absolute amount produced or constitute technical limitations.

On the bais of literature data, as well as own experience, several different situations can be distinguished, each one requiring a different strategy for optimization of the conditions of preparative-scale separation:

(1) When high selectivity is unobtainable and the resolution, R_s, determined under analytical conditions is less than 1.5, even when $N > 5 \cdot 10^3$. In such a case the rules for optimization of separations given by Martin *et al.*[3–5] are of special utility.

(2) $R_s \approx 1.5$. The relationships derived on the basis of the model of Haarhoff and Van der Linde[6] can be very useful, although the studies of Coq and co-workers[7–9]

* Presented at the *5th Danube Symposium on Chromatography, Yalta, November 11–16, 1985*. The majority of papers presented at this symposium have been published in *J. Chromatogr.*, Vols. 364 and 365 (1986).

0021-9673/86/$03.50

seem to be the most helpful, especially when considering the agreement between their calculations and experimental data.

(3) $R_s \gg 1.5$. The manner of optimization depends on the solubility of the substances in the mobile phase: (a) in the case of low solubility, $c < 10^{-4}$ M, only a typical volume overloading of a column[7–9] is possible, (b) in the case of high solubility, $c > 10^{-1}$ M, a mass overloading of the system is possible, enabling up to a five-fold increase in the column efficiency as compared to the case of volume overloading[10]. Gareil *et al.*[10–12] described the derivation of a function representing a chromatographic peak of a single substance. However, this does not enable simultaneous consideration of the presence of other constituents of the mixture. The conclusion that the influence of the column efficiency is not significant also seems doubtful; (c) intermediate situation, when the solubility ranges from 10^{-4} to 10^{-1} M. This is not explicitly described in the literature, and requires simultaneous application of mass and volume overloading.

In all of the situations discussed additional complications are possible, such as non-linearity of sorption isotherms over the entire range of concentrations.

The present research was aimed at optimization of the technical and economic parameters of industrial separation of a cardiac glycoside, lanatoside C, from an industrial waste originating from earlier stages in the production of this substance from an extract of *Digitalis Lanata* by different methods. Results on the selectivity were reported previously[13]. In the presented state of the art, it was impossible to employ existing optimization procedures, because this separation problem belongs to group 3(c) mentioned above, with polyfunctional sorption and predominant desolvation kinetics as additional complications.

Every optimization of the isolation conditions in liquid chromatography constitutes primarily an economic problem. The purpose of optimization is to ensure the minimum cost of obtaining unit mass of a product of desired purity in the shortest time possible. However, depending on the local conditions, the cost contributions arising from the packing material, mobile phase, instrumentation, labour, energy, etc., may be different. For this reason, a monetary unit per unit mass is not a useful criterion for evaluation and comparison of technologies, particularly those developed in different countries. We propose to use the following three parameters which characterize from different standpoints the effectiveness of the technology: a relative loading of a system

$$W = m/m_s \tag{1}$$

the time productivity of a column calculated per unit cross-section, A

$$P_t = m/t_c A \tag{2}$$

volume productivity of a column

$$P_v = m/V_c \tag{3}$$

where m = mass of mixture or of substance separated during a single cycle of separation, m_s = mass of stationary phase in the column, V_c = volume of the mobile phase used during a single separation cycle and t_c = duration of a single cycle.

The knowledge of the values of these three parameters enables a simultaneous evaluation of the production output and the degree of utilization of the phases. The primary advantage of such an approach is the fact that these parameters are independent of the column diameter, which enables evaluation of a given technology and comparison of various technologies at a preliminary stage.

Optimization of the conditions for production of a substance i by means of liquid chromatography requires the usage of a different method of determination of the maximum amount of the sampled mixture than those available in the literature[14,15]. The mass of a mixture, $m_{mix} = c_{mix}V_{mix}$, for which the product of the degree of recovery, r_i, of an isolated component of assumed purity, p_i, and the mass of this component present in a sampled mixture, m_i, is maximized was assumed as the maximum amount of the sampled mixture. A basic process parameter is the purity, p_i, of the isolated substance, which is associated with its further utilization. Monitoring of the purity is achieved by experimental determination of collected fractions. Intermediate fractions containing the remaining mass of a component i can be added to the separated mixture after their concentration.

The above definition can be written as

$$|m_{mix}| \overset{max}{\Leftrightarrow} \left\{ \begin{array}{l} |m_i r_i| \\ p_i = \text{const.} \end{array} \right\} \rightarrow \text{maximum} \qquad (4)$$

where c_{mix} = concentration (w/v or M) of sampled mixture, V_{mix} = sample volume, $m_i = V_{mix}c_{mix}a_i$ = mass of substance i in sampled solution and a_i = weight or mole fraction of substance i in the separated mixture. Other designations are explained in the text.

Besides the three parameters mentioned above, additional factors which should be taken into account during the final selection of the conditions for separation are technical limitations encountered while scaling up the separation process. Only some of them are of economic character.

An increase in column diameter is considered to be a principal method for scaling up this process. The main technical problem associated with this is maintaining both an high flow-rate and an high pressure at the pump and over the entire system. This involves increasing cost and increasing difficulties in preventing leakage, particularly of the column and sampling valves, as well as of increasing demands on the mechanical strength of materials.

The development of an effective and reproducible technology for packing of efficient and stable columns, particularly with diameters larger than 100 mm, is also essential. Another difficulty associated with the system scale-up is that of constructing devices which distribute liquid evenly over the entire cross-section of the packing.

EXPERIMENTAL

Materials

The column packings were Kieselgel Si 60 (E. Merck), d_p = 40–60 and 75–102 μm, obtained by sieve fractionation of the fraction having d_p = 0.063–0.2 mm, LiChrosorb Si 60, $\bar{d}_p$ = 10 μm, and LiChrosorb Si 100, $\bar{d}_p$ = 5 and 10 μm.

The mobile phase for preparative and industrial separations were dichloromethane–methanol–water (91.2:8:0.8, v/v) and for analysis of the composition of the collected fractions was dichloromethane–methanol–water (90:9:0.9, v/v). Dichloromethane and methanol were obtained from POCh Gliwice (Poland).

The mixture to be separated comprised a 15% (m/v) solution in the mobile phase of an industrial waste product containing 52, 26 and 13% (m/v) on average of lanatosides A, B and C, respectively, as the main components.

Instruments

The liquid chromatographic system consisted of a three-head membrane pump (output up to 13 ml/min at a pressure of 350 bar), a six-port sampling valve equipped with a sampling loop (1.5 mm I.D., volume 12 ml) with the possibility of quantitative sampling of smaller samples without the necessity of their transport along the entire loop, an UV detector operating at 254 nm (sensitivity 0.01 a.u.f.s., 10-mm 8-μl absorption cell; COBRABiD, Warsaw, Poland), a y–t strip-chart recorder and steel columns (6 mm I.D. × 200, 250, 400, 800 and 1200 mm) designed according to the principle of Wolf[16]. The pump, the sampling valve and the columns were designed and constructed in the Institute of Inorganic Chemistry and Technology, Gdańsk, Poland.

A technical liquid chromatograph, designed and constructed in the same in-

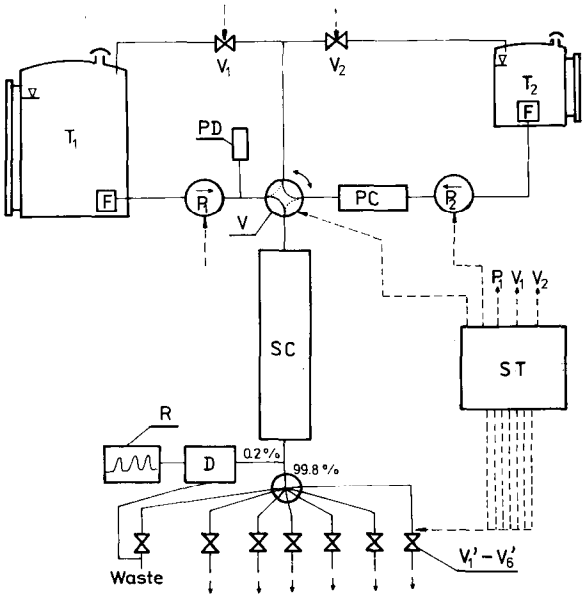

Fig. 1. Diagram of the industrial scale chromatograph (from ref. 19). P_1, P_2 = Four-head piston pumps Type NDA 25RS (ZDZ, Toruń, Poland), W_{max} = 6 dm^3/min, P_{max} = 60 bar; F = filters for liquids; PD = pulse damper; T_1 = mobile phase container; T_2 = container for the solution of the substance to be separated; V_1, V_2 = check valves; V = four-part valve; SC = 800 mm × 150 mm I.D. separating column designed according to the principle described in ref. 20; PC = 1000 mm × 100 mm I.D. pre-column packed with Kieselgel Si 60, d_p = 0.06–0.2 mm; D = UV detector (254 nm), volume 8 μl, optical path length 10 mm; V_1'–V_6' = electropneumatic valves of the fraction collector; R = y–t strip-chart recorder; ST = control system; ——, tubing for transport of liquids; – – – – –, controlling signal leads.

TABLE I

THE PARAMETERS OF THE COLUMNS USED

No.	d_p (μm)	d_c (mm)	L_c (mm)	u = 0.25 cm/s		u = 1 cm/s		φ**	ΔP (bar) at u = 0.25 cm/s
				N_0§	H_0* (μm)	N_0§	H_0* (μm)		
1	75–102	6	800	$1.4 \cdot 10^3$	600	$0.7 \cdot 10^3$	1200	1560	1.4
2	75–102	6	1200	$2.1 \cdot 10^3$		$1.0 \cdot 10^3$		1620	2.2
3	40–63	6	400	$1 \cdot 10^3$		$0.56 \cdot 10^3$		1680	3.1
4	40–63	6	800	$2.1 \cdot 10^3$	380	$1.2 \cdot 10^3$	670	1540	5.6
5	40–63	6	1600	$4.2 \cdot 10^3$		$2.4 \cdot 10^3$		1600	12
6	10	6	250	$3.4 \cdot 10^3$	75	$2.05 \cdot 10^3$	120	1280	35
7	10***	6	200	$3.0 \cdot 10^3$		$1.8 \cdot 10^3$		1300	26
8	10***	6	400	$5.9 \cdot 10^3$		$3.9 \cdot 10^3$		1210	48
9	5***	6	250	$15 \cdot 10^3$	17	–	–	1320	132
10	40–63	150	800	$2.2 \cdot 10^3$	364	–	–	2300	12
11	40–63	100	550	$1.5 \cdot 10^3$	375	–	–	3200	15

* HETP calculated for the peak of benzene, determined under analytical conditions.

** φ = Reduced permeability of column.

*** LiChrosorb Si-100; other packings, Si-60.

§ $N_0 = \frac{L_c}{H_0}$.

stitute, is illustrated in Fig. 1. Industrial chromatographic columns of diameters, d_c = 100 and 150 mm were made of acid-resistant steel with PTFE seals. The columns had two identical heads: distributing and collecting liquid. Both heads could be moved along the column axis and pressed against the surface of the packing by means of screws attached to the column body. The internal diameter of the steel tubing connecting the heads with the sampling valves was 4 mm. Each head was provided with a perforated and grooved steel plate, a wire gauze and a sintered metal disk sealed relative to the head casing. The rôle of the plate and gauze was to distribute liquid evenly over the packing cross-section. It was established experimentally for well packed columns that the heads resulted in the HETP values, determined under "analytical conditions", $H_0 \leqslant 100$ μm for mean particle size, $\bar{d}_p$ = 30 μm for column length, L_c = 250 mm at linear velocity, u close to optimum.

Methods

Research columns (6 mm I.D.) were packed using a slurry method ($\bar{d}_p$ = 5 and 10 μm) and using a dry impact method (d_p = 40–63 and 75–102 μm). Production columns (100 and 150 mm) were dry packed[17].

The efficiency of each column was characterized by the height equivalent to a theoretical plate (HETP) as a function of the mobile phase linear velocity, using 1–20 μl of a 10% solution of benzene in methanol in the case of 6 mm I.D. columns and 0.5 ml pure benzene in the case of technical columns; HETP was calculated on the basis of the peak widths at half height.

Table I lists the data for the research columns and the results of their performance (H_0, N_0), as well as the reduced permeability, φ.

The purity of collected fractions was examined by a chromatographic method.

RESULTS AND DISCUSSION

Because of the complex retention mechanism of lanatosides in the applied chromatographic system[13], the experimentally obtainable separation parameters, as well as the manner in which they change with column loading, differ both from theoretical values[10–12] and from experimental data obtained for explicitly defined systems. This is illustrated by chromatograms obtained during preliminary research with packings of various particle sizes (Fig. 2). The shape of the zones of all three lanatosides is asymmetric on all the columns, even when the column loading was of the order of $5 \cdot 10^{-5}$ g/g and "analytical" volumes of a solution were sampled. However, with benzene as a test substance the peaks were almost ideally symmetrical

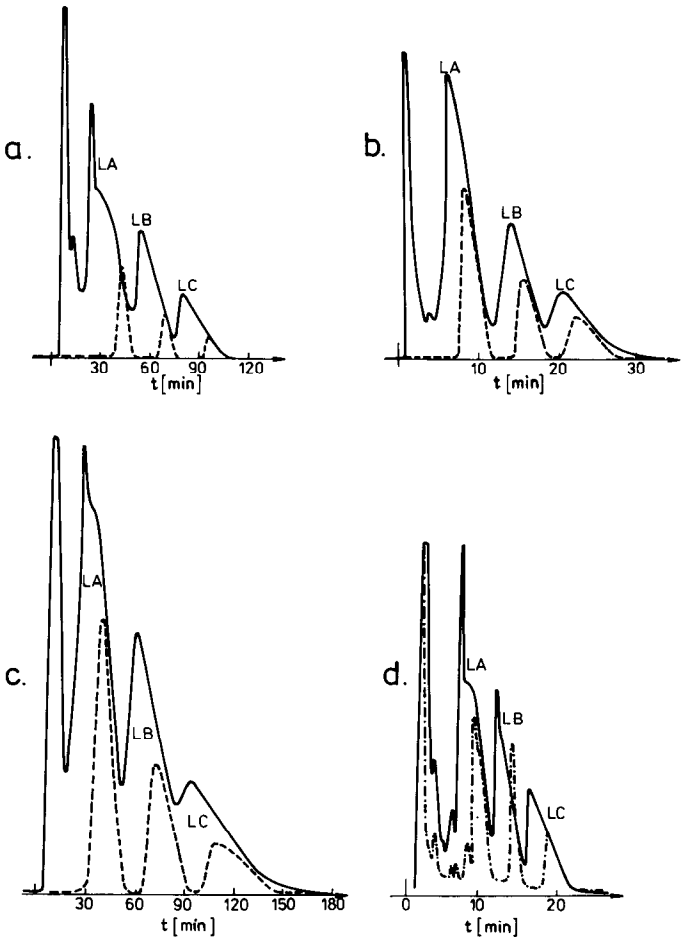

Fig. 2. Chromatograms obtained for 6 mm I.D. model columns under loadings of *ca.* $2 \cdot 10^{-2}$ g/g (——) and $5 \cdot 10^{-5}$ g/g (– – –). Mobile phase: dichloromethane–methanol–water (91.2:8:0.8, v/v). Detector (UV, 254 nm) sensitivity: 0.01–1.28 a.u. LA, LB, LC = Lanatosides A, B and C, respectively. (a) Column 4, V_i = 2 cm³, u = 2.5 mm/s; (b) column 4, V_i = 1 cm³, u = 10 mm/s; (c) column 2, V_i = 4 cm³, u = 2.5 mm/s; (d) column 9, V_i = 0.2 cm³, 0.05 cm³ (– · –), u = 3.1 mm/s.

(asymmetry factor at 0.1 peak height was $0.97 < As_{0.1} < 1.1$). This shows that the sorption isotherms are non-linear over the entire range of concentrations of the sampled solution. It may be due to the complex mechanism of sorption of lanatosides resulting from the specificity of the employed chromatographic system and from the multifunctional structure of lanatoside molecules, consisting of glycoside and aglycons fragments.

The mobile phase used in the separations, dichloromethane–methanol–water (91.2:8:0.8, v/v), is almost saturated with water. Under these conditions a water–methanol microlayer is probably formed at the surface of the silica gel. In addition to the predominant adsorption of a glycoside entity of the lanatoside molecules on the surface of this microlayer, the lanatoside molecules may also dissolve in the microlayer. Adsorption at the silica gel/water–methanol microlayer interface cannot also be precluded. The occurrence of such phenomena can be shown by the fact that the width of the lanatoside zones under "analytical" conditions is much larger than could be predicted from the column efficiency determined in a conventional manner for benzene (Fig. 3).

Figs. 4–7 present diagrams enabling the evaluation of the influence of the column length, particle size, specific area of packing as well as the linear velocity of the mobile phase on the effectiveness of separation of lanatoside C. Each experimental point in Figs. 4–7 corresponds to the maximum amount of the sampled mixture, as discussed in the Introduction. It was found that as a result of the introduction of the maximum amount of a sample for lanatoside C of purity higher than 99.5% (w/w) the R_s factor decreases to 0.6, and the efficiency of all the columns to a level of 50 theoretical plates. Under such conditions, 15–20% of lanatoside C is collected in the intermediate fraction, which, after concentration, was added to the separated mixture, in which the lanatoside C content was lower than the average.

It follows from Figs. 4–7 that the increase in the column length in the investigated range influences the values of all the three parameters determining the effectiveness of production, W, P_t and P_v. The greatest effect occurred with columns packed with the largest particles.

Fig. 4 shows that the relative loading of a system with a sample, W, increases

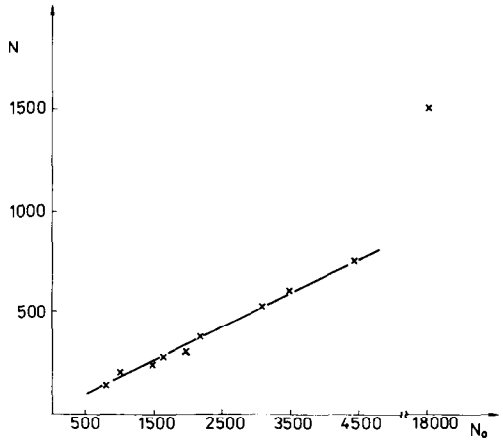

Fig. 3. Dependence of N measured for the lanatoside C peak on N_0 for benzene.

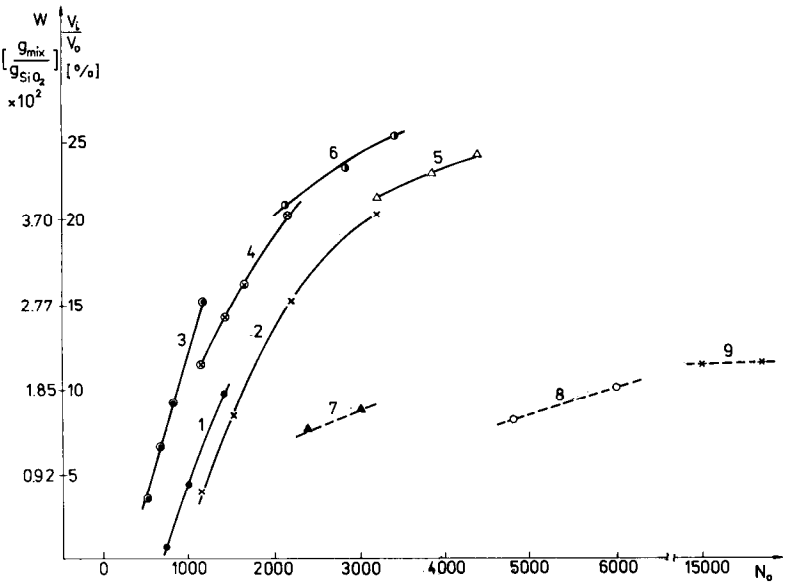

Fig. 4. The dependence of the maximum relative loading of the columns on their efficiency. Columns numbered as in Table I; mobile phase as in Fig. 2.

asymptotically with increasing of column efficiency due to an increase in column length or to a decrease in the mobile phase velocity. The curves reach a plateau at $N_0 > 4000$.

A great number of curves in Fig. 4 show that, under our conditions (mass

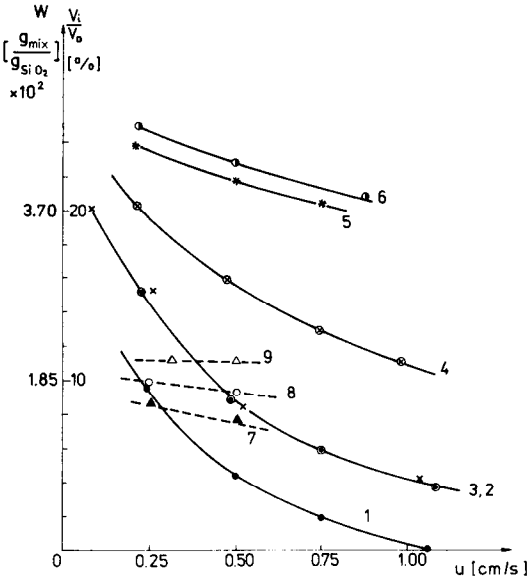

Fig. 5. The dependence of the maximum relative loading of the columns on the linear velocity of the mobile phase. Conditions as in Fig. 4.

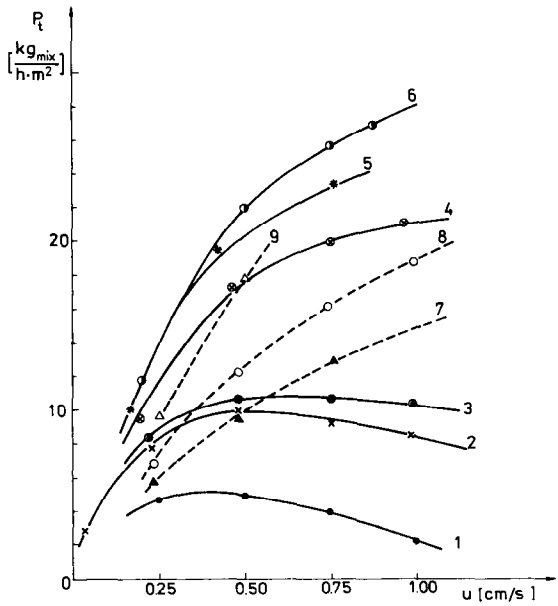

Fig. 6. The dependence of the productivity, P_t, on the linear velocity of the mobile phase. Conditions as in Fig. 4.

overloading), W depends not only on N_0, but also on H_0. If W depended only on N_0, then all points in Fig. 4 corresponding to the packing Si 60 (solid line) would lie on one curve. At the same time, it can be concluded on the basis of data from Fig. 4 and the values of H_0 from Table I that for the columns exhibiting constant N_0 but differing in H_0 their relative loading is inversely proportional to H_0.

A significant effect of the specific area of a column packing was also observed. The "plateau" level for gels of specific area *ca.* 300 m^2/g (Si 100) is about half that

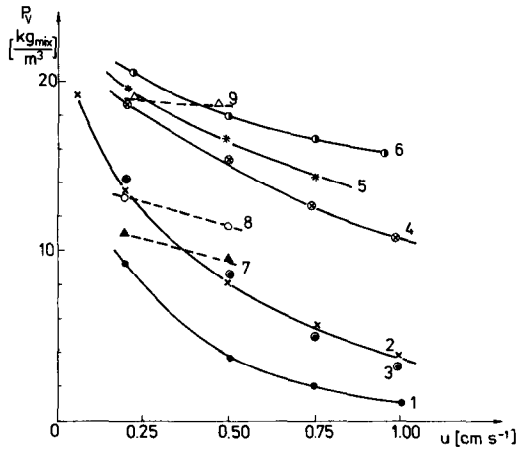

Fig. 7. The dependence of the productivity, P_v, on the linear velocity of the mobile phase. Conditions as in Fig. 4.

of gels of *ca.* 500 m^2/g (Si 60). The optimum value of W, equal to 75% of the maximum value, was achieved at N_0 within the range 1800–2500.

Inspection of the plots shown in Figs. 5–7 [W, P_t, $P_v = f(u)$] allows us to make the following conclusions pertaining to the effect of the mobile phase velocity, u, on the effectiveness of preparative-scale liquid chromatography.

There exists an optimum value of the mobile phase velocity, u_{max}, corresponding to the maximum time producitivity, P_t, of a column (Fig. 6). For instance, the experimental values obtained for lanatoside C were $u_{max} = 0.3$–0.45 cm/s for columns with $d_p = 90$ μm and $u_{max} = 0.4$–0.7 cm/s for column 3 with $d_p = 50$ μm. On the other hand, for longer columns with $d_p = 50$ μm and for packings having $d_p < 50$ μm, u_{max} will exceed 1 cm/s and will increase with the column length and particularly with decreasing d_p. However, for technological reasons, such conditions will hardly be achievable in industrial columns with diameter, $d_c \geqslant 100$ mm and packed with small particles.

The dependence between the productivity, P_t, and u and L_c is in agreement with the results of theoretical studies by Hupe and Lauer[15]. These authors predicted the existence of an optimum velocity of the mobile phase and the dependence of this velocity on the column length when d_p is constant. Comparison of our experimental results with the thereotical predictions of Hupe and Lauer can only be qualitative due mainly to a basic difference in the definition of the maximum sampled volume. According to Hupe and Lauer, this corresponds to the volume which ensures $R_s = 1$, while in our studies R_s was a result of sampling the volume defined by eqn. 4.

The effects of the velocity of the mobile phase on the relative loading of the stationary phase, W, and the volume productivity of a column, P_v, illustrated in Figs. 5 and 7 exhibit a distinct similarity, since the relationship

$$P_v = KW, \text{ where } K = k \cdot \frac{\rho_w}{1 + k'} \tag{5}$$

is valid, where K and k are proportionality constants, ρ_w is the density of the column packing and k' is the capacity factor. Despite this, the use of the two parameters W and P_v is justified from an economic point of view.

A decrease in u results in an increase in the parameters W and P_v. Apparently, this is valid for u values higher than u_{opt}, for which H_0 has a minimum value. Hence, the maximum utilization of the stationary phase and minimum consumption of the mobile phase occur when the time productivity of a column is not maximal. When the stationary or mobile phase is very expensive it may be advantageous to compromise between P_t and W or P_v through a reduction in the u to below u_{max}. Under normal conditions one should aim to maximize P_t.

The effect of u on W and P_v decreases with the particle diameter of the column packing, while the column productivity, P_t, increases with u.

The effect of the column length on P_t, P_v and W is clearly seen in Figs. 5–7 and 8. An increase in column length results in an advantageous increase in all these parameters. However, the dependences are asymptotic and the increase in column length required to attain a productivity higher than 70–80% of the maximum would result in a disproportionately higher increase in the system pressure (Fig. 8). This is in full accord with the predictions of Hupe and Lauer[15].

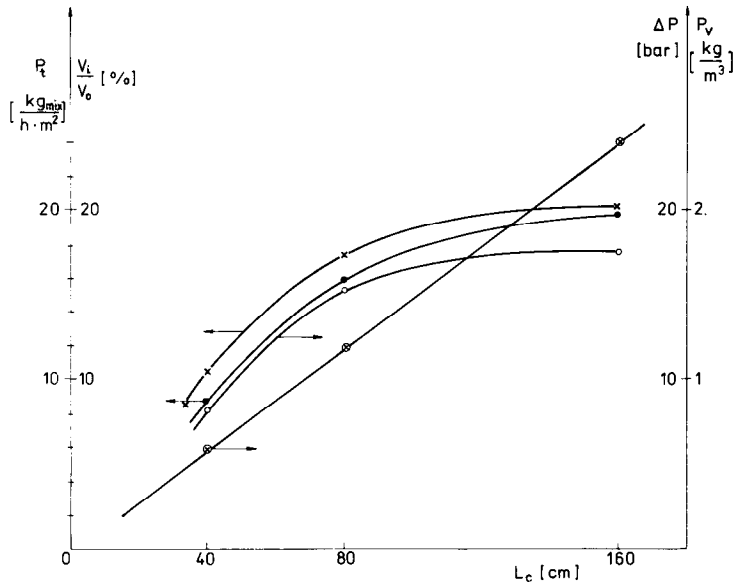

Fig. 8. Dependence of the relative loading (●), productivity, P_t (×), P_v (○), and preasure drop, ΔP (⊗, on the column length for $\bar{d}_p$ = 50 μm and u = 0.5 cm/s.

It follows from the data in Figs. 5–7 and Table II that under the conditions of mass overload of a column there are a range of possibilities for selection of combinations of d_p, L_c and u ensuring the achievement of desired values of P_t, P_v and W. On the other hand, taking account of only technical limitations results in the establishment of optimum conditions for the use of preparative chromatography.

The similarity of curves 2 and 3 in Figs. 5–7 indicates that the basic parameter which should remain constant when simultaneously increasing d_p and L_c is $\sqrt{L_c}/d_p$. Columns 2 and 3 have the same value of this factor. Under such conditions, identical values of P_t, P_v and W are probable. However, this postulate has not been verified experimentally and further investigations are necessary.

On the basis of Figs. 4 and 6, the maximum value of P_t for a packing of the

TABLE II

COMPARISON OF SEPARATION PARAMETERS FOR COLUMNS HAVING EQUAL TIME PRODUCTIVITIES

P_t (kg mix/ h · m²)	P_v (kg mix/ m³)	$W \cdot 10^2$ (g mix/ g SiO_2)	$\bar{d}_p$ (μm)	L (m)	u (cm/s)	ΔP (bar)
20	1.8	4.2	10	0.25	0.43	60
20	1.3	2.6	50	0.80	0.70	16
20	1.6	3.9	50	1.6	0.48	23
20*	1.6	3.9	90	4.0	0.30	10

* Value estimated on the basis of the position and shape of curves corresponding to columns 1 and 2 in Figs. 4–7.

TABLE III

THE CONDITIONS FOR INDUSTRIAL OPERATION OF TECHNICAL COLUMNS DURING SEPARATION OF A 15% (v/v) SOLUTION OF LANATOSIDES DESIGNED TO YIELD LANATOSIDE C, 99.5% PURE

P_t^{LC} = Time productivity of lanatoside C, V_i = sampled volume; v = flow-rate; V_0 = column dead volume.

d_p (μm)	m_s (kg)	V_0 (dm^3)	v (dm^3/min)	t_c (min)	ΔP (bar)	V_i (dm^3)	P_t (kg mix/$h \cdot m^2$)	P_t^{LC} (kg LC/$h \cdot m^2$)
40–63	7.9	9.9	2.8 (u = 0.35 cm/s)	50	10–16	1.85	18.8	2.43
	2.4	3.0	1.5 (u = 0.45 cm/s)	32	15–20	0.50	17.9	2.33

smallest applicable particle size, d_p = 10 μm, is *ca.* 28 kg separated mixture per hour and m^2 column cross section (kg mix/h · m^2). The optimum value, equal to 75% of the maximum, can also be achieved under the alternative conditions listed in Table II.

Taking into account the discussed experimental results and technical problems connected with the design of an industrial chromatographic system, as well as with achieving stable and efficient columns, columns packed with particles of d_p = 50 μm were chosen for the scale-up procedure. Additional reduction of the fraction evaporation efficiency made us decrease the mobile phase velocity, u, to 0.5 cm/s. On the basis of Fig. 8, an 80-cm column was chosen as optimal. The production of lanatoside C in columns of 100 mm, and especially 150 mm diameter, was carried out under the conditions listed in Table III. The chromatograms obtained are presented in Fig. 9.

It follows from a comparison of the data listed in Tables II and III that the productivity obtained during preliminary research using 6 mm diameter columns and

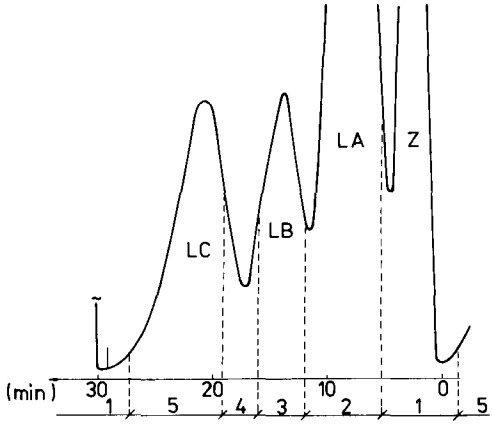

Fig. 9. An example of a chromatogram obtained during a single cycle of production of lanatoside C from one of the batches of a production waste containing lanatosides A, B, C and impurities Z. Conditions: mobile phase as in Fig. 3; column 11 (Table III); UV detector (254 nm), 0.64 a.u.f.s. -------, Limits of collection of fractions 1–5.

with production columns is similar. This suggests that in the case of columns of similar efficiency the entire design and selection of conditions for a separation process can be completed by using model columns, and the generally known rules of scale-up following from the geometrical proportions of the systems can be applied. However, based on our experience, it is of utmost importance that the columns should be packed in such a way that no "tailing" or "fronting" of peaks occurs at the baseline, and that they should be highly stable. These requirements can be achieved much more easily when packings of not too small a particle size are used.

CONCLUSIONS

(1) The maximum values of the parameters determining the effectiveness of the production of lanatoside C from the mixture used are $W = 2.6 \cdot 10^{-2}$ kg mix per kg SiO_2, $P_t = 28$ kg mix/$h \cdot m^2$ and $P_v = 20$ kg mix per m^3 mobile phase.

(2) The values of these parameters depend not only on N_0 and the specific area of the packing, but also on H_0. Hence the most effective columns are those packed with particles of the highest specific area and the smallest particle size. The case can occur, however, that the pressure drop along the column is not taken into account.

(3) An increase in the column length results in an asymptotic increase in W, P_t and P_v. These dependences are in qualitative agreement with theoretical considerations of Hupe and Lauer[15], although there are basic differences between the assumptions of the theoretical model and the present work.

(4) Similar agreement with Hupe and Lauer was found in the case of the dependence of the time productivity, P_t, on the mobile phase velocity.

(5) The conditions for production of lanatoside C assumed to be optimal on the basis of economic criteria and technical limitations assure a time productivity of 70–80% of the maximum.

(6) The type of chromatographic system employed consisting of silica gel and an hydrophobic mobile phase saturated with water has many technical advantages, *e.g.*, high volatility, low viscosity, easy regeneration and long column life, and reveals good selectivity towards many compounds whose molecules contain hydrophilic and hydrophobic parts[18]. Thus it can be considered as nearly as universal preparative-scale LC with reversed phases, but inexpensive and with higher column stability.

REFERENCES

1 A. M. Cantwell, R. Calderone and M. Sienko, *J. Chromatogr.*, 316 (1984) 133.
2 B. Śledzińska, J. Klawiter, M. Kamiński, R. Dobielewski, C. Nakonieczny, Z. Majer, M. Klauze and T. Wiwała, *Proceedings of the Symposium Advances of Chromatography in Poland in Last Years, Lublin, September 1984*, M. Curie-Sklodowska University, Lublin, 1984, pp. 60–65.
3 M. Martin, C. Eon and G. Guichon, *J. Chromatogr.*, 99 (1974) 357.
4 M. Martin, C. Eon and G. Guichon, *J. Chromatogr.*, 108 (1975) 229.
5 M. Martin, C. Eon and G. Guichon, *J. Chromatogr.*, 110 (1975) 213.
6 P. C. Haarhoff and H. J. van der Linde, *Anal Chem.*, 38 (1966) 573.
7 B. Coq, G. Cretier and J. L. Rocca, *J. Chromatogr.*, 186 (1979) 457.
8 B. Coq, G. Cretier and J. L. Rocca, *Anal Chem.*, 54 (1982) 2271.
9 G. Cretier and I. L. Rocca, *Chromatographia*, 18 (1984) 623.
10 P. Gareil, C. Durieux and R. Rosset, *Sep. Sci.*, 18 (1983) 441.

11 P. Gareil and R. Rosset, *Analusis*, 10 (1982) 397.
12 P. Gareil and R. Rosset, *Analusis*, 10 (1982) 445.
13 B. Śledzińska, *J. Chromatogr.*, 303 (1984) 179.
14 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 185 (1979) 445.
15 K. P. Hupe and H. H. Lauer, *J. Chromatogr.*, 203 (1981) 41.
16 I. P. Wolf, III, *Anal Chem.*, 45 (1973) 1248.
17 J. Klawiter, M. Kamiński and J. S. Kowalczyk, *J. Chromatogr.*, 243 (1982) 207.
18 B. Śledzińska and I. Pecka, *Proceedings of II Symposium "Advances in Chromatography in Poland", Lublin, September 1985*, M. Curie-Sklodowska University, Lublin, 1985, pp. 111–116.
19 K. Gazda, J. S. Kowalczyk, J. Klawiter and M.Kamiński, *Pol. Pat. Appl.*, P-240027.
20 J. S. Kowalczyk,M. Kamiński and J. Klawiter, *Pol. Pat. Appl.*, P-238461; *Eur. Pat. Appl.*, EP 108 242.