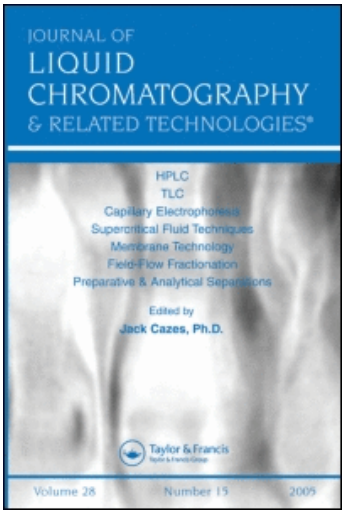

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Pretorius, Victor , Van Rossum, P. G. and Berry, V.(1991) 'A Simple Solvent Programmer for Liquid Chromatography. I', Journal of Liquid Chromatography & Related Technologies, 14: 13, 2429 — 2444
To link to this Article: DOI: 10.1080/01483919108049331
URL: http://dx.doi.org/10.1080/01483919108049331

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(13), 2429-2444 (1991)

A SIMPLE SOLVENT PROGRAMMER FOR LIQUID CHROMATOGRAPHY. I.

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SUMMARY

Equations have been derived from which the dimensions of a solvent gradient generator, coupled to open tubular, micro-packed, semi-micro packed or conventional HPLC columns, may be calculated for a desired gradient volume. Packed and open-tubular generators have been considered. Calculations, using the derived equations, predict that a generator of particular dimensions is needed for each column type. These dimensions are practically feasible for all column types except the conventional column.

INTRODUCTION

Solvent programming (gradient elution) is an established and important technique in liquid chromatography [1]. Judiciously employed it can improve resolution, separation time and detection sensitivity. The principles of the technique are well-known and have spawned a plethora

of devices - some excellent and many commercially available. Most exploit proven - if obvious - engineering practice and tend to be expensive.

A novel solvent programmer has been described by Berry et al [2]. It involves forming a sharp interface, between two solvents, by passing it through a bed of particles. The S-shaped gradient thus formed is quasi-linear and, together with two isocratic portions constitutes, a simple solvent program. The program cannot, of course, exploit the intrinsic potential of the column to the extent attainable with more sophisticated systems [3] but its simplicity offsets, to some extent, its limitations and makes it a useful technique for many separations. The purpose of this communication is therefore to

(a) elucidate the processes involved in its operation,
(b) derive equations relating the characteristics of the programmer to those of the program,
(c) extend the scope of the original concept,
(d) provide data to design and construct a working device.

1. Qualitative Description of the Programmer and the Program

The programmer consists of a gradient generator, G (a cylindrical tube which may be empty or packed with inert particles), pumps P_A and P_B and valves V. (Figure 1).

Valve V_i, is switched to deliver alternate pulses of solvents A and B to the generator such that the interfaces between the pulses are sharp. The volumes of the pulses are V_A^i and V_B^i and are generally different. These pulses constitute the input solvent program (ISP)

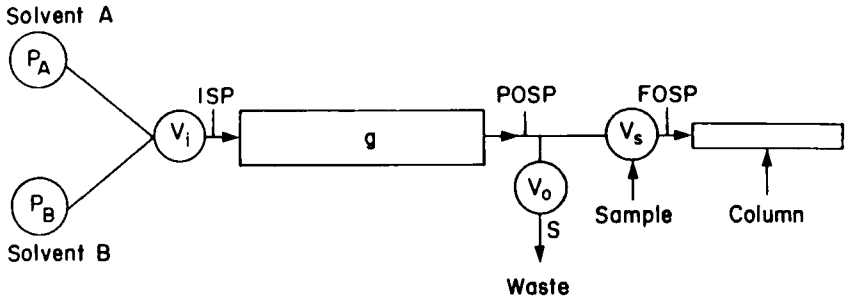

FIGURE 1 Schematic diagram of the solvent programmer

(Figure 2a.) As the pulses pass through the generator the interfaces are spread by processes discussed below and the pulses emerge from the generator in S-shaped forms as shown in Figure 2b by the dotted line which can be approximated by the solid line. These pulses constitute the primary output solvent program (POSP).

A fraction of the POSP may be split off (vented) and the remainder, the final output solvent program (FOSP) passed into the chromatographic column. The POSP and the FOSP differ in volume but not in shape. The POSP and the FOSP consist of repetitive solvent program elements each containing an isocratic region A, a gradient region G, an isocratic region B and a recovery region R.

The sample is injected, through valve V_s, into the solvent stream so that it coincides with the start of the weaker (A) isocratic portion of the program. This enhances solute focussing.

2. Quantitative Description of the Solvent Program Element

2.1 The Gradient Profile

The S-shaped gradient is identical to the familiar breakthrough or frontal analysis curve in chromato-

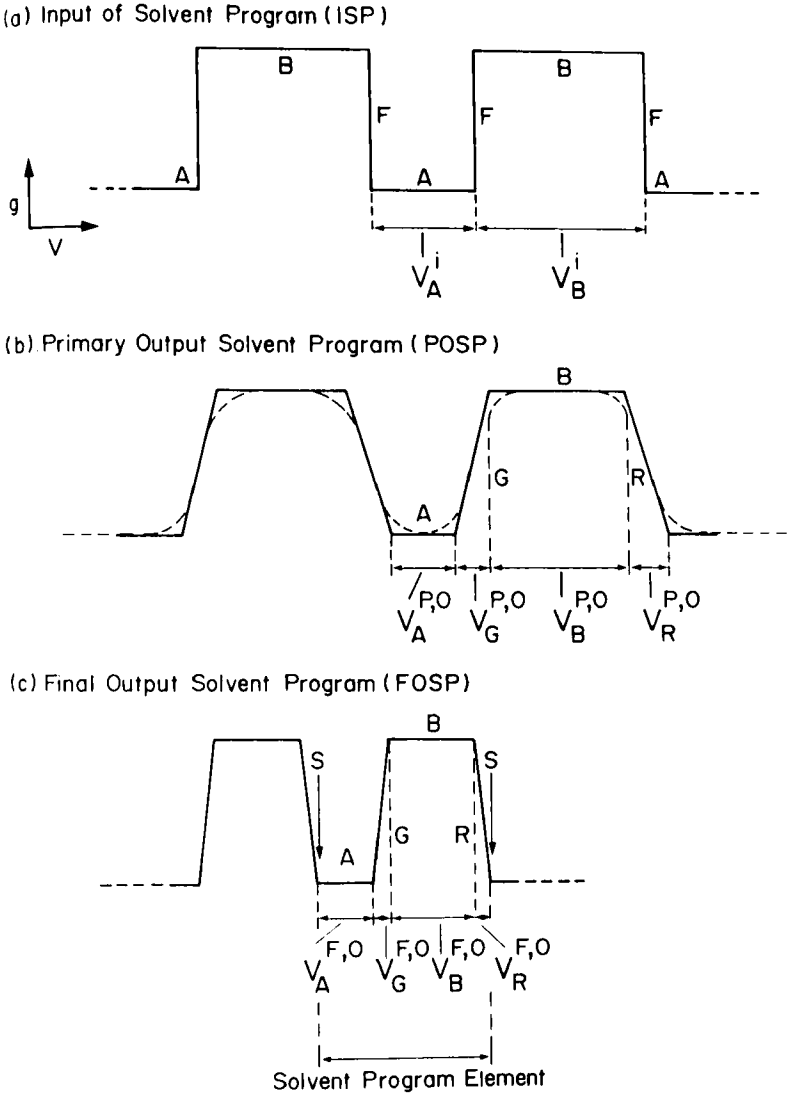

FIGURE 2 Solvent programs

graphy. The equation of the curve is given by [5]

$$F(t) = \int_{-\infty}^{t} f(x)\,dx \qquad \ldots\ldots (1)$$

where

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}}\, e^{-1/2\left(\frac{x}{\sigma}\right)^2} \qquad \ldots\ldots (2)$$

Equation 2 is the well-known Gaussian distribution in its normalised form and is shown in Figure 3(a). σ is the standard deviation of the curve where 2σ is the width of the curve at the inflection points.

Equation 1 has been plotted in Figure 3(b). The width, w, of the gradient - ie the S-shaped portion - is approximately:

$$w = \frac{h}{\tan \alpha} \qquad \ldots\ldots (3)$$

However $h = 1$ and

$$\tan \alpha = \frac{d}{dt}\,(F(t))\ at\ t = o$$

$$= f(x) \quad at \quad x = o$$

$$= \frac{1}{\sqrt{2\pi}\sigma}$$

Thus

$$w = \sqrt{2\pi}\sigma \qquad \ldots\ldots (4)$$

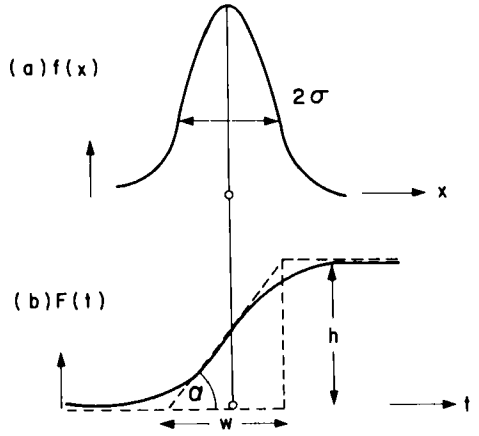

FIGURE 3 Gaussian (a) and frontal analysis (b) curves

2.2 The Isocratic Regions ($V_A^{F,O}$ and $V_B^{F,O}$)

From Figure 2b, 2c it follows that

$$V_A^{F,O} = V_A^{P,O}(1-S) \qquad \ldots\ldots (5)$$

where

$$S = \frac{V_A^{P,O} - V_A^{F,O}}{V_A^{P,O}} \qquad \ldots\ldots (6)$$

and

$$V_A^{P,O} = V_A^{i} - V_G^{P,O} \qquad \ldots\ldots (7)$$

From Eqs. 5,7

$$V_A^{F,O} = (V_A^{i} - V_G^{P,O})(1-S) \qquad \ldots\ldots (8)$$

Similarly $V_A^{F,O} = (V_A^i - V_G^{P,O})(1-S)$ ..... (9)

$V_A^{F,O}$ and $V_B^{F,O}$ may be varied over a wide range by varying V_A and V_B respectively since $V_G^{P,O}$ and S will be fixed by other considerations (see paragraph 4).

3. The Gradient Region

Gradient elution has been elegantly dealt with by Snyder [3] who has introduced the concepts of linear solvent strengths and gradient steepness. This approach will undoubtedly appeal to the chromatographic sophisticate but we shall adopt a more naive view which is in keeping, so we are told, with common practice in which the gradient volume $V_G^{F,O}$ is chosen in terms of a number of column volumes viz.

$$V_G^{F,O} = nV_c \quad \text{..... (10)}$$

where n = a number greater than unity and typically between 2 and 10
V_c = column volume

Since $V_G^{F,O} = V_G^{P,O}(1-S)$ ..... (11)

it follows from Eqs. 10, 11 that

$$V_G^{P,O} = \frac{nV_c}{(1-S)} \quad \text{..... (12)}$$

However $V_c = \pi r_c^2 \epsilon_c L_c$ ..... (13)

where r_c = column radius
ϵ_c = porosity of column
L_c = column length

so that from Eqs. 12, 13

$$V_G^{P,O} = \frac{n}{(1-S)} \cdot \pi r_c^2 \epsilon_c L_c \quad \text{..... (14)}$$

It now remains to relate $V^{P,O}$ to the generator characteristics. We start by noting that from Eq. 4

$$V_G^{P,O} = \omega \pi r_g^2 \varepsilon g \quad \text{..... (15)}$$

$$= \sqrt{2\pi^3}\sigma r_g^2 \varepsilon g \quad \text{..... (16)}$$

where σ = standard deviation of the gradient curve
ϵ_g = porosity of generator
r_g = internal radius of generator

Furthermore

$$\sigma = (H_g L_g)^{1/2} \quad \text{..... (17)}$$

where H_g = plate height of generator
L_g = length of generator

Expressions which relate H_g to the generator characteristics may be obtained from the Golay [5] equation for an open tubular generator and from the van Deemter [6] equation for a generator packed with solid particles. In both equations the partition ratio k = 0 since no stationary phase is present. Thus

$$H_g^{OT} = \frac{2D_m}{U_g} + \frac{r_g^2 U_g}{24 D_m} \qquad \text{..... (18)}$$

and

$$H_g^{p} = 2d_p + \frac{2D_m}{U_g} + \frac{d_p^2 U_g}{96 D_m} \qquad \text{..... (19)}$$

where H_g^{OT} = plate height of open tubular generator
H_g^{p} = plate height of packed generator
d_p = diameter of particles
D_m = inter diffusion coefficient of solvents
U_g = axial linear velocity of solvents in generator

The value of U_g in Eqs. 18 and 19 is determined by the fact that the volume flow rate of the solvent, after splitting, must be equal to that passing through the column.

Thus

$$\overline{V}_g = \frac{\overline{V}_c}{(1-S)} \qquad \text{..... (20)}$$

where V_g = volume flow rate through generator
V_c = volume flow rate through column

and $$U_g = \frac{\overline{V}_g}{\pi r_g^2 \varepsilon_g} \qquad \text{..... (21)}$$

From Eqs. 20 and 21

$$U_g = \frac{\overline{V}_c}{(1-S)\pi r_g^2 \varepsilon_g} \qquad \text{..... (22)}$$

$$\frac{nV_c}{(1-S)} = \sqrt{2\pi^3}\,(r_g^o)^2 \varepsilon_g^o \sqrt{L_g^o}\; x \left[\frac{2D_m(1-S)\pi\,(r_g^o)^2 \varepsilon_g^o}{\overline{V}_c} + \frac{\overline{V}_c}{24\,(1-S)\pi \varepsilon_g^o D_m}\right]^{1/2} \qquad \text{..... (23)}$$

and for packed generators

$$\frac{nV_c}{(1-S)} = \sqrt{2\pi^3}\,(r_g^p)^2 \varepsilon_g^p \sqrt{L_g^p}\; x \left[2d_p + \frac{2D_m(1-S)\pi\,(r_g^p)^2 \varepsilon_g^p}{\overline{V}_c} + \frac{d_p \overline{V}_c}{96 D_m (1-S)\pi\,(r_g^p)^2 \varepsilon_g^p}\right]^{1/2} \qquad \text{..... (24)}$$

where r_g^o, L_g^o and ϵ_g^o refer to open tubular generators and r_g^p, L_g^p and ϵ_g^p to packed generators.

We now consider a packed and an open tubular generator coupled to each of four column types listed in Table 1.

TABLE 1

Column Characteristics.

Column type	L_c(cm)	r_c(cm)	V_c($cm^3 s^{-1}$)	V_c(cm^3)+
open tubular	2000	10^{-3}	$2x10^{-6}$	$6.28x10^{-3}$
micro packed	25	$1.25x10^{-2}$	$1.5x10^{-4}$	$4.91x10^{-3}$
semi-micro packed	25	0.05	$2.4x10^{-3}$	$7.85x10^{-2}$
conventional	25	0.23	$5x10^{-2}$	1.66

+ Calculated from Eq. 13

The values of $\bar{V}_c$ and V_c from Table 1 have been substituted into Eqs. 23, 24 together with a typical value $D_m = 1.28x10^{-5} cm^2 s^{-1}$ [4] and $\epsilon_g^o = 1$ and $\epsilon_g^p = 0.4$. In addition we have set $d_p = 0.4 r_g^p$ to keep the pressure-drop across the generator as small as possible. Finally we have set S = 0 and n = 10 to correspond to the situation where the splitter is closed and the solvent gradient is 10 column volumes. The value of L_g and r_g has then been calculated. Values for which the number of theoretical plates, N_g, of the generator are less than 50 have been rejected to ensure a symmetrical gradient profile [6]. Values for which the pressure-drop across the generator are greater than one-tenth of that across the column have also been rejected. These pressure-drops have been calculated from the Poiseuille equation [6]

$$V_g^o = \frac{\pi \Delta P_g^o (r_g^o)^4}{8 \eta L_g^o}$$

for open tubular generators and from the Carman-Kozeny

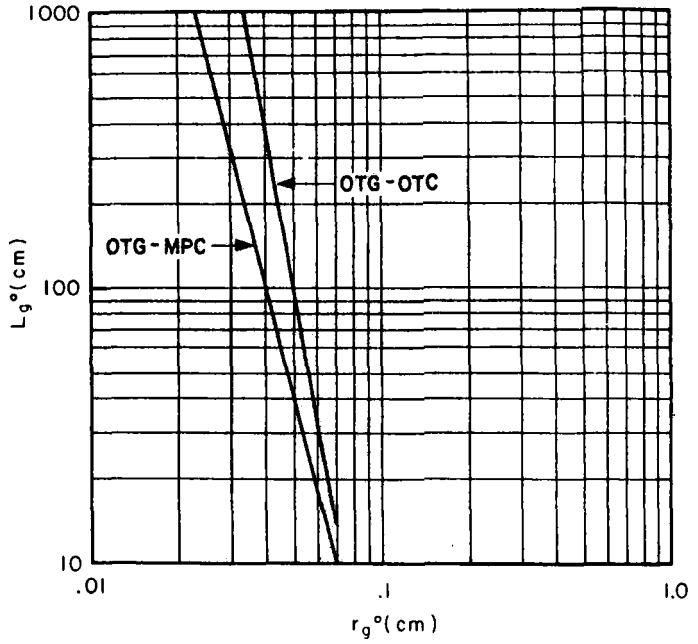

FIGURE 4 Relation between open tubular generator (OTG) length (L_g°) and radius (r_g°) for $N \geq 50$ n=10 and S=0

equation [6]

$$V_g^p = \frac{\pi (r_g^p)^2 (\varepsilon_g^p) d_p^2 \Delta P_g^p}{180 \eta L}$$

for packed generators.

where V_g = volume flow rate
Δ_p = pressure-drop
η = viscosity of solvent (typically 7×10^{-3} poise)

The results are shown in Figs. 4-5. It will be noted that the curve corresponding to an open tubular

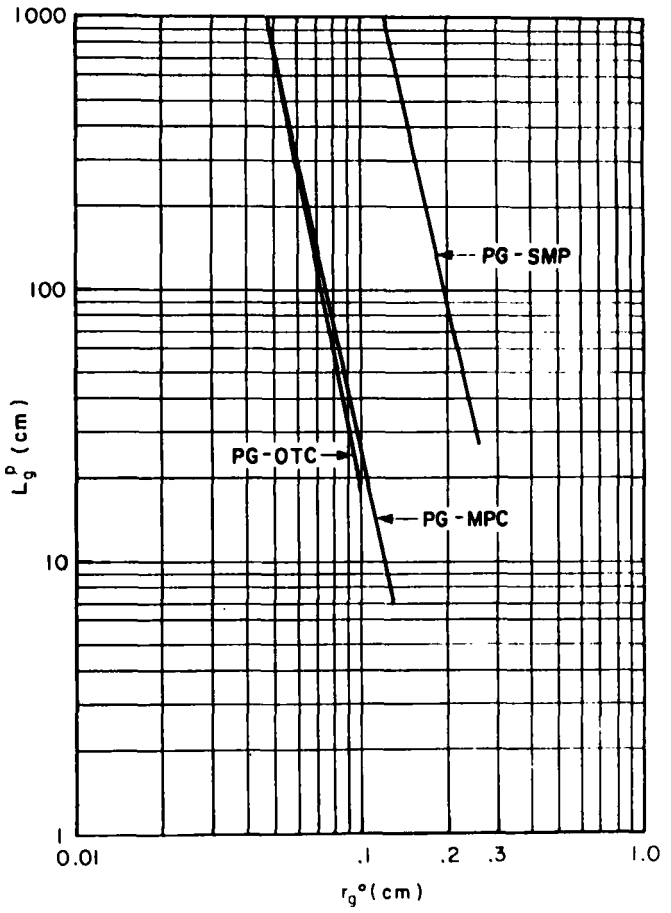

FIGURE 5 Relation between packed generator (PG) length (L_g^v) and radius (r_g^a) for N≥50 n=10 and S=0

generator and a semi-micro packed column is absent as are those pertaining to conventional column and both types of generator. The reason is that impractically long generator lengths are needed to meet the constraint $N_g > 50$ within the range 0.01 cm < r_g < 1.0 cm.

The data in Figs. 4-5 offers a choice of generator lengths and radii with S = 0 and n = 10. In practice a

TABLE 2

Selected generator geometries.

		L_g(cm)	r_g(cm)
open tubular generator	(OTG)	100	0.05
open tubular column	(OTC)		
open tubular generator	(OTG)	100	0.04
micro-packed column	(MPC)		
packed generator	(PG)	100	0.073
open tubular column	(OTC)		
packed generator	(PG)	100	0.075
micro-packed column	(MPC)		
packed generator	(PG)	100	0.198
semi-micro packed column	(SMPC)		

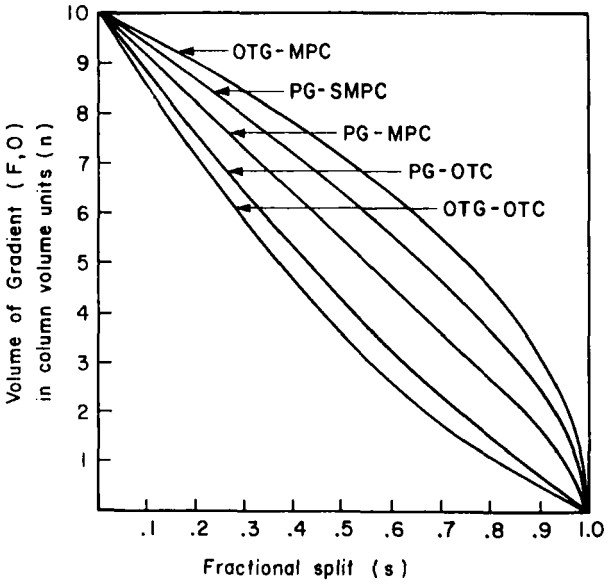

FIGURE 6 Number of column volumes of gradient as a function of the fractional split (s) for the gradient generator lengths and radii listed in Table 2

particular length and radius would be chosen for a particular column type and one would need to know how the split fraction, S, affects the gradient volume measured in column volumes.

Let us now consider the question of the choice of the generator type and dimensions. From Figs. 4-5 it is firstly evident that packed generators are more versatile than open tubular generators in that the former can be used for semi-micro packed columns. Secondly there is no one convenient generator geometry which can be used for all column types which means that a generator must be constructed for each column type. We have chosen to fix the generator length at 100 cm and to determine the radius in each case from Figs. 4-5 (Table 2).

By replacing the left-hand term, 10 V_c, in Eqs. 23 and 24 by nV_c the value of n may be calculated for various values of S using the values of $\bar{V}_c$ and V_c listed in Table 1 and the values of the other parameters given in the text. The results are shown in Fig. 6.

Finally to a given generator/column system and a chosen value of n the value of S may be obtained from Fig. 6 and the value of $V_G^{P,O}$ may be estimated from Eq. 11. The values of V_A^i and V_B^i may be obtained from Eqs. 8 and 9 for chosen values of $V_A^{F,O}$ and $V_B^{F,O}$.

CONCLUSIONS

The theory developed here predicts the generator characteristics needed to produce repetitive isocratic and gradient solvent program elements for the commonly used column types with the exception of conventional columns. Most significantly it suggests a practical

means of obtaining solvent programs for open tubular columns.

A working system based on the results published here is presently being constructed.

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