

Optimization of industrial-scale high-performance liquid chromatography applications using a newly developed software

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ABSTRACT

Industrial-scale HPLC applications have been evaluated and optimized to give the desired purity and throughput at the lowest total cost in the HPLC step by the use of a newly developed software, including the algorithm Nirvana. The algorithm, capable of handling self-displacement effects, has been utilized to simulate band profiles and yield in the optimization process. It is shown that this method can be used successfully for finding the optimal chromatographic conditions also for applications with self-displacement.

INTRODUCTION

The high costs involved in industrial HPLC separations makes it highly desirable to make preliminary calculations with a high degree of accuracy. However, the non-linearities of the models have made this task quite time-demanding up to now. The aim of this work was to show that accurate calculations can now be done on a small computer within a reasonable amount of time.

The preparative LC separation of two compounds was studied. Experimental chromatographic data were evaluated in order to obtain a relevant model for estimation of band profiles. Model simulations were performed using the new software package KromaGuide 2.0, incorporating Nirvana (New, Iterative, Relative Velocity-based Algorithm for Non-linear Adsorption) [1]. KromaGuide is a computer program developed by Eka Nobel; it is used as a service tool in customer support and is not available for

purchasing. An economic optimization was then performed with the use of yield estimates from the simulations.

Nirvana was developed for simulating preparative LC considerably faster than existing algorithms^{*}. A fast algorithm is necessary for being able to optimize an HPLC separation on a personal computer (PC), since a series of simulations with different loadings and column efficiencies has to be run.

The results obtained with the Nirvana algorithm agree well with those obtained with existing methods, *e.g.* Craig. A unique feature is that the computational time required for a simulation is almost independent of the number of theoretical plates, and that approximate solutions can be obtained very fast. Each simulated profile in this work was obtained in less than 15 min, typically in about 5 min (50 MHz 486 PC).

Execution time is shown in Table I and Fig. 1

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^{*} The development was initiated by Eka Nobel AB and realized at the Department of Chemical Reaction Engineering at Chalmers University of Technology, Gothenburg, Sweden.

TABLE I

SUMMARY OF SIMULATIONS

Adapted from ref. 1. Q_1 and Q_2 are loadings relative to column saturation capacity for substances 1 and 2; N is the number of theoretical plates.

Simulation	$Q_1 \cdot 10^2$	$Q_2 \cdot 10^2$	$N \cdot 10^{-3}$	Execution time (s)	
				Nirvana	Craig
a	1	1	1	6	129
b	1	1	4	6	2 070
c	1	1	10	6.5	12 900
d	1	1	25	11	80 700
e	3	3	1	9	159
f	3	3	4	29	2540
g	3	3	10	57	14 300
h	3	3	25	109	89 100

for a set of simulations with varying column efficiencies and loadings.

EXPERIMENTAL

Equipment

The HPLC system used consisted of a Waters (Millipore, Marlborough, MA, USA) 600-MS solvent-delivery system and system controller, a Waters 486 tuneable absorbance detector, a

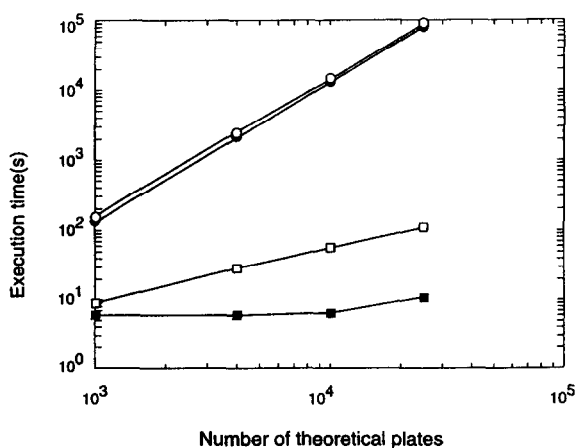


Fig. 1. A comparison between the execution times of Nirvana and Craig programs. Nirvana (\square) is considerably faster than the Craig algorithm (\circ) at high plate numbers. Filled symbols denote the lower loading, empty ones the higher.

Waters 746 data module, a Waters fraction collector and a Waters 717 autosampler. Columns were 250 mm \times 4.6 mm I.D. packed with Kromasil (Eka Nobel, Bohus, Sweden) 100 Å 10 μ m C_4 -, C_8 - and C_{18} -derivatized spherical silica. Detector wavelength was 254 nm. The solutes were benzyl alcohol (E. Merck, Darmstadt, Germany) and 2-phenylethanol (Sigma, St. Louis, MO, USA), as pure substances and as a 50:50 mixture. The mobile phase was acetonitrile–water at various compositions. Acetonitrile used was provided by Labscan, Dublin, Ireland.

Solid–mobile phase selection

Initial experiments were carried out at analytical loadings for various mobile phase compositions and with C_4 , C_8 and C_{18} stationary phase to determine the combination giving the highest throughput.

It was shown that the C_{18} column gives the best selectivity (Fig. 2a), and that approximately acetonitrile–water (30:70) will be the optimal mobile phase composition. The higher separation factor, α , at acetonitrile–water (20:80) will not compensate for the much longer retention time (cf. Fig. 2b), and throughput will go down. To avoid volume overload the samples had to be dissolved in a somewhat modified mobile phase (acetonitrile–water, 65:35), which could possibly affect the separation. However, since the volume was small and heavily overloaded, this influence was neglected, and modelled within the adsorption isotherm.

Procedure

The solutes were run at analytical loadings and at different flow-rates for determining the Knox parameters A , B and C in the plate height equation. Knowing these, the kinetic dispersion can be calculated for various flow-rates by the computer program in the optimization process. The plate height curve for one of the substances, 2-phenylethanol, is shown in Fig. 3.

Overloaded injections were performed both for the pure substances and for the 50:50 mixture. For the overloaded runs fractions were collected every 4th second, and analyzed on the same equipment.

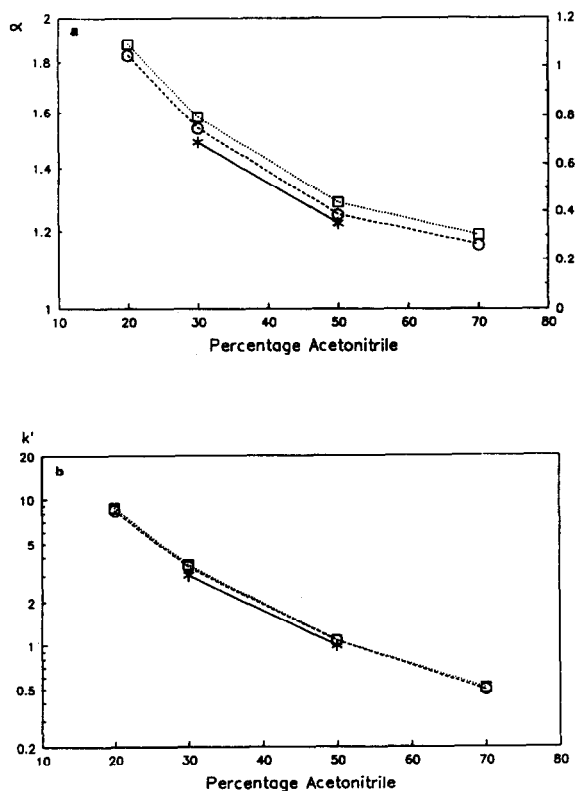


Fig. 2. (a) Separation factor, $\alpha = k'_2/k'_1$ (2-phenylethanol/benzyl alcohol) vs. mobile phase composition (acetonitrile-water). Stationary phase was C_4 - (*), C_8 - (O) or C_{18} - (□) derivatized silica. (b) Capacity factor, k' (2-phenylethanol) vs. mobile phase composition (acetonitrile-water). Stationary phase was C_4 - (*), C_8 - (O) or C_{18} - (□) derivatized silica.

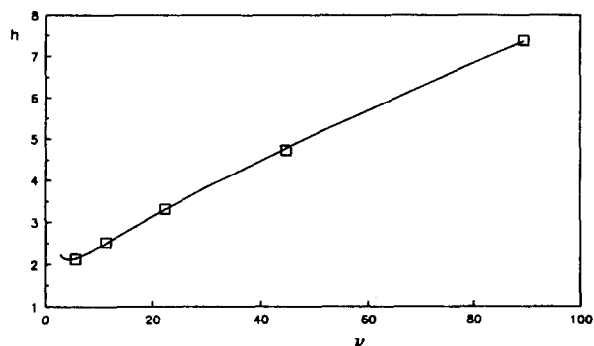


Fig. 3. Reduced plate height ($h = H/d_p$) vs. reduced flow velocity ($\nu = ud_p/D_m$) for 2-phenylethanol in the column used. The Knox equation, $h = A\nu^{1/3} + B\nu^{-1} + C\nu$, holds, with $A = 0.8$, $B = 2.7$ and $C = 0.043$.

MODEL

Initially, the experimental data were fitted to a Langmuir competitive isotherm with species-dependent saturation capacities:

$$c_i^S = \frac{k'_i c_i^M}{1 + \sum_{j=1}^n k'_j c_j^M / c_j^{S, \max}} \quad (1)$$

However, the peak shapes (Fig. 4) and consequently yield-purity relations were not satisfactory. To fit the model to the fraction analysis the peak broadening was studied, *i.e.* the deviation in band front times between experiment and estimate were minimized in a least square sense. These times are readily determined by hand from experimental chromatograms (or, in heavily overloaded cases, from fraction analyses of band profiles), and by the computer program from the simulated band profiles. The algorithm used for all parameter fitting was the MATLAB Optimization Toolbox implementation of Nelder-Meads simplex search [2]. (MATLAB from MathWorks, Natick, MA, USA)

The adsorption capacities so estimated were quite high for the first peak (610 mg/g packing) and quite low (220 mg/g) for the second. It seems that the displacement accounted for in the model is incorrect (*cf.* ref. 3), which called for the introduction of competition factors Γ_{ij} (*cf.* ref. 4):

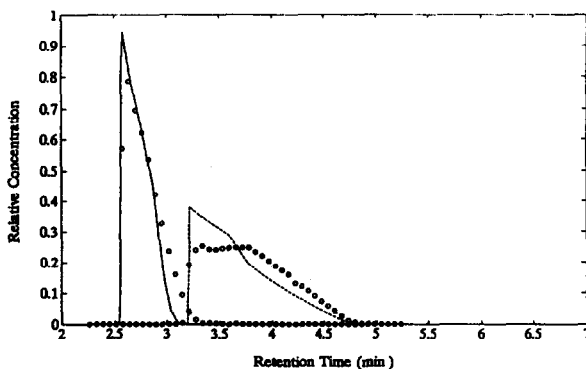


Fig. 4. Fraction analysis (circles) and simulated profiles using the isotherm model eqn. 1. The first simulated band is too narrow, and the second one is not enough tagged along compared to the experimental data.

$$c_i^S = \frac{k'_i c_i^M}{1 + \sum_{j=1}^n \Gamma_{ij} k'_j c_j^M / c_j^{S, \max}} \quad (2)$$

The expression $\Gamma_{ij} k'_j c_j^M / c_j^{S, \max}$ can be interpreted as the degree of blockage of the stationary phase by substance j from the viewpoint of substance i . Γ_{ij} is by definition equal to 1 for $i=j$. With $\Gamma_{ij}=1$ for all i and j , i.e. equal competition between all species, we obtain eqn. 1. With $\Gamma_{ij}=1$ for $i=j$ and $\Gamma_{ij}=0$ for $i \neq j$ we get the non-competitive Langmuir adsorption isotherm:

$$c_i^S = \frac{k'_i c_i^M}{1 + k'_i c_i^M / c_i^{S, \max}} \quad (3)$$

This isotherm was assumed to hold for the single-component cases, and adsorption capacities were determined from experiments with 20 mg/substance injected. These were esti-

mated (by studying peak broadening as in the above case) to be $c_1^{S, \max} = 320$ mg/g and $c_2^{S, \max} = 310$ mg/g packing material (Fig. 5a and b). The idea was to examine how these capacities agreed with the optimal parameters of eqn. 2.

In order to quantify the deviation between experimental and simulated band profiles, a few different criteria were used. The band front times used above were not enough to accurately describe the peak shapes and positions, as the extended model (eqn. 2) was used. Instead, the deviations in retention times for a number of points on the cumulative elution profile were studied. These deviations were also minimized in a least square sense, and the zone where the bands overlap was emphasized through the choice of the set of characteristic times. The estimate precision in this zone is crucial for accurate yield-purity predictions. By varying the adsorption capacities and the competition factors an optimum was found. The adsorption capacities were $c_1^{S, \max} = 340$ mg/g and $c_2^{S, \max} = 310$ mg/g, and the competition factors $\Gamma_{12} = 0.57$ and $\Gamma_{21} = 1.32$ (Figs. 6 and 7).

OPTIMIZATION OF PREPARATIVE LC SYSTEM

To perform a preparative HPLC separation at the lowest total cost we have focused on maximizing cost benefit, which is defined as

$$\text{Cost benefit (g/US\$)} = \frac{\text{Throughput (g/h)}}{\text{Total cost (US\$/h)}} \quad (4)$$

with

$$\begin{aligned} \text{Total cost} = & \text{cost}_{\text{solvent}} + \text{cost}_{\text{silica}} + \text{cost}_{\text{system}} \\ & + \text{cost}_{\text{labour}} + \text{cost}_{\text{lost crude}} \quad (5) \end{aligned}$$

Solvent cost includes recovery and make up. Silica cost is calculated from its purchasing cost and lifetime of the packing material, which might not be known at the planning stage, but must be estimated. The cost of system includes all fixed costs (preparative LC delivery system, column and premises). Labour cost could for example be the cost of one full-time technician.

Pricing of lost crude product is a way of specifying the acceptable yield in the LC step. Often, most of the product is not really lost, but can be recycled or repurified, repurifying in an

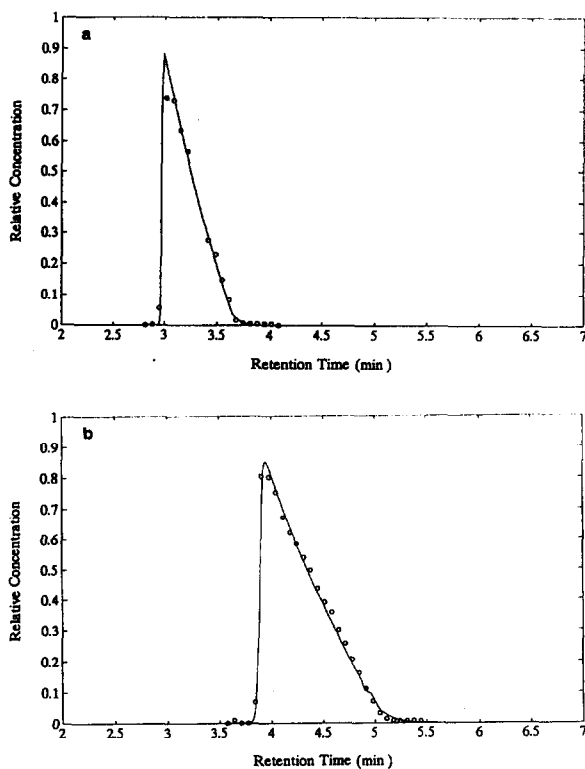


Fig. 5. Simulation and fraction analyses of single-component injections. (a) Benzyl alcohol, $c_1^{S, \max} = 320$ mg/g; (b) 2-phenylethanol, $c_2^{S, \max} = 310$ mg/g.

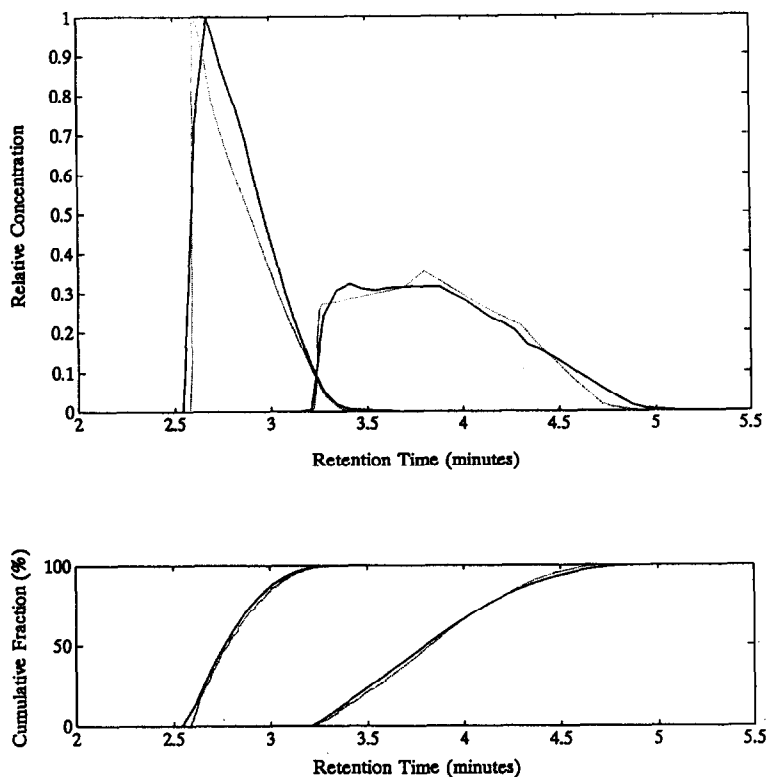


Fig. 6. The result of the parameter estimation: $c_1^{S,max} = 340$ mg/g, $c_2^{S,max} = 310$ mg/g, $\Gamma_{12} = 0.57$, $\Gamma_{21} = 1.32$. The solid line is a linear interpolation obtained via fraction analysis, the dotted line is the simulation. The upper diagram is a band profile, the lower is a cumulative elution profile.

isocratic system possibly involving an additional step to achieve a concentration increase, such as evaporation or precipitation. The cost should then be an estimate of the cost for this step plus the cost of the actual loss. Preliminary studies have shown that for difficult separations (with a separation factor, α , of below about 1.3) optimum yield for a single preparative LC run can be surprisingly low, sometimes in the range 30–50%.

By doing a series of simulations for different loadings and column efficiencies, and calculate and store the estimated yield, one obtains a table from which yield can be interpolated for any set of column and operational parameters that gives a loading and column efficiency within the table. This approach makes the optimization very fast, and one is thus able to interactively test the influence of various parameters on the cost benefit optimum.

AN OPTIMIZATION CASE

The isotherm parameters of eqn. 2 were determined as described above, and these data were used in a series of simulations. For each simulation, yield was calculated for the specified 99.5% purity of the first-eluting substance. Fig. 8 shows the simulation results, and how yield improves with the number of theoretical plates, for a few different loadings. The most favorable yield will depend on separation factor, α , cost for lost crude etc., and will vary considerably depending on these parameters.

A 300 mm I.D. column with axial compression, i.e. with a variable bed length, maximum bed length 1200 mm was considered. The minimum bed length was set to 200 mm for practical reasons. The maximum pressure drop was set to 70 bar. In the calculations no limit has been set for pump capacity.

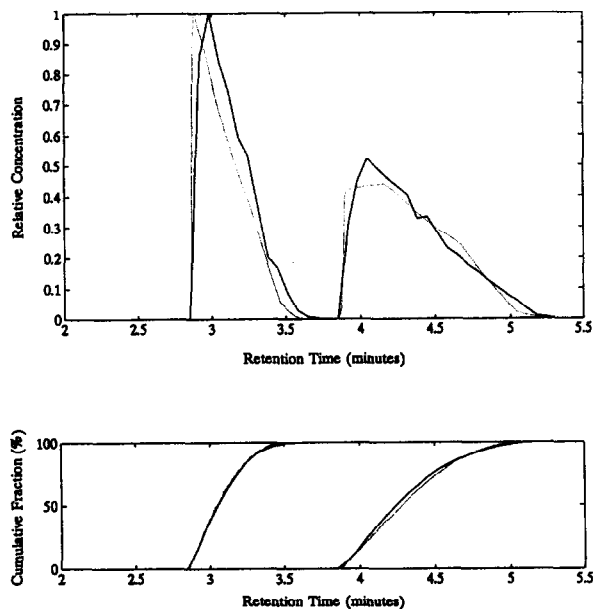


Fig. 7. The simulation result for the parameters in Fig. 6, but with an injection of 20 mg per substance. The simulated band (dotted) agrees well with the experimental one (solid), but extrapolation must be done with care, since there are always model discrepancies.

TABLE II

COSTS USED FOR THE CALCULATIONS

Solvent cost	1 US\$/l
Silica cost	
5 μm , C_{18}	2.6 US\$/kg,h
7 μm , C_{18}	2.2 US\$/kg,h
10 μm , C_{18}	2.0 US\$/kg,h
13 μm , C_{18}	1.8 US\$/kg,h
16 μm , C_{18}	1.7 US\$/kg,h
20 μm , C_{18}	1.6 US\$/kg,h
System cost	65 US\$/h
Labour cost	40 US\$/h
Lost crude cost	2 US\$/g

For the silica cost in Table II it is assumed that the lifetime of the packing material is 2000 h. The system is utilized 2000 h per year, with 5 years depreciation of the equipment.

For the separation a cycle time of 1.2 times the retention time of the last-eluting substance is considered.

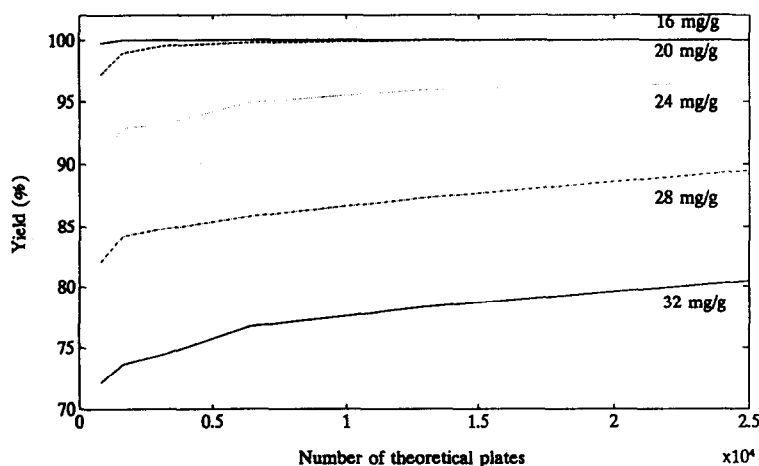


Fig. 8. The simulated yield used at optimization. By interpolation, yield can be obtained for any point within the limits. The curves represent total loadings of (from top downwards) 16, 20, 24, 28 and 32 mg/g packing material, respectively. A total of 49 simulations were run.

RESULTS

It was proved that the model (eqn. 2) could be successfully utilized to fit simulations to experimental data, and to use this model for further simulations at other operating conditions. In this case the interactions in the binary mixture could be described by the competition factors of eqn. 2. The values of $c^{S,max}$ are virtually constant compared to the single-component case.

Results from the economic optimization shows that a particle size of 13 μm will be the best

choice, with a cost benefit of 2.05 g/US\$, and with a bed length of 167 mm. However, we choose 16 μm in this case, having essentially the same cost benefit, 2.04 g/US\$, and a bed length of 242 mm. This bed length will be easier to pack to obtain a high efficiency. The running conditions are shown in Table III.

Influence of bed length on cost benefit and the resulting throughput are shown in Fig. 9, and the relative costs are shown in Fig. 10. Note that for an industrial system, with its relatively low fixed costs, the maxima do not coincide.

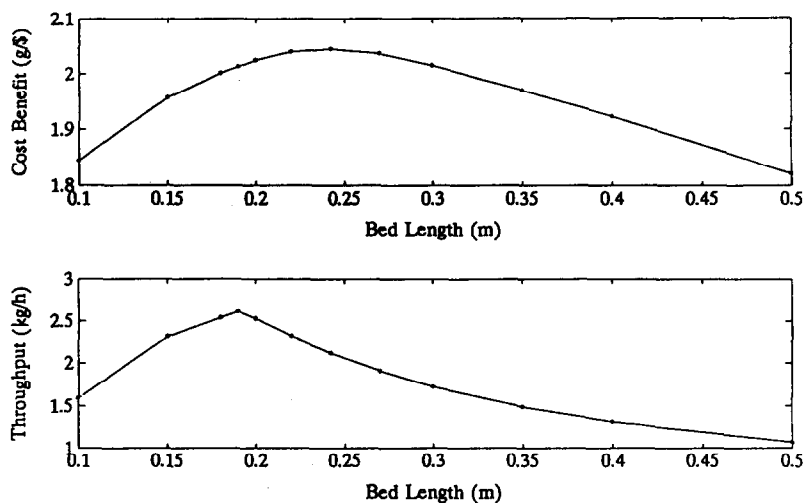


Fig. 9. Optimal cost benefit for different fixed bed lengths at a packing material diameter of 16 μm . Top: cost benefit vs. bed length; bottom: throughput vs. bed length.

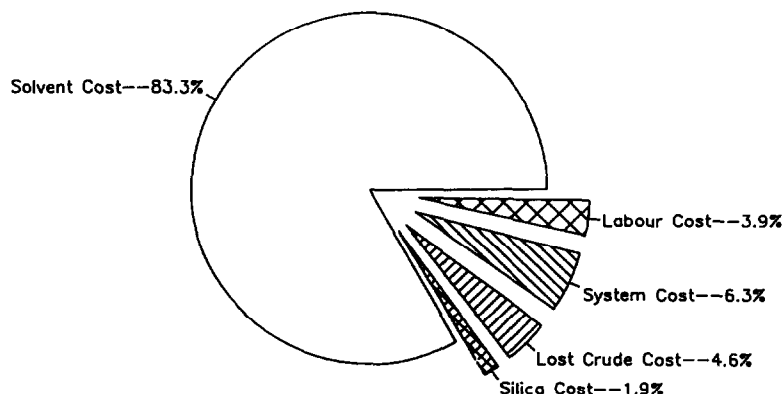


Fig. 10. Relative costs for separation at optimal running conditions as in Table III. The predominant cost is solvent, with over 80% of the total cost. System, labour and lost crude product each contribute with about 5%, and silica with about 2%.

TABLE III
RESULT OF ECONOMIC OPTIMIZATION

Particle size	16 μm
Bed length	242 mm
Flow-rate	860 l/h
Loading/injection:	117 g/substance
Total loading:	20.1 mg/g packing material

DISCUSSION

High-speed simulations are a necessity for optimization. With the help of KromaGuide 2.0 separation problems can be analyzed and optimized for best cost benefit (or highest throughput) in a reasonable amount of time on a PC. The optimal running conditions, *i.e.* column dimensions, particle size, flow-rate etc. can be readily obtained.

Initial experiments should preferably be carried out in analytical (or semi-preparative) scale, provided that the column system planned for in large scale is capable of producing the same column efficiency. For the overloaded experiments providing data for the isotherm model, heavy overload is recommended, giving higher accuracy.

It is apparent that a crucial task is to correctly determine the isotherms for the solutes involved, and to describe the interaction. We will continue looking into this problem more closely, and hope to develop more general models, making it possible to simulate and optimize a wide range of preparative LC separations in the future.

SYMBOLS

c^M	mobile phase solute concentration
c^S	solid phase solute concentration
$c^{S,\max}$	maximum adsorption capacity for a single substance
D_m	diffusion coefficient
d_p	particle diameter
H	theoretical plate height
h	reduced plate height
k'	analytical column capacity factor
n	number of substances
u	linear eluent velocity
α	separation factor, ratio between capacity factors
Γ_{ij}	competition factor (eqn. 2)
ν	reduced velocity

When a quantity refers to a specific substance, it is denoted by a subscript number or a variable i or j .

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