Time-Pulsed Vacuum and Time-Pulsed Alternating Pressure Chromatography

Mary Ann A. Endoma-Arias, Ian D. Brindle, Tomas Hudlicky*

Chemistry Department and Centre for Biotechnology, Brock University, 500 Glenridge Avenue, St. Catharines, Ontario, L2S 3A1, Canada
Fax +1(905)9844841; E-mail: thudlicky@brocku.ca

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Abstract: A comparison in separation efficiency between conventional chromatographic methods and newly devised time-pulsed methods (driven by either negative or positive pressure) is made. The methods are compared in terms of adsorbent amounts, solvent volumes, separation efficiency of a binary mixture, and the total time required for separation. It is found that alternating pressure or pulsed methods are superior on all counts compared to the conventional processes. This is supported by discussion of the principles as well as by the provision of detailed parameters for all experiments.

Key words: chromatography, solvent flow, time-pulsed vacuum, time-pulsed alternating pressure, new seperation methods

Introduction

The separation of mixtures by adsorption on chemically inert surfaces was invented (or discovered) more than 150 years ago.[1] Over the past 50 years, column chromatography methods have undergone numerous improvements. One of the major innovations in this field was the development of flash column chromatography as reported in 1978 by Still[2] (as of November 2013, this paper has been cited more than 8048 times according to the ISI Web of Knowledge). Other forms of column chromatography such as dry column chromatography,[3] and vacuum chromatography[4] have also been developed for separation. In addition, methods that change some of the physical parameters of separation and solvent flow, such as spinning disk chromatography (Chromatotron), have been used. In this paper, we report a new application of chromatography under reduced pressure with intentional time-pulsed interruptions that have a marked effect on difficult separations. Additionally, the technique uses less solvent and takes less time than conventional methods. As shown by the analysis of the principles involved, it should also be applicable to medium- and high-pressure chromatography, where pressure would be applied to the stationary phase from two directions in programmed time intervals.

Results and Discussion

We compared conventional gravity chromatography, flash column chromatography, and suction filtration chromatography with a suction filtration protocol under timed interruptions of pressure. The procedure is described in the experimental section. The results are summarized in Table 1, with additional details in the experimental section. In each case, two grams of a mixture of acetanilide (Acet) and *N*-methyl *p*-toluenesulfonamide (Nmet) (1 gram each) (R_f = 0.42 and 0.64, respectively; hexane–EtOAc, 1:1 elution solvent) was used. These results clearly indicate that the time-pulsed interruption of pressure has a beneficial effect on the separation. In dry column vacuum suction or vacuum liquid chromatography (references 3 and 4, respectively) effort was made not to interrupt the vacuum during fraction collection. In the case of time-pulsed vacuum filtration, the intentional interruption of vacuum sends a pressure wave backwards through the separation bed. Thus the components of the mixture travel down an active surface for time = t_1 and distance = d_1, then, as the pressure is interrupted, travel backwards over a partially saturated surface, modified by the presence of the adsorbed component, for time = t_2 and distance = d_2. The net effect that the pulsing has on the separation is that the components spend more time on an 'apparently' longer bed of adsorbent ($T = t_1 + 2t_2$ and $D = d_1 + 2d_2$). The separation process is therefore the function of the initial negative pressure, the reverse pressure gradient, the time regime of pulsing and the change in the characteristics of the stationary phase, caused by the adsorption of the solute (see the next section for a detailed explanation of the theory).

From the data in Table 1, it is clear that this method is superior to other forms of chromatography in terms of adsorbent loading, time of separation, and the volume of solvent used – all of which parameters are much more effective than those in the two standard methods used for comparison.

Repetition of the original vacuum dry column method published in 2001[3] produced results that were most similar to the pulsed vacuum protocol, except that it required ~20% more solvent (silica = 36 g; no. of fractions = 20; solvent = 600 mL; time = 14 min). This observation makes sense as the vacuum had to be interrupted in the original procedure for fractions to be collected. The advantages of the pulsed vacuum protocol over dry column vacuum chromatography are the adjustability of the time regime of the pulses and the lower volumes of solvent used (especially at larger scales).

In addition, we have designed a method of pressure-

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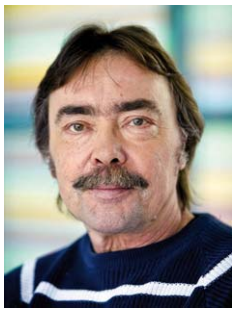
pulsed chromatography that combines the principles of flash chromatography with time-pulsed vacuum suction filtration. The pressure is applied in one direction for time = t_1, interrupted, and reapplied in the opposite direction for time = t_2. The principle is identical to that described for pulsed vacuum chromatography, except that positive pressure is employed to move the mixture through the adsorbent bed in an oscillating manner. The

Biographical Sketches

Mary Ann A. Endoma-Arias was born in 1969 in Rizal, Philippines. After completing her BSc (1990) at the University of the Philippines Diliman, in Quezon City, Philippines, she started her PhD studies at Virginia Tech, in Blacksburg, VA, under the supervision of Professor Tomas Hudlicky, in 1992. She moved with Professor Hudlicky in 1995 to the University of Florida, in Gainesville, FL, where she completed her PhD in 1997. Upon completion of her PhD, she returned to the University of the Philippines where she rose to the rank of Associate Professor. Her main interest during her research career in the Philippines focused on the preparation and conjugation of compounds with medicinal properties for targeted delivery. During this time she continued to work closely with the Hudlicky Research Group. She has published 19 papers and four patents. In 2010, she joined the group of Professor Hudlicky at Brock University in St. Catharines, ON, Canada as a Research Associate.

Ian Brindle, BSc, UMIST (University of Manchester Institute of Science and Technology), MSc Brock University, DSc, UMIST, FRSC (UK) is Professor of Chemistry and Chancellor's Chair for Research Excellence at Brock University. He is the author of more than 90 publications on analytical chemistry, archaeometry, and the development of assistive devices for handicapped chemists. He recently stepped down from his position of Vice-President, Research; previously he was Dean of Mathematics and Science at Brock University. His research interests cover the fields of inorganic and organic trace analysis. His work on arsenic, tin, lead, selenium, etc., and his pioneering use of l-cysteine in the determination of these elements led to it being called the "Brindle Reagent". The reagent is used worldwide to measure these elements that are toxic at very low concentrations. He has been involved in research programs aimed at understanding the processes that lead to accumulation of persistent organic pollutants, particularly in the Great Lakes. Currently, he is engaged in the development of improved methods (faster, safer, and automated) to prepare mineral and ore samples for the mining industry.



Tomas Hudlicky was born in 1949 in Prague, Czechoslovakia, where he received his elementary and middle school education. After several years of working as a process chemist apprentice and in other odd jobs in pharmaceutical chemistry, it became apparent that higher education opportunities were closed to him. In 1968, he emigrated to the U.S. with his parents and sister. Hudlicky's educational experience continued at Blacksburg High School, from which he dropped out in the spring of 1969. Accepted as a probational student at Virginia Tech the following autumn, he received his BS in chemistry in 1973, and went on to pursue graduate studies at Rice University under the direction of Professor Ernest Wenkert in the field of indole alkaloid total synthesis, earning his PhD in 1977. He then spent a year at the University of Geneva working under the late Professor Wolfgang Oppolzer on the synthesis of isocomene. In 1978, he joined the faculty at the Illinois Institute of Technology as an Assistant Professor, and began the first phase of his research career in the field of general methods of synthesis for triquinane terpenes and other natural products containing five-membered rings by [4+1] cyclopentene, pyrroline, and dihydrofuran annulation methodologies. He returned to his alma mater, Virginia Tech, in 1982, and rose to the rank of Professor in 1988. One year later, at the 20-year class reunion of the Blacksburg High School class of 1969, he received his High School Diploma. The next phase of his research involved the investigation of *cis*-cyclohexadienediols in enantioselective synthesis. In 1995, he moved to the University of Florida in Gainesville. In 2003, Dr. Hudlicky accepted an offer from Brock University where he currently holds a position as Canada Research Chair. His current research interests include the development of enantioselective synthetic methods, bacterial dioxygenase-mediated degradation of aromatics, the design and synthesis of fluorinated inhalation anesthetic agents, the synthesis of morphine and Amaryllidaceae alkaloids, and the design of unnatural oligo-saccharide conjugates with new molecular properties. His hobbies are skiing, hockey, martial arts, and music.

results are shown in the last column in Table 1 for comparison with the vacuum pulsed protocol.

Discussion of the Principles Involved

The separation of the mixture is a function of the free energy of adsorption of the components and, in accordance with the Van Deemter equation (Equation 1):

$$H = A + \frac{B}{u} + C \cdot u$$

Equation 1

is also a function of the velocity of flow over the adsorptive surface, where H = theoretical plate height, and u = velocity of the mobile phase. The coefficients A, B, and C represent, respectively, tortuosity, longitudinal diffusion, and the mass transfer coefficient between the mobile and stationary phases. It is the C factor, the mass transfer coefficient, that is most significant in this application. In time-pulsed chromatography, the additional parameters that need to be considered are the pressure gradients and time.

Consideration of equilibrium thermodynamics allows an equation (Equation 2) to be developed that relates pressure and the distribution coefficient for the solute between the mobile and stationary phases.[5] The term, ΔG_{dist} is the Gibbs free energy of binding of the solute to the stationary phase, K_{dist} is the distribution coefficient for the solute, ΔV is a measure of the pressure-induced change in the partial molar volume, and Δn is the change in the coefficient of the solute between the mobile and stationary phases; κ_s is the isothermal compressibility of the solvent:

$$\left(\frac{\partial \Delta G_{dist}}{\partial P}\right)_T = -RT\left(\frac{\partial \ln K_{dist}}{\partial P}\right)_T + \Delta n RT\kappa_s = \Delta V$$

Equation 2

Equation 2 can be developed to show the relationship between pressure and the selectivity factor α (Equation 3):

$$-RT\ \frac{\Delta \ln \alpha}{\Delta P} = \Delta V$$

Equation 3

This effect will be small at the modest pressures used in this technique. The reversal of the direction has a twofold effect: one is the virtual lengthening of the column, as outlined above; the second is that the reversal of the flow would have the effect of reconcentrating, or focusing the solutes on the stationary phase that is partially covered with the solute.

Stationary phases with adsorbed solute would be expected to have a greater affinity, and thereby would increase the distribution coefficient. The consequence of this change and reconcentration would be that the adsorption profile would be sharpened, and the width of the peak narrowed, which would, in turn, affect the number of theoretical plates, as we can see from Equation 4, where N = number of theoretical plates, t_R = retention time, and w = peak width.

$$N = 16\left(\frac{t_R}{w}\right)^2$$

Equation 4

The resolution (R) between two eluting peaks is a function of the number of theoretical plates (N), the selectivity coefficient (α), and the retention factor (k_B') of the later-eluting peak, as shown in Equation 5:

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B'}{1 + k_B'}\right)$$

Equation 5

In addition, the retention factor depends upon the time taken by the solvent and the solute to pass through the column, as shown in Equation 6:

$$k' = \frac{t_R - t_M}{t_M}$$

Equation 6

Table 1 Comparison of Separation Methods[a]

	Gravity		Flash	Vacuum, time-pulsed[b]		Pressure, time-pulsed[c]	
Silica (g)	150	36	150	**36**[d]	36[e]	36[f]	36[g]
No. of fractions	38[h]	20[i]	36[j]	**20**[k]	20[l]	20[m]	20[n]
Solvent (mL)	1140	615	1065	**510**	525	550	550
Time (min)	73	75	38	**14**	12	32	43

[a] Average of several trials. [b] The pump used generated 650 mm/Hg, hence a reverse ΔP gradient of ~100 mm/Hg. [c] The nitrogen pressure was 8 psi. [d] 5 second pulse. [e] 10 second pulse. [f] 5 second alternating pulse. [g] 10 second alternating pulse. [h] Fraction volume = 30 mL. [i] Fraction volume = 25 mL. [j] Fraction volume = 30 mL. [k] Fraction volume = 25 mL. [l] Fraction volume = 25 mL. [m] Fraction volume = 25 mL. [n] Fraction volume = 25 mL.

These times will depend upon the programming of the pressure regime, as described above, and will be consistent with the notional longer bed.

The other important factors to be taken into consideration, and that have an impact on the peak width, are the changes in the retention factors for the eluents as they undergo reversal of flow direction. This phenomenon is described by Poole in terms of Secondary Chemical Equilibria.[6] Although it might seem that the reversal of flow will merely remix the components, in fact, the change in distribution coefficients, or retention factors, as mentioned above, will, in fact, be altered three times at each reversal event:

1. The solute passes down the column with no adsorbed solute, k_1.

2. At the first reversal, the solute in the mobile phase will be more strongly retained on the stationary phase since the solute modifies the surface, which is thus partially saturated. For this situation, k_2 will be greater than k_1.

3. The second reversal returns the solute over the stationary phase that has adsorbed even more of the solute, thereby increasing further the value of the retention factor, k_3.

Once the third step is achieved, the process can begin again, resulting in further sharpening of the elution profile.

Thus, with the temporarily increased retention factor of the column, at the point where the solute can be more strongly adsorbed, results in the focusing, alluded to above. This focusing is analogous to cold-trapping in gas chromatography, as described by Poole,[7] in which a 'temporary increase of the retention power' of the column is a recognized mechanism for focusing analytes, thereby reducing band-broadening and narrowing the width of the peak. From the equations above, it will be clear that reducing the peak width will result in a greater number of theoretical plates, which in turn, will mean a greater resolution, since resolution is proportional to the square root of the number of theoretical plates.

The practical implications of this improved resolution are that: i) satisfactory resolution of closely-eluting solutes can be achieved with a shorter column bed; ii) following from (i), smaller volumes of eluting solvent will be required; iii) a smaller number of fractions will be needed to achieve functional separation of solutes, and iv) separations can be achieved in a shorter time-frame.

Proposed Design of Instruments

We propose the following instrumental design that would allow quantitative control and programming of all crucial parameters. Figure 1 shows a diagram for suction filtration with a device that allows accurate control for the duration of time that suction is applied and the time of interruption (and therefore equilibration of pressure on the column). Figure 2 shows the design for a medium- to high-pressure application, where positive pressure, rather than vacuum is used, with the pressure being applied in pulsed intervals in opposite directions.

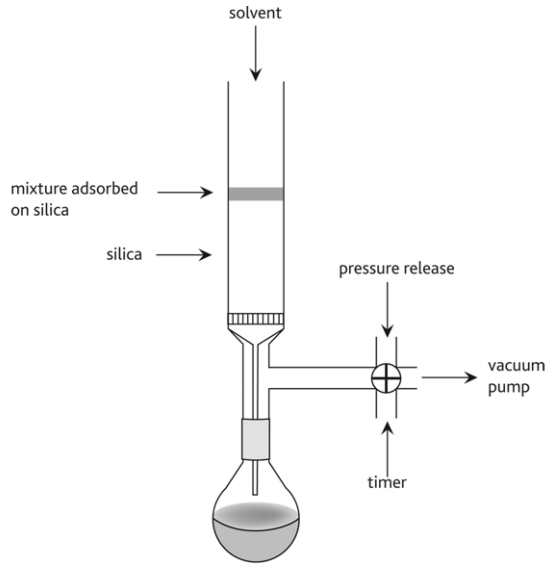


Figure 1 Design for time-pulsed vacuum chromatography

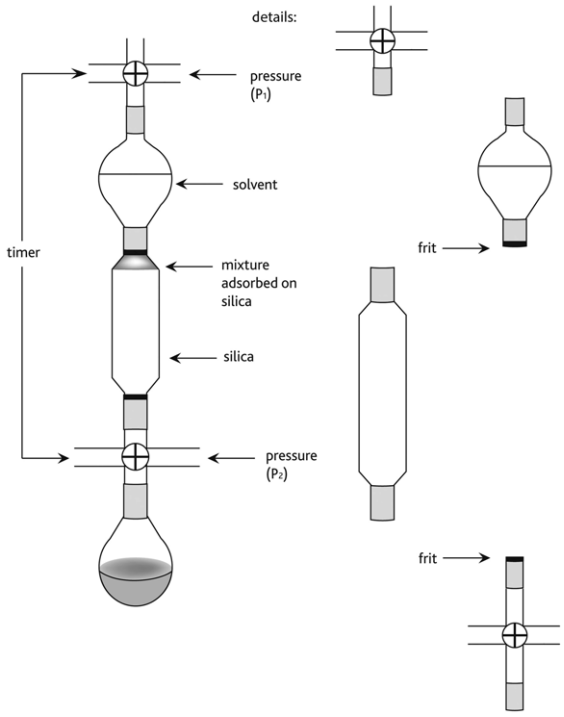


Figure 2 Design for alternating time-pulsed pressure chromatography

Conclusions

We have demonstrated that time-pulsed or alternating pressure chromatography provide separations that are more effective that those acquired by conventional methods. The vacuum time-pulsed method as well as the alternating pressure method both use less solvent, less

adsorbent, take less time to complete and provide comparable or better separation of standard binary mixtures compared with either gravity or flash column chromatography. These improvements are especially valuable for applications at larger scales, where the amount of adsorbent and the volume of solvent used are substantial and contribute to the increased costs of separation. With the use of the time-pulsed method, we have been able to separate routinely large amounts of mixtures with ΔR_f = ~0.1 with the ratio of silica to compounds of ~20:1 (as compared to ~100:1 for conventional methods). It is especially useful for repetition of separation protocols with the same mixtures (as is often the case in projects dealing with total synthesis), where the first experiment is used as a trial run to determine more optimal conditions with regard to the regime of time-pulse and fraction collection.

In addition, both the time-pulsed vacuum method and the alternating pressure method are expected to be amenable to computer interface driven automation for the purpose of precise timing, variable vacuum and pressure adjustments, monitoring of separation, and fraction collection. Future endeavors in this area will be focused on the development of instrumentation that allows quantitative control of the experimental parameters in these separations.

Experimental

Time-Pulsed Vacuum Chromatography

The column is packed dry and the mixture of compounds is pre-adsorbed on silica, and the dry silica placed on top of the column bed. The less polar component of the solvent system is used to begin the elution under vacuum for gradient elution (~600–650 mm/Hg), whereas a 1:1 mixture of hexanes and EtOAc was used for isocratic elution. The vacuum is interrupted at specified intervals (usually after 5 or 10 seconds of flow). The shock wave travelling back up the column is visible and after the pressure equilibrates, the vacuum is applied again (Table 2). The fraction volume depends on the scale of separation (details of all other separation protocols are listed in the Tables in the Supporting Information).

Parameters that are constant for all the runs:

R_f (hexanes–EtOAc, 1:1): Acet = 0.42, Nmet = 0.64.

Mixture of Acet–Nmet: 2 g (1 g each).

Application of the mixture on silica: dissolved in EtOAc (7.5 mL), sonicated for 1 min, adsorbed on silica (4 g).

Amount of silica: 36 g.

Volume of each fraction collected: 25 mL.

Vacuum strength: 650 Torr.

Isocratic elution (hexanes–EtOAc, 1:1).

Pulse: 5 s.

Table 2 Time-Pulsed Vacuum Chromatography

Trial 1		
Total time for fraction collection	16 min	–
Number of fractions	21	–
Elution volume of Nmet	75 mL	950 mg
Elution volume of Acet	225 mL	970 mg
Total volume of eluent	525 mL	–
Trial 2		
Total time for fraction collection	12 min	–
Number of fractions	20	–
Elution volume of Nmet	75 mL	980 mg
Elution volume of Acet	225 mL	980 mg
Total volume of eluent	500 mL	–

Time-Pulsed Alternating Pressure Chromatography

The column is packed dry and the mixture of compounds is pre-adsorbed on silica and, the dry silica placed on top of the column bed. A 1:1 mixture of hexanes and EtOAc was used for isocratic elution under 8 psi nitrogen gas pressure. Nitrogen gas pressure is applied in opposite directions (Figure 2), indicated by P_1 and P_2 at specified intervals (usually 5 or 10 seconds of flow). Bubbling is visible once the pressure is applied from the bottom of the column (Table 3). The fraction volume was based on initial results with time-pulsed vacuum chromatography (details of all other separation protocols are listed in the Tables in the Supporting Information).

R_f (hexanes–EtOAc, 1:1): Acet = 0.42, Nmet = 0.64.

Mixture of Acet and Nmet: 2 g (1 g each).

Application of the mixture on silica: dissolved in EtOAc (7.5 mL), sonicated for 1 min, adsorbed on silica (4 g).

Amount of silica: 36 g.

Volume of each fraction collected: 25 mL.

Isocratic elution (hexanes–EtOAc, 1:1).

Pressure of nitrogen gas: 8 psi.

Forward nitrogen pressure for 5 seconds, backwards nitrogen pressure for 5 seconds.

Table 3 Time-Pulsed Alternating Pressure Chromatography

Trial 1		
Total time for fraction collection	35 min	–
Number of fractions	20	–
Elution volume of Nmet	50 mL	960 mg
Elution volume of Acet	325 mL	960 mg
Total volume of eluent	550 mL	–
Trial 2		
Total time for fraction collection	30 min	–
Number of fractions	20	–
Elution volume of Nmet	50 mL	940 mg
Elution volume of Acet	325 mL	920 mg
Total volume of eluent	550 mL	–
One tube contained a mixture of Nmet and Acet = 25 mL	–	100 mg

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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