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EXTRACTION DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING SOLVENT SEGMENTATION OF THE COLUMN EFFLUENT

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SUMMARY

A high-performance liquid chromatographic post-column detection system based on ion-pair extraction in a solvent-segmented stream is described. Weakly basic compounds eluting from a reversed-phase RP-2 or CN column are mixed with a fluorescent counter ion (9,10-dimethoxyanthracene-2-sulphonate). The ion pair is then continuously extracted into an organic solvent and detected fluorimetrically. The natures of the organic solvent and the ion pair strongly influence the detection sensitivity. A comparison of the solvent-segmentation and air-segmentation approaches showed no significant difference with regard to band broadening. The solvent-segmentation principle, on the other hand, offers advantages with regard to simplicity of apparatus and handling and is less sensitive to temperature and flow variations.

INTRODUCTION

Post-column reaction detectors in high-performance liquid chromatography (HPLC) have gained increased popularity in recent years[1,2]. For relatively fast reactions with reaction times of a few seconds to several minutes, capillary reactors or bed reactors have been recommended[3,4]. For longer reaction times (> 5 min), the air-segmentation principle used in AutoAnalyzers has been adopted to avoid excessive band broadening[5]. The theoretical aspects of band broadening in such systems have been treated by Snyder[6] and Snyder and Adler[7,8].

Recently, we have shown[9] that the air-segmentation system can also be used to advantage for fast reactions mainly in cases where the excess of reagent interferes in the detection process. In this work, an ion-pairing phenomenon was used to study two tertiary amine drugs, chloro- and bromopheniramine. A fluorescent counter ion,

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9,10-dimethoxyanthracene-2-sulphonate (DAS), proposed earlier by Westerlund and Borg[10], was adopted and the excess of DAS was removed by using the dynamic micro-extraction principle well known with AutoAnalyzers and used successfully for DAS ion pairs by Gfeller and Frey[11].

The results of this work were encouraging and band broadening was kept below 20% using standard Technicon equipment. On the other hand, one is still dealing with a relatively complex three-phase system, which makes the phase-separator unit a critical part of the extraction detector. Continuous extraction without air segments has been used successfully by Karlberg and Thelander[12] for micro-extraction of caffeine in a continuous-flow system. Band broadening was, of course, not as critical in this system as it would be in HPLC detection, but it was reported to be small. This paper describes results obtained with this solvent-segmentation approach and discusses its potential and limitations for detection in HPLC.

EXPERIMENTAL

Reagents

Fig. 1 depicts the structures of the test compounds. Stock solutions of these compounds (except HT) were prepared in distilled water at a concentration of 1 mg/ml. Aliquots of these solutions were diluted to yield working solutions, the concentrations of which varied according to the compound. HT was dissolved in methanol to give a 0.1 mg/ml solution, which was diluted with water to produce a working solution. The counter ion was sodium 9,10-dimethoxyanthracene-2-sulphonate (DAS; Sandoz, Basle, Switzerland) dissolved in water at a concentration of $1 \cdot 10^{-4}$ *M*. The organic solvents studied (Table I) were of analytical-reagent quality (J. T. Baker, Deventer, The Netherlands), except for trichloroethylene, which was of reagent-grade quality.

High-performance liquid chromatography

A Perkin-Elmer Series 2 liquid chromatograph was employed. The column (10 cm × 3 mm I.D.) was home-packed with an experimental batch of CN-bonded 10-μm silica (Merck, Darmstadt, G.F.R.). A 10 cm × 3 mm I.D. LiChrosorb RP-2 column was also used. The mobile phase consisted of 25% methanol in 0.1 *M* sodium dihydrogen orthophosphate at a flow-rate of 1.0 ml/min. The column outlet was connected to the post-column reactor system by means of $^1/_{16}$-in. stainless-steel capillary tubing.

Ion-pairing detection system

Fig. 2 shows the arrangement of the AutoAnalyzer system (Technicon, Tarrytown, N.Y., U.S.A.) as set up for solvent segmentation. A Perkin-Elmer Model 204A fluorescence spectrophotometer was used for detection of the fluorescent ion pair (excitation 383 nm, emission 452 nm). To study band broadening, a variable-wavelength UV detector (Pye Unicam, LC-3, Philips, Eindhoven, The Netherlands) was placed between the post-column ion-pair detection system and the analytical column. Band broadening of the ion-pair detector was measured as the increase in peak width (at half-height) in seconds relative to the UV signal.

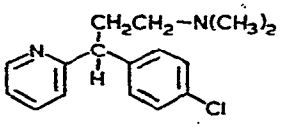
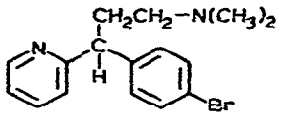
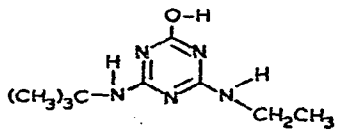
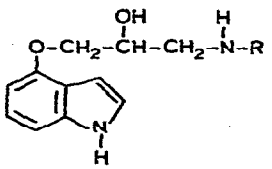
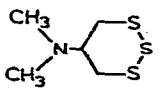
Name	Structure
CPA	
BPA	
HT	
HM	
SAN	

Fig. 1. Structures of compounds.

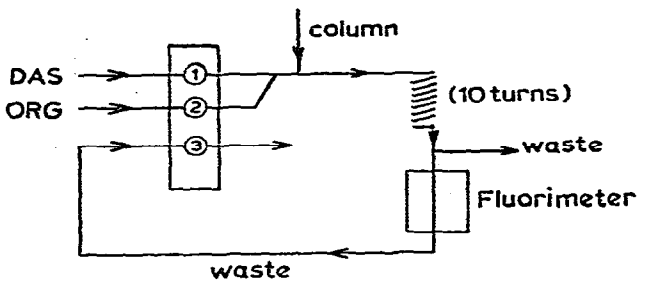


Fig. 2. Post-column ion-pair extraction system. Flow-rates (ml/min): DAS, 0.32; organic solvent, 0.92; through the flow cell, 0.34; HPLC, 1.0.

RESULTS AND DISCUSSION

The reaction unit

Examination of the reaction and extraction unit (Fig. 2) reveals the simplifications that have been introduced in comparison with the set-up used earlier in connection with the three-phase system[9]. The ion-pairing process occurs instantaneously so that no reaction coil is needed. The DAS solution is therefore segmented with the extraction solvent prior to adding it to the column effluent immediately after the column. The extraction takes place in a 10-turn glass-coil (2 mm I.D.) and is of about the same efficiency as the previously used 20-turn coil in the three-phase system[9]. This corresponds to about a 1.4-min extraction or residence time. Doubling this extraction time by using a 20-turn coil resulted in a less than 5% increase in the signal. A reduction of the extraction unit to a 5-turn coil under otherwise identical flow conditions resulted in a 25% drop in the fluorescence signal. The phase separation was carried out with a conventional Technicon phase separator with a PTFE insert. In the present set-up, only about one third of the organic phase is drawn into the detector cell, the reason for this being the capillary inlet to the cell which at higher flow-rates caused too much back-pressure. This can be changed with a re-design of the cell compartment.

Band-broadening effects

The influence of the extraction detector units on band broadening was also investigated. In Fig. 3, the use of UV and fluorescence (for ion pairs) detection in the separation of chloro- and bromopheniramine is compared.

The band broadening observed for the extraction detectors in comparison with direct UV detection was of the order of 12 sec, or less than 20%, for the two pheniramine peaks. As band broadening was shown to be less than 2 sec per 10-turn coil, it can be assumed that the phase separator is still the critical unit in the extraction detector. In addition, the band broadening caused by the ion-pair extraction technique was found to be identical for the present solvent-segmented two-phase and the previously used air-segmented three-phase system, the former moreover seemingly giving a more consistent phase separation. As a consequence, the warm-up time in the two-phase system, which is about 10 min, was considerably shorter than in the three-phase system, where about 1 h was needed.

Regarding the dependence of band broadening and extraction efficiency on the ratio of aqueous to organic phase, a ratio close to unity was found to be optimal. Hence, most of the work was carried out under such conditions, which correspond to a rate of about one extractant segment per second. However, we have observed that the ratio of aqueous to organic phase can be varied from about 0.5 to 1.5 without seriously increasing band broadening (12 ± 2 sec) or reducing the extraction efficiency.

Influence of extraction solvent

Obviously the choice of suitable organic solvents, pH and buffer concentration are of prime importance for an efficient extraction and phase separation. All of these parameters have been studied extensively under batch conditions and in dynamic systems for the pheniramine–DAS system[16]. In the present study, six conventional

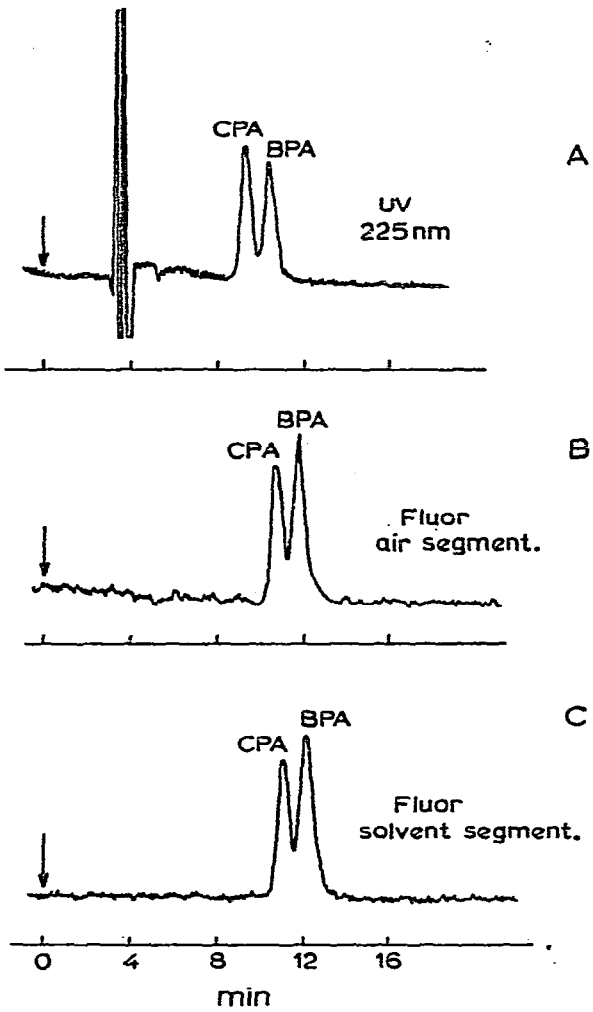


Fig. 3. Comparative chromatograms. (A) UV detection of chloro- and bromopheniramine (CPA and BPA, respectively) at 225 nm. (B) Fluorescence, air segmentation. (C) Fluorescence, solvent segmentation. Organic solvent, chloroform. Detector conditions as in Fig. 2. HPLC conditions: CN column, 25% methanol–0.1 *M* NaH_2PO_4 as mobile phase, flow-rate 1.0 ml/min.

chlorine-containing solvents were studied directly in the solvent-segmented dynamic system with regard to their suitability for this detection approach. The pH and the buffer concentration were kept optimal (pH $\approx$ 4) as determined from earlier batch studies[16]; all other conditions were also kept the same. The results for four different compounds (*cf.*, Fig. 1) are shown in Table I as relative peak heights for the fluorescence signal.

Firstly, it should be mentioned that band broadening was the same with all solvents tested; *i.e.*, in general, reasonable flexibility in selecting a suitable water-immiscible solvent can be expected. On the other hand, as expected, the choice of solvent has a pronounced effect on the extractability of the various ion pairs. Tetra-

TABLE I

EFFECT OF THE ORGANIC SOLVENT ON POST-COLUMN EXTRACTION OF DAS ION PAIRS

Solvent	*Relative peak height*			
	CPA	*HT*	*HM*	*SAN*
1,2-Dichloroethane	46	10	14	6
Dichloromethane	40	18	15	3
1,1,2,2-Tetrachloroethane	36	68	57	11
Trichloromethane	24	11	15	2
Tetrachloromethane	0	0	0	0
Trichloroethylene	0	0	0	0

chloromethane and trichloroethylene do not extract the ion pairs. This agrees with the results of Schill *et al.*[17], who observed at least a 1000-fold difference between chloroform and tetrachloromethane for the extraction of various ion pairs. The formation of hydrogen bonds with the ion pair may explain this phenomenon. Dichloromethane causes problems owing to its high volatility, which results in bubble formation and, therefore, disturbances in the detector. The other three solvents showed satisfactory performance, 1,1,2,2-tetrachloromethane giving the best overall results. The relative signals obtained for the different compounds obviously reflect the relative differences in structure and extractability of the ion pairs.

The addition of alcohols is known[17] to enhance the extractability of compounds containing hydroxy groups, the reason being improved solvation on the ion pair. Results obtained in the present study are shown in Table II. The extraction of HT and HM was significantly enhanced, particularly with the higher alcohols, whereas practically no effect was observed for SAN and CPA. The addition of up to 20% (v/v) of the alcohols to chloroform did not have any effect on band broadening. However, one can expect problems to occur at still higher percentages, owing to the better solubility of DAS in the organic phase and consequently a higher fluorescence background. Changes in solvent density may also lead to a poorer phase separation.

TABLE II

EFFECT OF ADDITION OF ALCOHOLS (20%, v/v) TO THE ORGANIC SOLVENT ON ION-PAIR EXTRACTION

Solvent	*Relative peak height*			
	CPA	*HT*	*HM*	*SAN*
Chloroform	15	9	9	4
Ethanol	11	11	8	3
Propanol	12	21	19	4
n-Butanol	19	52	49	—
n-Octanol	22	46	45	9

Analytical data

The reproducibility of the detection system based on the solvent-segmentation principle is at least as good as that for the three-phase air-segmentation system. The

results of repetitive injections of a mixture of the four compounds tested are shown in Fig. 4. The quantitative reproducibility is better than $\pm$ 3.5% (relative standard deviation) ($n = 4$).

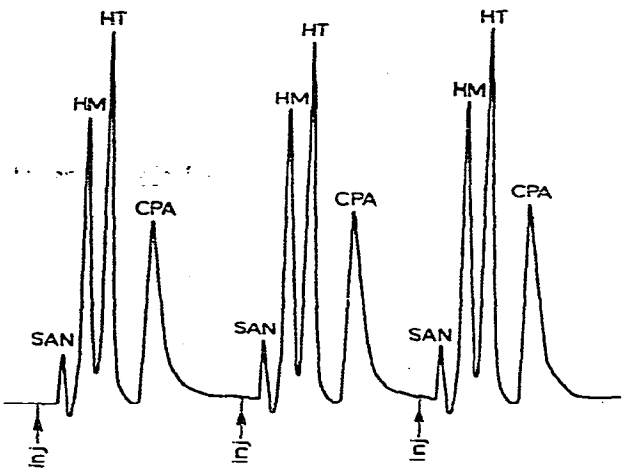


Fig. 4. Replicate injections of SAN (4 μg), HM (1.5 μg), HT (1.5 μg) and CPA (200 ng) on an RP-2 column; solvent segmentation. Conditions as in Figs. 2 and 3 with the exception that tetrachloroethane was used as organic solvent.

The approximate detection limits (signal-to-noise ratio 3:1) under the conditions described in Fig. 4 are 6 ng of CPA, 25 ng of HT, 30 ng of HM and 400 ng of SAN. The selectivity and the practical application to, for example, urine analysis have been demonstrated in a previous paper[3].

CONCLUSIONS

Under the conditions studied, the solvent-segmentation principle seems to be just as applicable to post-column reaction detection as an air-segmented system. It seems misleading to classify this principle as a flow-injection technique[13–15], such as Karlberg and Thelander[12] have done, as the band-spreading phenomena are different. In fact, it appears more logical to classify this approach under segmented-flow techniques where the air bubble has been replaced with an immiscible-solvent segment to reduce band broadening. Further studies on the band broadening and mixing phenomena in such systems will be necessary in order to be able to assess how they differ from descriptions given by Snyder[6] and Snyder and Adler[7,8].

The advantages of the present approach over the three-phase system used for HPLC detection earlier[9] are obvious. The design is simpler and the phase separation can be effected more conveniently. The residence time per unit spiral volume is longer, as no volume is occupied by air bubbles. The system is less temperature sensitive and more flexible with regard to flow variations owing to the absence of compressible gas bubbles. For the same reason, it should be possible to work at higher pumping pressures, so that standard HPLC pumps and lines could conceivably be used. Variation of the segmentation rate and the ratio of organic to aqueous phase can be

flexible, although a ratio close to 1:1 seems to be optimal, as found also by other workers[12]. The possibility for choosing an optimal organic solvent with regard to, *e.g.*, viscosity, solubility, density, vapour pressure and spectral characteristics seems to introduce another interesting aspect. As a consequence of all of these advantages it should be possible to minimize band broadening and to reduce background noise and, accordingly, also to improve the detection limits and the reproducibility of this detection mode.

Finally, it should be realized that the solvent-segmentation principle offers many interesting possibilities in the development of reaction detectors for HPLC including, in addition to ion pairing and complex formation, the whole range of relatively simple and fast chemical reactions where the excess of reagent would interfere in classical reactor approaches. On the negative side, it has to be admitted that the phase separation still contributes rather heavily to band broadening. Miniaturization and re-design of the mixing and reaction tracts and the phase separator are being studied with the aim of reducing band spreading further. Electronic segment suppression (analogous to electronic debubbling) may be another alternative.

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