Low Pressure Separations Using Automated Flow and Sequential Injection Analysis Coupled to Monolithic Columns

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Abstract

Automation is a key demand in modern analytical chemistry. Automated analytical schemes facilitate samples handling, and enable effective processes such as dilution, extraction, derivatization, and preconcentration to be carried out. Flow (FI) and sequential injection (SI) analysis are well-established and mature automated analytical techniques with more than 18,000 publications so far in all areas of analytical science. FI and SI offer significant advantages such as low instrumental and operational cost, widely available instrumentation, and effective automation of critical steps in the analytical process. Until recently, the main disadvantage of FI and SI was the inability of simultaneous determinations of more than two analytes in a single run. Due to the low pressure operation of these techniques, it was impossible for them to be coupled to conventional particulate-based separation columns that enable chromatographic separations. This drawback was overcome by the introduction of monolithic stationary phases. Monolithic columns are prepared from organic and silica monomers. Silica-based monoliths have small-sized skeletons and a bimodal pore size distribution with µm-sized throughpores and nm-sized mesopores. This gives silica-based monoliths favorable properties for high-efficiency fast separations, such as low-pressure drop across the column, fast mass transfer kinetics and a high binding capacity. They consist of a single rigid porous rod, enabling higher flow rates than particulate columns at reasonable back-pressures. These unique features of monolithic columns enabled their incorporation in low/moderate pressure setups, such as FI and SI, expanding dramatically their possibilities.

Introduction

Automation of the laboratory processes was initiated in the 1970s. Among the first approaches on the automation of chemical procedures was introduced by Ruzicka and Hansen who firstly developed the flow injection analysis (FIA) technique (1). In the years to follow, FIA became popular because it made it possible to automate routine procedures, providing simplicity, low operation and instrumental cost, reduction in sample consumption, and high sampling rates (2). Nowadays, FIA is a mature analytical technique as demonstrated by the over to 16,000 research articles, reviews, and several books that have already been published devoted in this topic (3).

The second generation of flow injection analysis [sequential injection analysis (SIA)] has been proposed and developed by Ruzicka and Marshall in 1990 (4). The introduction of SIA marked a new era in the development of flow analysis, overcoming some significant limitations of the FIA technique. Compared with FIA, the economy in reagents, the reduction in waste generation, the computer-controlled experimental parameters and the simple singled-line manifold are the main benefits of the SIA technique.

Generally, FIA and SIA techniques have one important drawback (they cannot primarily provide the separation procedure and analysis of multi-component samples). However, simultaneous determination of two or three compounds without a separation step has been accomplished using FI or SI setups (5–8). Due to the low pressure operation of these techniques, it was impossible to be coupled to conventional particulate columns that enable chromatographic separations. This "weak point" was solved by the coupling of a short monolithic column with FIA and SIA manifold (9,10). The new technique is called sequential injection chromatography (SIC), and up until now, it has been used for the analysis of multi-component samples (mainly pharmaceuticals) (11). In an alternative and straightforward approach, the SIA manifold was directly connected to the injection valve of the HPLC instrumentation performing on-line solid phase extraction (12), derivatization (13,14), or on-line dilution of the sample (15) prior to separation.

The monolithic columns are usually made of single rigid porous silica, which was initially called "silica rod" (16). Silica-based monoliths provide favourable properties for high-efficiency fast separations, such as low-pressure drop across the column, fast mass transfer kinetics, and a high binding capacity. The main characteristic of monolithic columns is that it allows higher flow rates than particulate columns at reasonable back-pressures, expanding the possibilities of FI and SI techniques.

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Recently, a review article on the application and methodology of SIC has been reported by Chocholous et al. (11). The article was focused only on the SIC-based applications and did not include FI-based approaches. The purpose of this article is to present a more updated review on this topic, summarizing the applications of both flow and sequential injection chromatography.

Flow injection analysis

FIA is characterized by an automated, continuous flow approach to perform chemical analysis, based on injecting a small, well-defined volume of sample into a continuously flowing carrier stream (to which appropriate auxiliary reagent streams can be added) whereby a concentration gradient of the sample is created (17). The sample zone is gradually dispersed into the carrier by axial and radial diffusion as it is moved through the channel under laminar flow conditions. Reagents may be added at various confluence points and mix with the sample zone under the influence of radial dispersion to produce reactive or detectable species. The obtained peak height or area can be used to quantify the analyte (18). The whole procedure, including sample injection, transport, reaction, and detection, can be performed in a relatively short time. A typical FIA configuration is depicted in Figure 1A.

Sequential injection analysis

A basic SIA setup (Figure 1B) consists of a bi-directional propulsion device (e.g., peristaltic or syringe pump), a holding coil, a multi-position selection valve, a detector, tubing adequate for unifying all different components of the system, and a computer. The computer synchronises the pump and the multi-position selection valve in such a way to define the volume and direction of the stream of the different solutions.

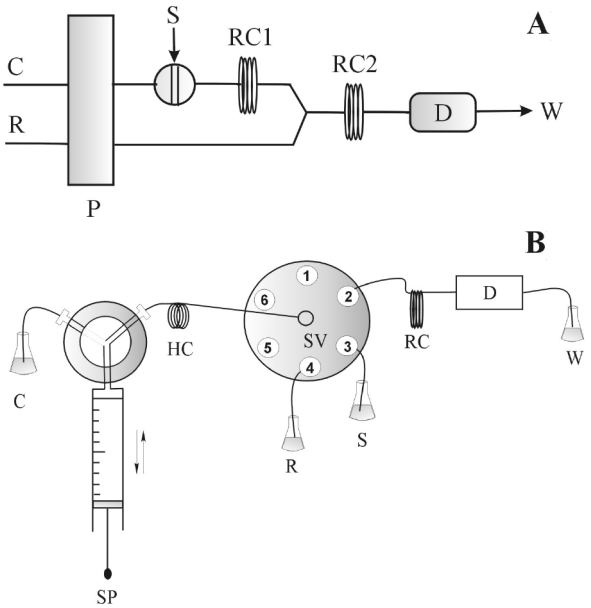

Figure 1. Typical FI (A) and SI (B) instrumentation setup. Where: C, carrier; R, reagent; S, sample; RC, RC1, and RC2, reaction coils; HC, holding coil; SP, syringe pump; P, peristaltic pump; SV, selection valve; D, detector; W, waste.

In a typical analytical cycle, pre-defined volumes of sample and reagents are aspirated in an exact and reproducible way through the appropriate ports of the selection valve and positioned into the holding coil. By reversing the direction of the carrier stream, the zones of the sample and reagents are overlapped due to the radial and axial dispersion processes. When this overlapping zone is directed to the detector, a transient signal is obtained of a magnitude proportional to the concentration of the species (3,19,20).

Monolithic columns

In the last years, many researchers have been focused on the development of new materials, which are used in HPLC columns. One important direction of this research area is the development of monolithic columns as new separative tools used in HPLC.

Historically, Nakanishi et al. synthesized the first porous silica monolith material based on the simultaneous hydrolysis and condensation of tetramethoxysilane (TMOS) in the presence of polyethylene oxide (PEO) under acidic conditions (21). Up to now, several research articles (22–24) and reviews

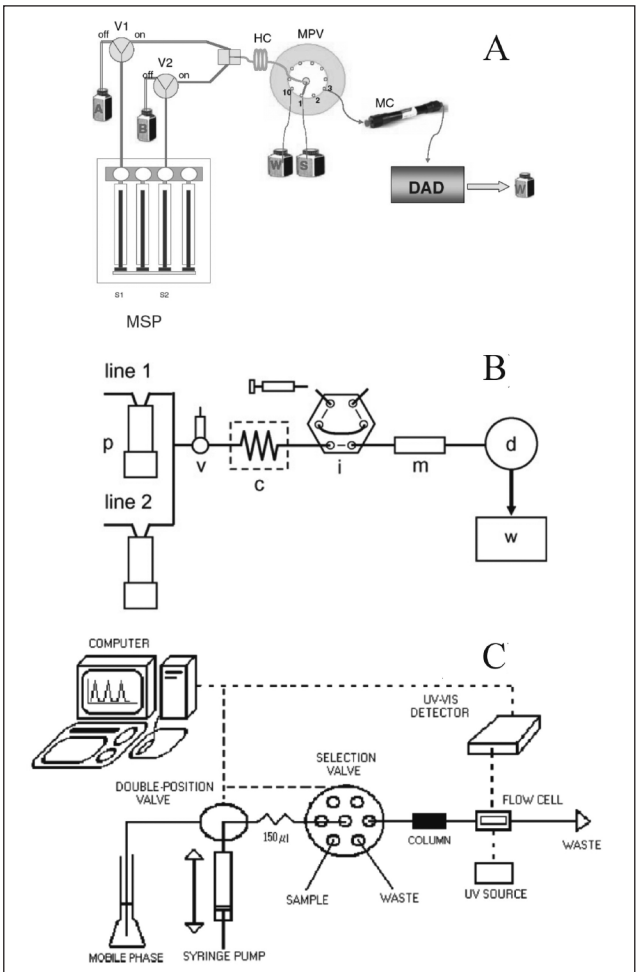

Figure 2. A scheme of MSFIA (A) (29), hybrid FIA/HPLC (B) (30), and SIC (C) (10) instrumentation, where: V, V1, and V2, valves; P, pump; MSP, multisyringe pump; HC, holding coil; C, coil; MPV, multi-position valve; m or MC, monolithic column; d or DAD, diode-array detector; W, waste; S, sample; i, injection valve.

(25–27) have been reported regarding monoliths preparation and its applications.

Monoliths provide favorable properties for high-efficiency fast separations, such as low-pressure drop across the column, fast mass transfer kinetics, and a high binding capacity. The main characteristic of monolithic columns is that they allow higher flow rates than particulate columns at reasonable backpressures. The most important features of these materials are the high porosity resulting from the network of macropores and the structure of the stationary phase skeleton (28). These two structural characteristics permit the combination of a low resistance of the column to the mobile phase and an enhancement of the mass transfer rate of the analyte molecules through the material. These unique features of monolithic columns enabled their incorporation in low/moderate pressure setups, such as FI and SI, dramatically expanding their possibilities.

Flow and sequential injection chromatography

The concept of flow injection chromatography was first introduced by Miguel et al. using multi-syringe flow injection analysis (MSFIA) coupled with a short monolithic column for the separation of β-lactam antibiotics (29). A typical MSFIA-column setup consisted of a multi-syringe module, three solenoid valves and a two-way connector, reaction coils, a multiposition selection valve, and a monolithic column (Figure 2A). The "marriage" of MSFIA instrumentation with monolithic column provides an outstanding tool to perform separations with low consumption of organic solvents without using an HPLC setup. Another approach has been recently reported by Adcock et al. who inserted the idea of hybrid FIA/HPLC to perform gradient elution on a monolithic column (30). A typical configuration of FIA/HPLC setup includes two MilliGAT pumps directly connected with a six-port injection valve, monolithic column, and a detector (Figure 2B). The gradient elution program was achieved by alteration of the flow of each pump.

Sequential injection chromatography SIC has been developed by Huclova et al. in 2003 (10). These researchers used a commercially available SIA manifold (FIAlab 3000) directly connected to a 25-mm long monolithic column prior to UV detector (Figure 2C). From an instrumentation point of view, SIC seems to be advantages over MSFIA and FIA/HPLC because it uses less instrumental parts. However, one of the main drawbacks of the SIC technique is its inability to perform gradient elution.

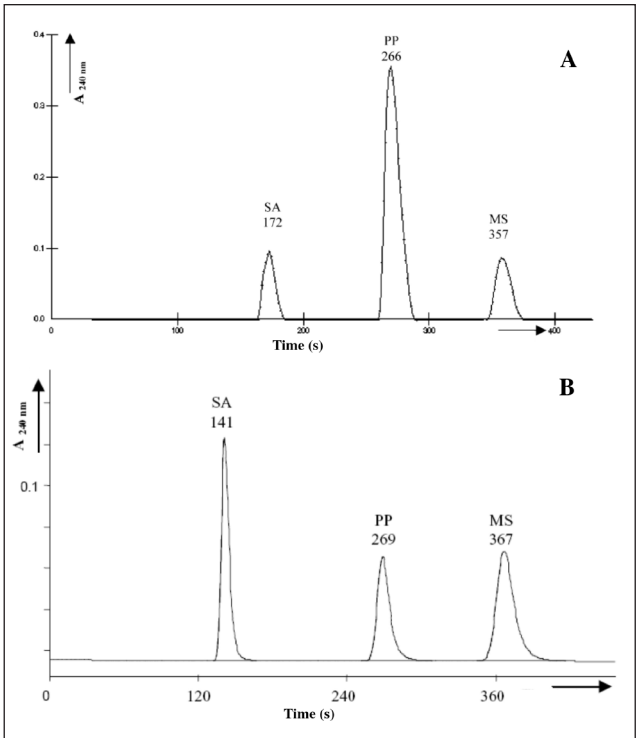

Figure 3. Typical chromatograms of the SIC (A) and HPLC (B) analysis of standard solution of salicylic acid (SA), propylparaben (PP), and methylsalicylate (MS) (10).

Applications

Sequential injection chromatography

The first report on coupling SI to monolithic columns for low pressure separation appeared on 2003 by Huclova and coworkers, which also introduced the concept of sequential injection chromatography (10). In that study, they reported the simultaneous separation/determination of salicylic acid and methylsalicylate using propylparaben as internal standard. Detection was carried out spectrophotometrically at 240 nm. An SI setup similar to that depicted in Figure 2C was used. The sample injection volume was 10 µL, and the mobile phase (35:65, v/v acetonitrile–water, pH = 2.5) was propelled through the monolithic column (50 × 4.6 mm i.d.) at a flow rate of 10 µL/s (0.6 mL/min) using a 10-mL syringe pump. Under these conditions, the analytes and internal standard were eluted in less than 7 min. Typical SI and HPLC chromatograms under the same running conditions are comparatively presented in Figure 3. The analytical figures of merit and chromatographic parameters of the developed assay were adequate for the quality control of pharmaceutical formulations.

The same research group used a shorter monolithic column (25 × 4.6 mm i.d.) to demonstrate its ability to separate four different compounds [methylparaben and propylparaben (preservatives), triamcinolone acetonide (active ingredient) and ketoprofen (internal standard)] in a cream formulation under SI conditions (31). UV detection was used, while separation was completed in ca. 6 min at a flow rate of 0.6 mL/min. The resolution factors of the peaks were higher than 3.9 and the number of theoretical plates higher than 2000 (except for methylparaben).

The same short monolithic column as above (Chromolith Flash RP-18e) was applied by Satinsky et al. to the separation of a similar mixture of pharmaceutical compounds by SIC (32). The mixture consisted of methyl- and propylparaben (preservatives), sodium diclofenac (active ingredient), and butylparaben (internal standard). An interesting feature of this study was the application of a flow rate gradient in the range of 8–20 µL/s, in order to accelerate the separation cycle. The flow gradient was performed sequentially in steps by increas-

ing the flow rate after each analyte elution. Under these conditions, separation of all four compounds was completed in less than 8 min.

Coupling of SI to monolithic stationary phases was also applied to the simultaneous determination of ambroxol hydrochloride and doxycycline in pharmaceuticals (33). Separation was carried out at with a Chromolith Flash RP-18e short monolithic column at a flow rate of 8 µL/s (0.48 mL/min) using a 5-mL syringe pump. The active pharmaceutical ingredients and the internal standard (ethylparaben) were detected in the low UV range (213 nm) within less than 9 min. The resolution factor was higher than 3 in all cases, while the number of theoretical plates was satisfactory. The analytical figures of merit (linearity, detection limit, precision, and repeatability) were adequate for the routine quality control of pharmaceutical formulations.

The same research group expanded its research on the determination of ambroxol hydrochloride by developing an SIC approach for the separation of ambroxol, methylparaben, and benzoic acid (34). The latter two compounds are preservatives that often co-exist with the active ingredient in the pharmaceutical formulations. Salicylic acid was used as the internal standard. The conducted experiments proved that the short monolithic columns reported in previous studies (e.g., Chromolith Flash RP-18e and Chromolith Speed RP-18e) provided insufficient efficiency for the separation of the four compounds. For this reason, the authors improved the resolution power of the Chromolith Speed RP-18e column by connecting it to a 10 × 4.6 mm i.d. monolithic pre-column. In this way, the 60 × 4.6 mm i.d. column provided adequate separation of the analytes with a resolution factor of higher than 1.7 in all cases. According to the authors, it was not possible to use combinations that resulted in longer columns [e.g. (50 + 25) mm × 4.6 mm i.d.] due to backpressure limitations. A mobile phase flow rate of 8 µL/s (0.48 mL/min) enabled completion of the analysis cycle in less than 11 min. The assay was applied successfully to the quality control of commercially available syrups and drops.

Another interesting application of sequential injection chromatography was reported recently by Klimundova and coworkers (35). SIC was coupled to a Franz cell in order to test the in vitro release of semi-solid dosage forms. A schematic representation of the automated setup is depicted in Figure 4. The developed approach enabled the simultaneous determination of lidocaine and prilocaine that were released from a topical pharmaceutical formulation over a period of 4 h, using automated sampling in 10.5 min intervals. The analysis cycle of the two active ingredients and the internal standard (trimecaine) was completed in less than 7 min. Such a combination was presented for the first time, while no human control was needed during the tests.

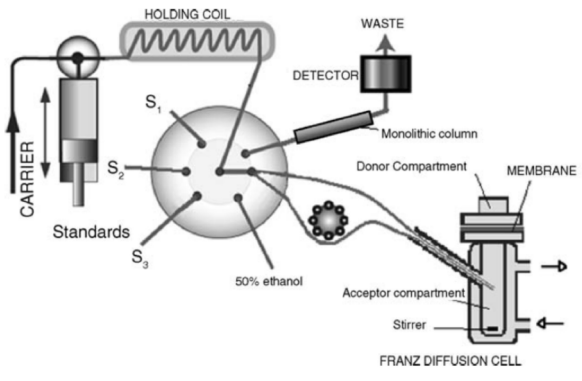

Figure 4. The scheme of SIC system with Franz cell for release test measurement (35).

Zacharis and co-workers coupled SI with polymeric-based monolithic material in the form of strong anion exchanger discs [convective interaction media (CIM)] to study drug– protein interactions (36). The separation principle was based on the affinity of such material to bio-macromolecules. On this basis, bovine serum albumin (BSA) was strongly retained on the monolithic strong anion exchanger while the free form of the drug was eluted and detected by fluorescence or simple UV. The protocol was applied to the study of the binding of ciprofloxacin, a potent antibiotic, under equilibrium and non-equilibrium conditions. Phosphate buffer saline was used as carrier/mobile phase, while 200 µL of 1 mol/L NaCl was used in order to elute the retained amount of protein after each analysis cycle (Figure 5). The obtained results (protein free fraction, binding constant) were evaluated versus data from the literature and by comparison to experimental data derived from ultra-filtration experiments.

Continuing their work on the application of SI to the study of drug–protein interactions, Zacharis et al. developed a sequential injection affinity chromatography system by immobilizing bovine serum albumin on epoxy monolithic discs (CIM) (37). Naproxen, a non-steroidal anti-inflammatory drug, was selected as a model compound. The principle of this study was based on the delivery/propulsion of a fixed volume of a naproxen (NAP) solution to the BSA-functionalized monolithic disk and subsequently the sensitive determination of the non-bound fraction of NAP by measuring its native fluorescence. The binding of NAP on BSA was investigated at various

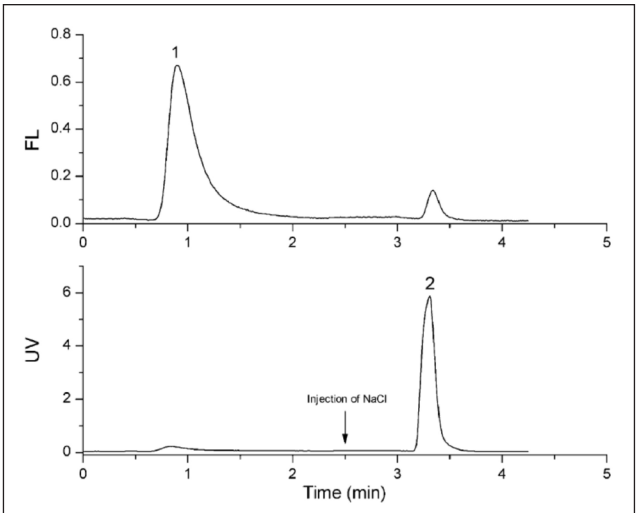

Figure 5. Chromatogram obtained from a mixture of ciprofloxacin and bovine serum albumin. Peak 1 corresponds to non-retained ciprofloxacin and peak 2 corresponds to the pulsed elution of the retained BSA using 1M NaCl (36).

temperatures and in varying incubation times. Affinity constants, the number of affinity sites, and thermodynamic parameters were determined using frontal analysis.

Another application of SIC to pharmaceutical analysis involves the separation and simultaneous determination of naphazoline and methylparaben using a short Flash RP-18e monolithic column (38). By employing a diode array detector (DAD), it was possible to achieve maximum sensitivity by monitoring the active ingredient at 220 nm and the preservative (methylparaben) and internal standard (ethylparaben) at 256 nm. The analysis time was less than 4 min, while the chromatographic resolution between peaks was higher than 4.0 in all cases. The overall analysis time was reduced, as only dilution of the nasal samples was required prior to injection in the SIC system.

In a similar manner, Satinsky et al. reported a sequential injection chromatographic assay for the simultaneous determination of chloramphenicol and betamethazone in pharmaceutical eye drops (39). Separation of the active ingredients and internal standard (propylparaben) through the 25 × 4.6 mm i.d. column was achieved in less than 8 min at a flow rate of 8 µL/s. Efficient separation of the analytes was confirmed by the values of the resolution factors that were higher than 2.1 in all cases. UV detection was performed at two wavelengths (i.e., 241 and 278 nm, absorption maxima of betamethasone and chloramphenicol, respectively).

Onyx monolithic columns are commercially available alternatives to the widely used Chromolith, although manufactured using the same patented technology. The first application of SIC using Onyx monoliths (50 × 4.6 mm i.d.) was reported to determine salicylic acid and triamcinolone acetonide simultaneously in pharmaceutical formulations (40). Under the optimal experimental conditions, the chromatographic separation of the two analytes and the internal standard was completed within 5.1 min with a resolution better than 4.5.

A shorter monolithic column by the same manufacturer (Onyx 25 × 4.6 mm i.d.) was also used in a more advanced study carried out by the group of V. Cerda (41). The authors coupled

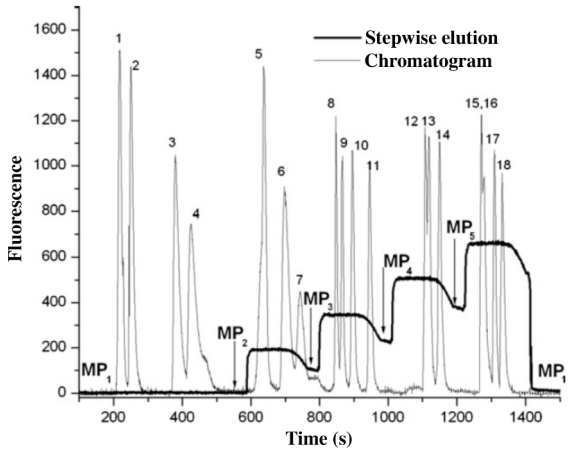

Figure 6. Separation of mixture of 18 amino acid under stepwise elution with SIC setup. Chromatographic peaks are as follows: Asp, 1; Glu, 2; Asn, 3; Ser, 4; Gln, 5; Gly, 6; Thr, 7; Ctr, 8; Arg, 9; Ala, 10; Tyr, 11; Met, 12; Val, 13; Phe, 14; Ile, 15; Leu, 16; Orn, 17; and Lys, 18 (44).

SIC to second-order multivariate regression models based on multivariate curve resolution-altering least squares in order to improve the resolution efficiency. In this way they achieved efficient separations of complex matrices in ultra-fast isocratic mode regardless of peak overlapping. Another interesting feature of the proposed protocol is the ability to carry out the analysis at elevated flow rates compared with previously-reported SIC methods. This was accomplished by removing the three-way solenoid valve from the head of the syringe pump and replacing it with a simple two-way connector. This modification enabled flow rates up to 3.0 mL/min with tolerable back-pressures. In terms of applications, a mixture of five phenolic compounds was selected, and the applicability of the procedure was proved by analyzing environmental water samples.

Another interesting protocol by the same research group involved the application of a step-wise gradient elution scheme in order to separate three vitamins (B1, B6, and B12) (42). Gradient elution allows the expansion of SIC to the analysis of more complicated samples, including mixtures of analytes with significantly different chromatographic behavior. This goal was accomplished by the programmable use of two syringe pumps for the delivery of two separate mobile phases. Mobile phase A (aqueous buffer) was propelled by the first syringe pump for a defined period of time (0–2.4 min), then by switching the three-way solenoid valves, mobile phase B (aqueous buffer–MeOH, 80:20) was propelled by the second syringe pump for the rest of the separation cycle (2.4–6.4 min). A flow rate of 0.5 mL/min enabled operation at low back-pressures while the gradient elution scheme provided improved peak shape and efficient separation of the vitamins in less than 10 min.

The concept of stepwise elution in SIC was also explored by Masini et al. in the analysis of complex mixtures of amino acids (43). In that approach, only a single syringe pump was used. The necessary mobile phases were aspirated through respective ports of the multi-position valve. An additional unique feature of the developed method was the on-line derivatization of the amino-acids by the well-known OPA reaction. The derivatization reaction was also carried out in the SIC setup prior to separation through the monolithic column. A total number of 18 amino acids were separated through the five-step elution program. The total analysis time was ca. 25 min, while a typical chromatogram can be seen in Figure 6. Detection was performed fluorimetrically at λex = 340 nm / λem = 450 nm. Compared to the previously mentioned approach (42), a potential advantage is that the step-wise elution is carried out using only one syringe pump. However, this mode of operation involves a discontinuous operation were the separation is interrupted periodically in order to fill the syringe with the various mobile phases.

Finally, Chocholous and co-workers reported the application of a typical SIC approach to the determination of two pesticides, namely fenoxycarb and permethrin (44). An interesting feature of this work is the adaptation of a flow rate gradient approach in order to speed-up the separation. Fenoxycarb was, therefore, eluted at a flow rate of 0.6 mL/min and permethrin at a flow rate of 1.2 mL/min. This approach enabled completion of the separation cycle within 6.5 min. UV detection at 225 nm was selected in all cases. The proposed method was

applied to the analysis of the pesticides in veterinary pharmaceutical foams and sprays. An overview of SIC applications was given in Table I.

Flow injection chromatography

There are fewer reports on coupling flow injection to monolithic-based columns compared to SIC. However, these reports do not lack novelty or innovation.

The group of Capitan-Vallvey used an ultra-short monolithic column to separate antioxidants, preservatives, and sweeteners in food samples under flow injection conditions (45). Under the selected experimental conditions they managed to simultaneously determine aspartame (AS), acesulfame (AK) / saccharin (SA), methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BP), propylgallate (PG), and butylhydroxyanysole (BA) using a single-channeled FI manifold. The mobile phases were propelled by an Ismatec gear pump at a flow rate of 3.5 mL/min. A volume of 125 µL of the samples was injected in the carrier stream using a PTFE low-pressure injection valve. Due to the different polarities of

Table I. Applications of Sequential Injection Chromatography

Analytes	Analytical column	Detection	Mobile phase	Flow rate (mL/min)	Application	Ref
Salicylic acid, methyl salicylate	Chromolith SpeedRod (50 × 4.6 mm i.d.)	UV at 240 nm	ACN–water (35:65) pH = 2.5	0.6	Pharmaceuticals	(10)
Methylparaben, propylparaben, triamcinolone acetonide	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 243 nm	ACN–MeOH–water (35:5:65), + 0.05 % nonylamine pH = 2.5	0.6	Pharmaceuticals	(31)
Methylparaben, propylparaben, diclofenac	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 275 nm	ACN–water (40:70) + 0.05 % triethylamine, pH = 2.5	0.48 – 1.2	Pharmaceuticals	(32)
Ambroxol hydrochloride, doxycycline	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 213 nm	ACN–water (20:90), pH = 2.5	0.48	Pharmaceuticals	(33)
Ambroxol hydrochloride, methylparaben, benzoic acid	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 245 nm	ACN–THF–0.05M CH_3COOH (10:10:90), pH = 3.75	0.48	Pharmaceuticals	(34)
Lidocaine, prilocaine	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 212 nm	ACN–0.05M phosphate (40:80) + 0.01 % triethylamine	0.6	In vitro release test	(35)
Ciprofloxacin	Strong anion exchange CIM disks	FL at λ_{ex} = 300 / λ_{em} = 460 nm	Phosphate buffer saline (PBS)	1.8	Drug-protein binding	(36)
Naproxen	Epoxy CIM disks	FL at λ_{ex} = 230 / λ_{em} = 350 nm	Phosphate buffer saline (PBS)	0.6	Drug-protein interactions	(37)
Naphazoline nitrate, methylparaben	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 220 and 256 nm	MeOH–water (40:65), pH = 5.2	0.9	Pharmaceuticals	(38)
Betamethasone, chloramphenicol	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV at 241 and 278 nm	ACN–water (30:80)	0.48	Pharmaceuticals	(39)
Triamcinolone acetonide, salicylic acid	Onyx C18 (50 × 4.6 mm i.d.)	UV at 240 nm	ACN–water (35/65)	0.9	Pharmaceuticals	(40)
Phenolic compounds	Onyx C18 (25 × 4.6 mm i.d.)	UV–vis at 210–400 nm	ACN–phosphate buffer (60/40), pH = 3.0	2.0	Environmental waters	(41)
Vitamins B1, B6, and B12	Chromolith FlashRod (25 × 4.6 mm i.d.)	UV–vis at 280:325:360 nm	0–2.4 min: CH_3COONH_4 (pH=7.0) > 2.4 min: CH_3COONH_4-MeOH	0.5	Pharmaceuticals	(42)
Free amino acids	Chromolith FlashRod (25 × 4.6 mm i.d.)	FL at λ_{ex} = 340 / λ_{ex} = 450 nm	Stepwise gradient elution	0.6	Green algae Tetraselmis gracilis	(43)
Fenoxycarb, permethrin	Chromolith RP-18 (10 × 4.6 mm i.d.)	UV at 225 nm	ACN–water (60:40)	0.6 & 1.2	Veterinary pharmaceuticals	(44)

the analytes, the simultaneous separation was accomplished by employing two mobile phases; ACN–phosphate buffer (4:96, v/v) in the range of 0–200 s and MeOH–water (30:70 v/v) in the range of 200–400 s. Detection was carried out spectrophotometrically using a photo-diode-array (PDA) detector.

Gradient elution is a valuable tool in HPLC for the efficient separation of complex mixtures of compounds with different polarities. All previously reported flow and sequential injection chromatographic methods that employed gradient elution were based on the formation of stepwise gradients by changing the mobile phases at pre-set time intervals. Formation of linear gradient profiles was investigated only recently by Adcock et al. (30). The authors combined two milliGAT pumps under precise computer-controlled operation conditions. The flow injection manifolds are shown in Figure 2B. The milliGAT pumps were delivering different solutions and by simultaneously accelerating one pump and decelerating the other, they achieved the generation of concentration gradients in a simple flow manifold. The reproducibility of these concentration gradients was confirmed by visual comparison of the profiles generated by combining acetone (100 mM, line 1) and water (line 2) and monitoring the absorbance of the solution at 235 nm. A variety of concentration gradients were produced by simply altering software settings, such as initial and final ratio of solutions and the total gradient time. In order to avoid destruction of the pumps due to overpressure, a pressure relief valve (100 psi) was introduced between the pumps and the injection valve. Additionally, a cooling coil was placed after the pressure relief valve in order to lower the temperature of the mobile phase prior to injection. The developed hybrid FI/HPLC system was applied to the analysis of opiate alkaloids and biogenic amines in biological samples using chemiluminescence detection.

An alternative approach to carry out medium-pressure separations using monolithic columns is by multi-syringe-flow injection analysis (MSFIA) (29). This technique combines the advantages of both FI and SI. The basic element of MSFIA is a multisyringe burette, which allows the simultaneous movement of four syringes. These syringes are connected in block to the same stepper motor. The three-way solenoid valve placed on the head of each syringe increases the flexibility of the technique and reduces sample and reagent consumption since reagents are injected into the system only when necessary. A schematic diagram of such a setup is depicted in Figure 2A. The chromatographic performance of the system was evaluated by a standard procedure involving the separation of a mixture of anthracene and thiourea using a mobile phase consisting of ACN–water (60:40, v/v) at a flow rate of 2 mL/min. Additionally, the authors developed methods for the determination of amoxicillin, ampicillin, and cephalexin using isocratic elution.

The same research group expanded the possibilities and applications of MSFIA by incorporating an automated solid-phase extraction (SPE) step prior to separation of the analytes through the monolithic column (25 × 4.6 mm i.d.) (46). By using a cation-SR sorbent material they managed to pre-concentrate hydrochlorothiazide and losartan from spiked water samples. Using 1-mL samples and detection at 226 nm, the achieved detection limits were 70 and 90 µg/L, respectively, and the recoveries from real samples were in the range of 95–118%.

Conclusions

In this review article, the application of chromatographic principles in SIA and FIA was described and discussed. The application of monolithic columns in the field of chromatographic separation has gained increasing interest during the last few years due to the distinct features of porous monolithic material (e.g., low-pressure drop, fast separation, fast mass transfer kinetics, high binding capacity). The coupling of monolithic columns to SIA and FIA manifolds is a promising area with the aim of performing separation in low-pressure mode using simpler and inexpensive instrumentation compared with HPLC. These techniques were proved to be an alternative to HPLC separation tools used in chemical analysis with satisfactory results.

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