Commentary on Advances in the Hyphenation of Flow Analysis Techniques with Liquid Separations for Pharmaceutical Analysis

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COMMENTARY

This commentary critically discusses the main advances in the field of flow analysis techniques hyphenated with liquid separations for pharmaceutical analysis over the last 15 years. Flow analysis techniques provide a convenient way to perform chemical assays with significant benefits in terms of rapidity, simplicity and cost of instrumentation, economy, precision, versatility in sample handling and potential for automation. The first-generation flow injection analysis (FIA), developed in the 1970’s, has been supplemented by a host of second-generation and third-generation flow methods developed in the 1990’s and 2000’s such as sequential injection analysis (SIA) and multi-syringe flow injection analysis (MSFIA) [1]. However, the main drawback of these techniques is their limited ability to simultaneously determine several analytes. Therefore, the hyphenation of flow techniques with separation techniques is a very attractive because it enables multi-component analysis. It was the introduction of monolithic columns [2] that has made possible the direct coupling of flow techniques with liquid separations platforms and has led to the development of hyphenated approaches such as FIC, SIC and MSC [3-5]. In all these techniques, a separation column is inserted in the flow path between the point of sample introduction and the detector; the main principles of operation can be found in the relevant literature [3-7]. FIC, SIC and MSC offer distinct advantages in terms of rapidity, simplicity, versatility and capital costs compared with high performance liquid chromatography (HPLC) and have been applied to the determination of a large number of pharmaceuticals [3-5]. When simple mixtures are considered, these hyphenated methods produce results that are comparable to HPLC [8,9]. The main advances in the hyphenation of flow analysis techniques with liquid separations for the purposes of pharmaceutical analysis are identified below.

From low-pressure to medium-pressure separations

The choice and matching of the appropriate components is an important prerequisite for coupling flow analysis techniques to separation columns. Traditional flow analysis techniques normally operate at low pressures (<100 psi) and, therefore, pressure problems are rarely encountered. Since the introduction of a separation column in the flow manifold causes an increase in the backpressure, the most critical aspect is the pressure specifications of the components to be used. FIC makes use of peristaltic pumps or the low-pressure variant of version of the MilliGAT pump which are limited to backpressure of around 100 psi [10-12]. Similarly MSC systems make use of low-pressure solenoid valves that also pose some limitations on the pressure that could be achieved [13]. Early work in SIC typically made use of medium-pressure syringe pumps which can withstand substantially higher back pressures (up to 500-700 psi) such as the commercial discontinued FIAlab® 3000 analyzer (which was modified in-house for SIC applications) which was superseded by the dedicated (also discontinued) SICHrom™ system (FIAlab Instruments, USA) [14]. Even higher pressures (up to 2000 psi) can be achieved with industrial syringe pumps recently introduced in SIC [15] or the new high-pressure version of the MilliGAT pump (rated at 1500 psi) as an alternative option [16]. Of course, the use of higher pressures also poses limitations on the valves used in the manifold; for instance the low-pressure variants of the widely used the Vivi-Valco injection and selection valves can withstand only up to 250 psi and switching to a higher pressure system would require replacement of the valve to one rated for higher pressures (e.g. suitable for HPLC tolerant to 5000 psi). This ultimate development of such a medium-pressure system is typified by the current commercial SICHromII™ system (FIAlab Instruments, USA) [17]. The ability of the manifold to handle higher pressures affords significant advantages as it enables the use of potentially longer and more densely packed columns (to enhance the separation efficiency) and higher flow rates (to speed up the analysis). However, high-pressure components involve substantially higher purchasing costs and one has to consider the relative benefit-to-cost ratio with regards to the intended application.

Separation columns

The backpressure limitations discussed above, especially in relation to the early-generation low-pressure systems, also impose some limitations in terms of the type and length of the separation column. Therefore, initially applications were limited to C18 reverse-phase monolithic columns, typically 5-25 mm long. The introduction of medium-pressure systems allowed the use of longer (e.g. 50 mm) monolithic columns [18]. More recently, short fused-core columns
with dimensions 5-30 mm have been successfully implemented in medium-pressure chromatographic systems [19,20]. In addition, other packings (such as a cyano 5 mm long monolithic column for sub-1 min separation of dyes in pharmaceuticals) [21] and RP-amide, F5 and phenyl-hexyl materials [19] have been introduced. An interesting approach is the use of two-columns of different length, each operating in an isotropic separation mode but with different compositions of mobile phase [22].

**Development of gradient elution protocols**

The majority of hyphenated flow analysis-separations systems in the literature make use of isotropic elution protocols. However, when the target analytes exhibit a wide variation in polarity, isotropic elution becomes inefficient. A step change of the eluent is the most widely applicable and easiest method to modulate the elution force of the mobile phase and involves sequential elution of the analytes with two or more separate solvents of different polarity [23,24]. Another alternative is the generation of a gradient profile in the holding coil of a SIC manifold by aspirating zones of different solvents with different polarities; the stacked zones are then directed to the column and cause the elution of the analytes in order of varying polarity [25]. Obviously, in this case the gradient profile is not step-like since the zones of the solvents undergo mutual dispersion and mixing. Recently, our group proposed a powerful method for the generation of a strictly linear two-solvent gradient elution profile by appropriate flow rate modulation of 2 pumps delivering solvent of different polarities [11].

**On-line sample pretreatment**

One of the main advantages of flow analysis methods is the exploitation of zone fluidics for on-line sample pretreatment. Essentially the same protocols can be adapted to hyphenated SIC and MSC systems for simple filtration and dilution [26] or more complicated operations such as solid-phase extraction using packed minicolumns [27-29] or the head of the separation column [30].

**Automation and computer control**

FIC manifolds with isocratic elution (which involve continuous and unidirectional flow of the mobile flow) do not normally require the use of computer control since both the pump and the injection valve can be manually controlled [12]. In this sense, these systems are uncomplicated, relatively inexpensive, simple to operate and also suitable for educational use but, on the other hand, their potential for automation is limited. Of course, when complex gradient elution protocols are required in FIC, the use of computer-controlled pumps and valves, as well as the relevant software, becomes necessary, resulting in fully automated FIC manifolds [11]. The introduction of SIC and MSC has also necessitated the use of computer control for solution handling; the controlling computer can also exploit for data acquisition. This trend has resulted in the development of hyphenated chromatography systems using commercial [17] or proprietary control and data acquisition software [11,13].

To summarize, in this commentary the key advances, advantages and limitations of FIC, MSC and SIC have been identified and the author's opinion that hyphenation of flow analysis techniques with liquid separations offers wide scope in pharmaceutical analysis applications and these systems can serve successfully as complimentary to HPLC for both separation and quantification purposes.

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