Bead injection with a simple flow-injection system: an economical alternative for trace analysis

S. Kradtap Hartwell, G.D. Christian, K. Grudpan

The use of beads for sample pretreatment, such as preconcentration, isolation, and separation, and for accommodation of chemical reactions has attracted increasing interest. Beads can be used as a removable solid surface in many analytical applications. It is very practical to use beads in a flow-based system, where beads can easily be moved in and out of the system using a flowing stream of reagent(s). This is called the bead-injection (BI) technique, which was first introduced to be used with a sophisticated computer-controlled sequential injection analysis (SIA) system. In many laboratories, a relatively high-cost SIA system is not available, whereas a simple, low-cost, first-generation flow-injection (FI) analysis system is easy to assemble. The development and applications of the BI technique coupled with a lower cost first-generation FI technique is then an alternative. This simple BI–FI system can be a useful tool for pretreatment of samples that are abundant and when there is no need for micro volume control.

S. Kradtap Hartwell*, K. Grudpan*
Department of Chemistry, Faculty of Science and Institute of Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand

G.D. Christian,
Department of Chemistry, University of Washington, P.O. Box 351700, Seattle, WA 98195-1700, USA

*Corresponding authors.
E-mail: kradtas@yahoo.com (S. K. Hartwell); kate@chiangmai.ac.th (K. Grudpan)

1. Introduction

Sample pretreatment is often a necessary step in analysis of real samples. Depending on the matrices and the sensitivity of the detection system, the type and the degree of sample pretreatment required will be different. In the flow-injection (FI) technique, continuous flow of solutions in a closed system enables on line sample pretreatment. Even though digestion, unlike other sample pretreatment methods, normally requires heating or harsh conditions, on-line sample digestion is possible with different techniques, such as microwave assisted digestion [1,2], stopped flow digestion [3,4] and on-line destruction of samples using radiation [5], oxidizing agents [6,7] and heat [8]. In some cases (e.g., when using a detection technique that has a relatively narrow dynamic range such as atomic absorption spectroscopy (AAS)), dilution of sample is actually needed prior to passing it to the detector. Since concentrations of samples are unknown, batch-wise dilution by trial and error before performing the analysis becomes very inconvenient. On-line dilution using the FI technique, either by sample dispersion [9] or by flow manipulation [10], helps avoid some of the tedious steps encountered in batch processes. In some cases, dilution can also be useful in extending the calibration working range, such as in the FI dialysis-ion chromatographic system (FID-IC) [11]. More commonly, preconcentration and preseparation are needed for samples with trace amounts of analyte, especially when the detector does not have extremely high sensitivity. Among the many preconcentration techniques available (e.g., column [12–17], precipitation [18,19] and dialysis [20,21]), the use of a column packed with materials that can selectively adsorb or absorb the analyte is probably the most widely employed [22]. The column technique allows the use of a single standard for construction of a calibration graph by varying the loading time [16,17]. This single standard calibration technique provides various flexibilities in operation, including eliminating or reducing prearation of a set of standard solutions, use of glassware and time consumption. Isolation and separation are other processes needed for samples that comprise complex matrices that can interfere in the analysis. Conventional solvent extraction requires large amounts of solvent and produces a lot of waste, and flow-based-column solid phase extraction systems are more efficient [23,24]. Extraction of sample can be carried out in a packed column of sorbant material [25], similar to the preconcentration column.

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However, there is a drawback of the packed column in that the surfaces of the sorbant material need to be regenerated after each analysis. It is very important to get rid of memory effects to ensure accuracy and precision. Even though it is useful to reuse the same column over again and again, the washing step is normally extensive (i.e. time consuming) and may require a strong washing solution [26,27], or, in many cases, a suitable washing solution is simply not available, so a replaceable solid surface becomes interesting in many studies [28]. Microbead particles have been used in drug discovery for a long time. Now that there are many different functionalized beads commercially available together with various detection techniques, the applications of beads for preconcentration and isolation of analyte arise [29–37]. For example, the use of beads in bead-based immunoassays can be accomplished using very small diameter beads (i.e. less than 10 μm) and in different forms, such as in a small-size droplet with microelectrodes [38,39] and in a flow system, such as flow cytometry [40] and spectrophotometry [41]. Size, material of beads and modification of functional groups on the bead surface can be selected according to the applications. In systems where direct measurement of analyte is done directly on the bead surface, the properties of the bead material become very important. Beads should not interfere in the analysis in any way (e.g., beads should not scatter or absorb light in the region where analyte is detected, and they should not be decomposed or have their properties changed in the solvent).

2. Bead injection

The bead-injection (BI) technique is the combination of the use of beads with a flowing stream of solution in a FI system. Beads are utilized as solid surfaces to preconcentrate or extract the analyte or to accommodate a chemical reaction. The flowing stream of solution is used to carry beads through the system. There is no need to regenerate the bead surfaces because they are discarded after each use and are replaced by fresh ones. This helps to reduce the risk of contamination, denaturation, and system fouling, and also makes it possible to operate BI in the continuous flow system even if no suitable eluent for bead cleaning is found possible [42].

Early BI techniques were coupled with the sequential injection analysis (SIA) technique [43]. Beads were retained in a specially designed jet ring cell that let only the solution pass through. SIA was introduced in 1990 by Ruzicka and Marshall [44]. The main features of the system are that it could precisely manipulate forward and reverse flow with micro-volumes of samples and reagents, so the sample and reagent flow path could be minimized between the injector and the detector with appropriate mixing. This is possible by using a multi-port valve and a bi-directional pump with computer control. Sample and reagent zones are drawn and stacked into a holding coil. Zones are penetrated and dispersed into each other by reverse flow on the way to the detector. SIA has an increasingly wide range of applications, such as trace chemical determinations, pharmaceutical studies and food chemistry [45–49]. There are two different forms normally employed. Micro-beads may first be trapped in a flow cell or column and then physically or chemically retain the analyte of interest. Upon eluting with a suitable solvent, the solution of the analyte is detected downstream. There are examples of applications with this form of detection using ETAAS [50,51]. Another form is on-bead detection, where the change on the surface of the beads after interacting with the analyte is monitored directly using the detector located at the flow cell, or by transporting the beads into the detector. There are a number of examples of this form of detection using fiber-optic spectrofluorometry [52], fluorescence microscopy [49], ETAAS [53] and Raman spectroscopy [54].

The SIA system has also been further developed into the lab-on-valve (LOV) system that comprises specially designed micro-channels on a piece of plastic integrated as a part of the multi-channel valve [55–57]. The BI technique can be coupled to the SIA–LOV system and can be used in various applications, such as environmental studies [58], immunoassays [59], and biological studies [60]. Beads can be trapped in an adjustable volume channel on the LOV. Now, a commercially available LOV can be adjusted into two different volumes or path-lengths by moving the optical fiber [61]. Detection can be done directly on the beads with the optical fibers that are made to fit the channel or can be done downstream via the eluted sample. The main advantage of the SIA–LOV–BI system is that the micro-miniaturization of on-line sample pretreatment and detection can be done automatically with various types of detectors [62–64].

3. Bead-injection–flow-injection system

In spite of the outstanding advantages of the SIA system, some laboratories cannot afford such relatively high-cost instrumentation, so a simpler FI technique is desirable. The development of a BI–FI system can be very useful for both general and trace analysis where samples and reagents are abundant and when there is no need of micro-volume control. Assays of some contents of environmental samples, foods and beverages, of which high quantities of samples are available, can be made using a simple FI system.

The BI–FI system can be constructed using common materials that can be found in the laboratory. A simple, cost-effective configuration of the cell and simple,

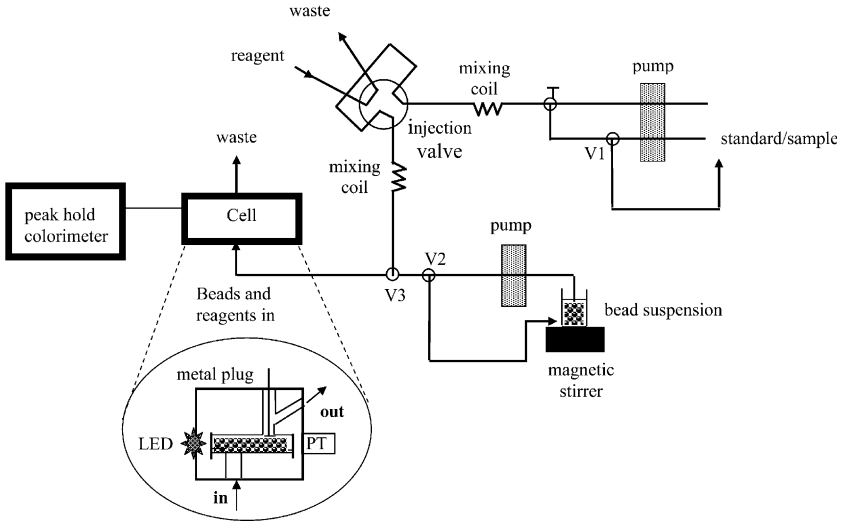

Figure 1. Bead injection with flow-injection system, adapted from Jitmanee *et al.* [65] (V1, V2 and V3 are three-way valves; and, T is a three-way connector).

unidirectional pumps can be used. The cell has the multi-purposes of bead retention, accommodation of chemical reaction (sorption for on-line preconcentration and/or separation and color development) and detection.

Our group has developed an FI–BI system [65], which is depicted in Fig. 1. The bead suspension is pumped into the cell and beads are retained while solution goes through the small space around the metal plug at the end of the cell. When a sample is passed through the cell, an analyte of interest, followed by a color reagent, is loaded onto the surface of the beads, and a color complex is formed. A suitable-wavelength LED and a phototransistor located opposite each other can monitor the intensity of the color complex directly from the surface of the beads. After each run, the used beads can be flushed out by moving the metal plug backward to open the outlet for the beads. The new analytical cycle can then be started. A time-based, single calibration can also be used with this system. There is no need for computer software control. It is also simple to operate and not expensive. The system design and the detector unit can be varied, depending on what components are available.

4. Examples of applications

To demonstrate the application of the BI–FI system, iron was first chosen as a model trace analyte to be used with the system shown in Fig. 1. Chelex-100 resin was used as solid phase and 1,10-phenanthroline was the color reagent used to form a red complex with Fe^{2+} that can be detected at 520 nm. The iron content of various types of water samples (tap, pond and drinking) were successfully determined with the proposed system. The lowest detectable concentrations of Fe^{2+} were 0.90 and 0.45 μmol/L for 3 and 5 min sample loading times, respectively. Both Fe^{2+} and Fe^{3+} were spiked for recovery studies. Fe^{3+} was reduced to Fe^{2+} with ascorbic acid before analysis. Total iron was found as 100–110% recoveries [65].

The application of the system was also extended to determine iron in a complicated sample, such as beer [42]. Even though an interference effect from tannic acid in beer was found, the results from the proposed BI–FI system agreed in all cases with the results obtained from the AOAC standard batch FAAS method and the FI–AAS within the tolerance limit of beer analysis when standard addition was performed.

The sampling rate was 10–20 analyses per hour, depending on sample loading time. The detection limit can be lowered by increasing sample loading time with, however, sacrifice of sample throughput. A precision of 4% RSD was achieved.

Another model trace element, chosen to express sensitivity and selectivity of the system, was copper. In the presence of other elements and matrices, determination of copper in swimming pool water and mineral supplement tablets was achieved with this system [66].

The FI–BI technique can be applied to analyze other ions in different samples by selecting suitable surface modified beads, color reagents and buffers [65–67].

Currently, the number of works published on FI–BI is far fewer than on SIA–BI. The main reason is possibly because SIA–BI was developed with high degree of automation and many applications were demonstrated by the developers to confirm the extended use of a commercial SIA system. However, the FI–BI system also

has potential as an alternative for versatile use in sample pretreatment and as a form of detection, similar to the SIA–BI system. The simpler, lower cost FI–BI system, having no fully automated computer control system, can be advantageous for many laboratories that do not have an SIA system. FI–BI should therefore be used more in the future, and many BI applications described above should be readily adapted to this simple system.

5. Important criteria

In the BI technique, the uniformity and the homogeneity of beads is very important for good precision. Beads should be sieved for suitable sizes and the bead suspension should be kept stirred at a fixed rate at all times of the analysis. The quantity of beads loaded into the cell or the bead loading time need to be optimized in line with the dimension of the cell. Excess beads outside the detection window will adsorb sample and reagent but cannot be detected, and that can yield false negative results, but too few beads cause space in the cell where light scattering may occur or color intensity measurement may not be effective. The concentration of the color reagent also needs to be sufficient for the range of sample concentrations. Bead- and sample-loading times for each analysis should be constant. Other important general guidelines for system design and operation involving the use of column sorption should be followed [22] (e.g., delivering sample by pumping it into the column located down stream is recommended instead of suction of the sample through the column). Tubing connecting from the pumps should be as short as possible to save reagents and to minimize the possibility of sample and reagent sorption on the tubing wall. Flow reversal should be considered to avoid tightening the column packing that may cause back pressure.

6. Conclusion

The BI technique can be used with the first-generation FI technique for successful trace-ion determination. The system is simple and economical without the need of computer technology. Contamination of samples from outside environment and from a previous run is very minute because it is a closed system, with a bead-discarding step done before a new analysis. It is suitable for the analysis of samples that are abundant and do not require micro volume control, such as environmental, food and beverage samples. The applications of the system can be extended using the suitable combination of surface-functionalized beads, buffer solutions and color reagents.

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