Novel Detector Cells for Flow Injection and Sequential Injection Analysis in Process and Biotechnology Measurements*

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Abstract

Novel detector/reactor cells have been developed for the measurement of soluble, gaseous and particulate analytes. The universal sandwich membrane cell is a versatile detector cell that can be used for absorbance and chemiluminescence measurements. Its unique design minimizes refractive index effects, and it can be used for extraction, gaseous diffusion, and kinetic measurements. The microvolumetric cell can be used for sequential stepwise reactions in a complex chemical system involving multiple reagents for sequential injection analysis. And the fountain cell provides the ability to perform precise perfusion measurements on living cells. The principles of these three flow cells are summarized and representative applications presented.

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The application of flow injection techniques in industrial and biotechnology processes requires detection systems that can operate reliably over wide concentration ranges and in a variety of measurement modes. Our laboratory has developed a variety of detector/reactor cells for the measurement of soluble, gaseous and particulate analytes. These include a universal optical detection cell, a microvolumetric flow cell for performing multiple chemical reactions, and a fountain cell for novel perfusion measurements on living cells. These flow cells are reviewed here, with some representative applications.

Universal Sandwich Membrane Cell. The sandwich cell (1) combines fiber optics, separation membranes, donor and acceptor flow channels, spacers, and reflecting surfaces into a sandwich design sensing unit. The membrane acts as a reflecting surface to perform absorbance measurements by reflecting transmitted light from a bifurcated optic cable back through the flowing solution to a detector. The optical path can be varied by means of the number of spacers. Chemiluminescent measurements can be performed using a single fiber optic cable to collect the light. A dialysis or gas diffusion separation step may be carried out using a suitable membrane by passing the sample (donor) solution on the side opposite the fiber optic cable and receiving the diffused species on the acceptor stream side of the membrane. The cell design minimizes refractive index effects, as clearly demonstrated by Dasgupta et al. (2), and air entrapment is also minimized. The utility of the cell was demonstrated by determination of Co(II) by chemiluminescence, in which the Co(II) sample was injected on the acceptor stream side and the luminol/hydrogen peroxide reagents were merged and flowed on the donor side (1). Hypochlorite was determined by gas diffusion-chemiluminescence by pumping luminol through the acceptor side and injecting the sample into HCl solution on the donor side. Ammonia was determined by gas diffusion by

injecting NH_4Cl solution into NaOH donor carrier stream, with a bromthymol blue acceptor stream; the flow of both streams was stopped in the cell, to record a diffusion-rate curve. Phenol was determined using the chemistry previously described (3), by either continuously pumping the aqueous phenol solution through the donor side or injecting it, and pumping the reagents through the acceptor side; the flow is stopped in the cell and the increase in absorbance with time is recorded. Trace phenols may be determined in kerosene samples by using a silicone rubber membrane for extraction -preconcentration (3). This cell has been used to measure volatile analytes such as ammonia and carbon dioxide by gas diffusion, including ammonia in a gas stream (4).

Microvolumetric Flow Cell. The technique of sequential injection analysis (SIA) is a single line system that is more rugged and reliable for process analyses (5, 6). However, the number of chemical reagents or reactions that may be employed is restricted. This limitation may be eliminated by utilizing a microreaction chamber to perform sequential reactions (7). A 750 μl volume flow cell is used, which incorporates a small magnetic stirring bar. Multiple reagents may be injected in sequence into the cell, allowed to react with the sample, and then propelled to the detector for measurement. The microreactor cell may actually serve as the detector by utilizing fiber optic cables mounted opposite one another to transmit light through the cell and collect it for the spectrometer. Such an arrangement has been used for the real time turbidimetric measurement of biomass in a fermentation process (8). An injected aliquot of the fermentation sample flows directly into the cell; automatic exponential dilution occurs as it flows through and exits the cell, allowing selection of the measurement time for optimal measurement. Precisions equal to or better than those obtained using a manual gravimetric method are achieved.

Fountain Flow Cell. The fountain cell utilizes a radial flow path to precisely control flow characteristics (9). The cell consists of a circular Teflon base plate with a central inlet port, surrounded by a ring-shaped outlet well with a drain hole. It is enclosed with a top circular microscope slide cover glass, with a small spacer defining the thickness of the radial flow path. The injected sample enters from the bottom and radiates in a circular pattern from the central inlet hole. The cell was designed as a perfusion chamber for the fluorescence microscopy study of living cells under very reproducible conditions (10). Cells may be perfused with a reagent with a reproducibility of 1% r.s.d.

The fountain cell has been modified to retain suspended microparticles in the optical path (a fiber optic cable is mounted over the inlet hole, or a microscope objective is focused on the inlet hole, where the particles are trapped for detecting via reflectance or luminescence) (11). The particles may be removed after monitoring and replaced with a new batch, for renewable reaction surfaces.

These three flow cell/detector systems provide a way for performing nearly all optical measurements, both absorbance/reflectance and luminescence, on a variety of sample types with optimal control of flow conditions with high precision.

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