A microcomputer system for the detection, integration and area apportionment of g.l.c. elution profiles

IAN G. GILES
Department of Biochemistry, University of Southampton, Southampton SO9 3TU, U.K.

The problems associated with collection and handling of data from liquid and gas chromatography are well documented (Fozard et al., 1972; Taylor & Davies, 1973; Fox & Wilkinson, 1976; Schomberg et al., 1972). The advent of low-cost microcomputers and analog-to-digital converters (ADC) now make these attractive alternatives to the dedicated hardwired electronic integrator. There are many problems associated with performing area apportionment in real-time, in that the setting of preset values is critical. Furthermore the existence of shoulders on a larger main peak very often confuse the algorithms used. This is especially true if the shoulder occurs on the leading edge of the main peak. The whole process becomes much simpler if the integration and area apportionment algorithms have the entire elution profile at their disposal. It is then just as simple to look forward in time from the current point on the profile, to see what is going to happen, as it is to look back to see what has already happened. No real-time system has this flexibility.

Programs to perform the integration of peaks from an amino acid analyser have previously been developed in our laboratory (Giles & Gore, 1983; Gore & Giles, 1983). The problems associated with data acquisition from this system (long separation times, wide peaks and two wavelengths to monitor) are somewhat different to the problems associated with g.i.c. and h.p.l.c. (short separation times, narrow peaks and sloping baselines). Consequently the previous programs have been reworked so as to be suitable for use with g.l.c.

The microcomputer used is the Apple IIe (or Apple II+/e). The programs are comprised of several compiled BASIC units, and machine code interfaces to the 12-bit ADC (conversion time 20μs) and real-time clock (resolution 1 ms) used. It was decided to use compiled BASIC to optimize the performance of the combined g.l.c.-microcomputer system. Compiled BASIC will run up to 10 times faster than the normal interpreted BASIC.

The system used comprises several autonomous subroutines. The first of these is the data collection routine. This operates by sampling the voltage from the g.l.c. detector as fast as possible over a 0.5s time window. The average value is then used as representative of the elution profile at that point. This box-car averaging technique serves to improve the signal-to-noise level of the electronic signal and to preserve information about the shape of the elution profile. Each point is already a partial integral of the entire curve.

This is particularly important when sharp peaks occur, as otherwise shape distortion can be introduced. Using compiled BASIC 150-170 samples can be initiated within each 0.5s window, and a 25min elution profile represented by 3000 averaged points.

The second major subroutine used is the integration and area apportionment routine. This operates on both the original data and the slope of the profile at each point. The procedures search for a peak start (looking ahead of the current point to preserve area information) and once found the area is accumulated using the trapezoid rule. This area accumulation continues until either a return to baseline occurs, or another peak follows in a multuplet. In the latter case the area accumulation continues, but in another integrator, until the baseline is found. That is the perpendicular drop method is used within a multuplet. Once it has been decided that a return to baseline has occurred, the area corresponding to that below the baseline is subtracted from that accumulated to give the true area of the peak. The program continues looking for peaks and integrating them until the end of the profile is reached. The data is then output in terms of retention time and peak area. A typical 25min g.l.c. separation is integrated in 10-12s depending on the number and nature of the peaks present. The elution profile, together with the regions integrated, can be viewed on request.

An alternative method of area apportionment is offered which the user can select if a shoulder peak occurs. This employs the tangential skimming algorithm (Fozard et al., 1972). As the entire profile is available the skimming can be applied to shoulders on both the leading and the trailing edges of a major peak. The difference in areas obtained by this method and the perpendicular drop method is added to the area of the major peak. Peaks that emerge near to the solvent front can be treated as shoulders, and valid areas obtained.

The programs have been in use in our laboratories for over 2 years and they have proved to be reliable and easy to use.