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EVALUATION OF SOME VOLUMETERS FOR LIQUID CHROMATOGRAPHY

T. MACKO

Polymer Institute, CCR, Slovak Academy of Sciences, 84236 Bratislava (Czechoslovakia)

O. CHIANTORE

Istituto di Chimica Macromolecolare, Università di Torino, 10125 Turin (Italy)

and

D. BEREK*

Polymer Institute, CCR, Slovak Academy of Sciences, 84236 Bratislava (Czechoslovakia)

SUMMARY

　　The reliability, precision and repeatibility of various siphon systems, drop counters and thermopulse capillary flowmeters were evaluated. Relationships were found between the particular volumeter response (dumpings of a siphon, number of drops or thermopulses), the true volume of liquid determined by weighing and the following operational variables: nature or composition of liquid, its flow-rate and temperature, as well as the spacial configuration or position of the volumeter. The response of each volumeter depended on at least two of the parameters studied. All devices yielded only relative values of the volume of the liquid, and calibrations were necessary to obtain absolute data. The repeatibility of measurement decreased in the series: thermopulse volumeter, cell volume 10 μl > drop counter > siphon systems > thermopulse volumeter, cell volume = 100 μl, being ± 0.14, ± 0.24, ± 0.45 and $\pm 1.16\%$, respectively.

INTRODUCTION

　　Precise and reliable mesurement of small volumes of flowing liquids is necessary in many areas of science and technology. The volume of liquid required to elute a sample component from a column is an important parameter in analytical liquid chromatography (LC). Knowledge of elution volumes in interactive LC is necessary for the identification of sample components, for the evaluation of LC columns and for quantitative treatment of separations based on peak areas. On the other hand, the retention volumes, V_e, together with the concentration profiles of polymers in the column effluent represent the basic data for calculation of the molar mass and molar mass distribution of macromolecular substances by means of gel permeation chromatography (GPC). It was shown that even small errors in V_e values influence substantially the precision of GPC results[1–3].

　　The pumping systems applied in low- and medium-pressure liquid chromato-

graphy often generate unstable flow-rates if the separation times are long (several hours), possibly due to the construction of pumps with large-volume piston chambers and damping systems. Therefore, it is necessary to measure the volume of effluent continuously. This is usually done by means of siphon systems. The electromagnetic[4] and mechanical flowmeters widely used in many fields of technology as well as procedures based on measuring the extent of cooling of a heated thermistor, immersed into a flowing liquid[5], have not yet been applied to LC. Evidently, such devices would be unable to measure precisely small flow-rates or their response would be influenced significantly by the nature of the liquid. The weighing of an effluent yields fairly precise data, but it is difficult to avoid the evaporation of eluent from a continuously operating instrument[6]. Moreover, instrumentation for automatic effluent weighing is not commercially available, and its construction seems to be too complicated for ordinary analytical laboratories.

In conventional GPC, siphon systems are widely used, the construction of which is simple and can be improvised in any laboratory. Each siphon dump activates a photoelectric circuit and the resulting electrical impulses can easily be counted and applied, *e.g.*, for the automation of multiple sample injections[7-9].

With the development of high-speed, high-performance and miniaturized liquid chromatographs, the demands on the precision of elution volume determination have increased. Most authors suppose that modern pumping systems generate strictly constant flow-rates and that it is sufficient to rely on the time-scale. However, it has often been observed, *cf., e.g.*, ref. 10, that due to either random or systematic changes in eluent viscosity, partial blocking of column end fittings or changes in the structure of the sorbent bed, the flow-rate can change, as a consequence of the compressibility of liquids even in advanced reciprocating pumps provided with feedback systems.

For these reasons, many attempts have been made to develop techniques for precise and reliable control and measurement of elution volumes in LC. In addition to the classical methods mentioned, the application of internal standards[11-13], improved drop-counter devices[14] and capillary volumeters[15,16] have been tested. However, the peaks produced by internal standards may interfere with the peaks of the sample components. If the retention volumes of the internal standards are at the extremes of a chromatogram, variations in the flow-rate during elution may be mutually compensated and thereby remain undetected[3]. In capillary volumeters, liquid flows through a capillary in which either the pressure drop is measured or the number or pulses of liquid that flow through the capillary is counted. However, the well known principle used in pressure-drop measurements, the Darcy law, cannot be applied to liquids with changing composition. The counting of capillary volumes assumes appropriate sequencing of the flowing liquid. This can be done by injecting air-bubbles[15] or mercury drops[16] into a capillary. The air-bubble or mercury drop activates a photoelectric circuit at the end of the capillary and this triggers another air or mercury injection. However, the gas bubbles tend to dissolve in the eluent and the data obtained are strongly dependent on the flow-rate and pressure within the capillary. On the other hand, the use of mercury in the laboratory is hazardous. Recently, an interesting liquid-sequencing procedure was proposed[17-19] and the corresponding volumeters became commercially available from Knauer (Bad Homburg, F.R.G.) and Molytek (Pittsburgh, PA, U.S.A.). The liquid within the measuring

capillary is sequenced by short thermal pulses at the entrance of the capillary. The increase in temperature is detected at the end of the capillary, and the impulses are counted. Miller and co-workers[18,19] and Chamberlin and Tuinstra[3] tested such "thermopulse volumeters" and found them to be very promising for application in high-performance liquid chromatography (HPLC).

The aim of the present study was to compare three kinds of commonly used volumeters, namely siphon systems, drop counters and thermopulse capillary volumeters, with respect to their precision, reliability and repeatability, as well as to changes in operational parameters.

EXPERIMENTAL

Instruments

The following volumeters were tested: two capillary thermopulse volumeters (Knauer), namely the older model with a capillary volume of about 100 μl (referred to as Knauer I) and the newer model, Type 68,00, with a 10-μl capillary (Knauer II); drop counter Models DC 1001 and DC 1002 (Laboratory Instruments, Prague, Czechoslovakia) with various drop orifices; glass siphons of various sizes and shapes. Preliminary measurements have shown that the siphon shape used in the Waters GPC apparatus, Model 200 (Waters Assoc., Milford, MA, U.S.A.)[8] was least affected by the formation of air-bubbles in the outlet tubing during siphon dumping. These bubbles cause large variations in the volume of liquid released by a siphon. The siphons were provided with a thermostating glass jacket and with a device preventing the evaporation of liquid during siphon filling[20] (Fig. 1).
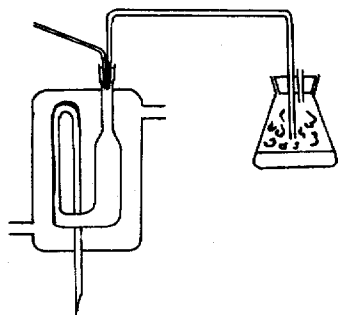

Fig. 1. Jacketed siphon with a device preventing the evaporation of liquid during siphon filling.

Reagents

All chemicals were obtained from Lachema (Brno, Czechoslovakia). Water was redistilled, tetrahydrofuran was distilled and stabilized by 0.01% of butylated *p*-cresol.

Method

The measured liquid was transported by a single-piston reciprocating membrane pump, VCM 300 (Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia), provided with a pulse dampener[21], or by a Siemens pumping system (LC chromatograph S100; Siemens, Karlsruhe, F.R.G.).

   The effluent from a LC column was collected in a vial and weighed with a precision of ±10⁻⁴ g. Whenever possible, measures were taken to prevent the evaporation of liquid during manipulation. Systematically, one dump of a siphon, 10 drops from drop counters and a volume corresponding to 100 or 400 thermopulses from capillary volumeters were considered. Prior to measurement, each liquid was thermostatted during passage through a LC column placed either in the column chamber of the S100 liquid chromatograph kept within ±0.5°C or in the water-bath kept within ±0.1°C.

RESULTS AND DISCUSSION

Effect of flow-rate and nature of the liquid
   The relationship between the true volume of liquid for a given number of events (siphon dumps, drops or thermopulses) and the flow-rate is illustrated for various liquids in Figs. 2–5.
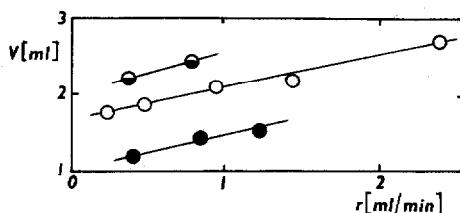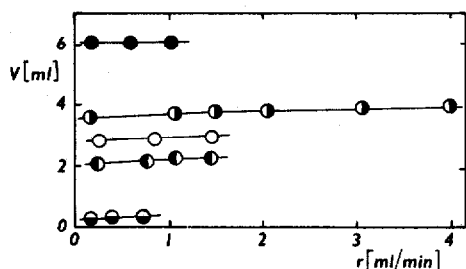

Fig. 2. Dependence of the true volume, V, of 100 drops of liquid on the flow-rate, r, for water (●), dimethyl formamide (◐), tetrahydrofuran (○), n-hexane (◐) and tribromomethane (◖).

Fig. 3. Dependence on the flow-rate of the true volume of liquids corresponding to then thermopulses in the Knauer I volumeter. Symbols as in Fig. 2.
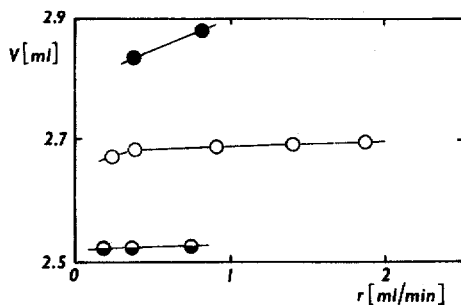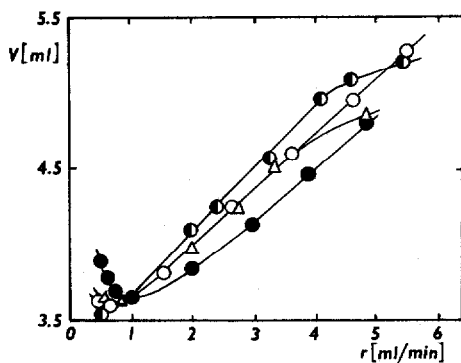

Fig. 4. Dependence of the true volume of liquids, corresponding to 400 thermopulses, on the flow-rate in the Knauer II volumeter. Symbols as in fig. 2 except for benzene (△).

Fig. 5. Dependence of the true volume of a siphon dump on the flow-rate of liquids. Symbols as in Fig. 2.

The effect of the nature of the liquid on the apparent volumes or the volumeter responses for drop counters and siphons was not unexpected. It is mainly a consequence of the differences in the surface tension, viscosity and density of the different liquids. Evidently, the larger the surface tension of a liquid, the larger are the drops in the drop counter and the larger is also the volume of liquid causing the siphon dump. Changes in the viscosity of liquids affect the time of the siphon dump and, consequently, the volume delivered, since liquid continues to fill the siphon while it is dumping. Changes in the density of liquids influence mainly the size of the drops in the drop counter.

The sensitivity of drop counters and siphon systems to the nature of the liquid can seriously affect the precision of measurements, not only when the composition of the eluent is altered systematically (gradient elution), but also due to the presence of samples. This is illustrated for a drop counter in Fig. 6, showing the influence of polymer samples present in the measured liquid on the mass of 100 drops.
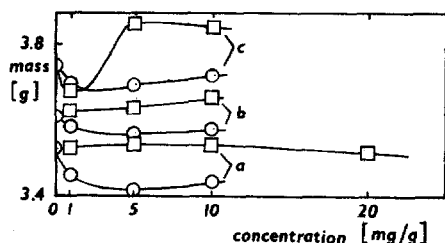

Fig. 6. Influence of polymer solutes in dimethylformamide on the mass of 100 drops at a flow-rate of 1 ml/min (a), 2 ml/min (b) and 4 ml/min (c). O, Polystyrene; □, polymethacrylate.

The sensitivity of the thermopulse volumeters to the nature of the liquid, although lower than in the case of siphons and drop counters, is somewhat surprising. It is probably brought about by the differences in both the specific heat and the thermal conductivity of the liquids and, consequently, by the differences in the rate of heat conduction, i.e., in the width of the heated zone. The rate of heat conduction is also influenced by the temperature difference between the heated zone and the surrounding cold liquid, i.e., it depends on the period of heating. The above effects are suppressed in the Knauer II device, where the volume of the measuring capillary was decreased and the measuring electronics was improved. With regard to the parabolic flow profile within the measuring capillary and to possible disturbances in the flow patterns, the position of the thermistor may also be important. In the case of Knauer II, the thermistor is located on the circumference of the capillary, while it is placed on the axis of the capillary in the Molytek[18] thermopulse volumeters. With the latter volumeter, Hoffman and Miller[19] observed an influence of the dissolved polymer in the measured liquid on the response.

The responses of all volumeters depend on the flow-rate; however, the thermopulse volumeters are the most sensitive to this parameter. For example, a ten-fold rise in the flow-rate increases the difference between the true and apparent measured volume of tetrahydrofuran in the following way: 50% for Knauer I, 40% for Knauer II, 10% for the drop counter and 1.7% for the siphon. This means that thermopulse

volumeters can be used for corrections of flow-rates only within a narrow range of flow-rates. Moreover, they do not produce reliable results at flow-rates lower than about 0.5 ml/min. This may be caused by the increasing role of counter-current axial diffusion of liquids at very low flow-rates and precludes the use of the existing thermopulse volumeters for microbore and capillary HPLC columns.

        Of the volumeters tested, only the drop counter can be used for measurements at very flow-rates, provided the evaporation of eluents from the surface of the drops is prevented.

Effect of temperature

        Except for the siphon, the tested volumeters cannot be thermostatted. The volume of tetrahydrofuran pumped at a rate 1 ml/min through a heated column was measured with all volumeters at ambient temperature and with the siphons also at the column temperature. Water was measured only with the Knauer II volumeter. The results are summarized in Fig. 7.
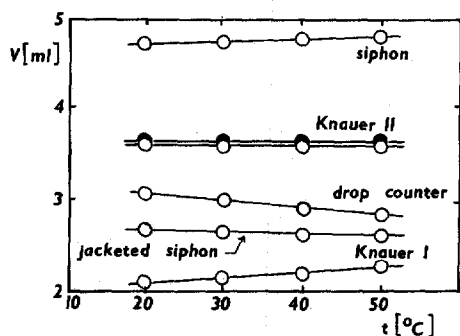

Fig. 7. Effect of temperature on the true volume corresponding to 1 siphon, 400 thermopulses (Knauer II volumeter), 100 drops, 1 thermostatted siphon and 10 thermopulses (Knauer I volumeter).

        The apparent volume of liquid measured by both the drop counters and the siphons decreases by about 7 and 1%, respectively, when the temperature is increased from 20 to 60°C. This is due to the decrease in density, surface tension and viscosity with temperature.

        In a non-thermostatted siphon filled with liquid, the extent of the decrease in temperature is a function of both the flow-rate and ambient temperature. The actual temperature of the liquid at the time of siphon dumping is not known. The changes in the true volume of the siphon due to the thermal expansion of glass are negligible.

        The Knauer I thermopulse volumeter showed a slight increase (about 1% within the given temperature range) in actual volume with temperature, probably as a result of the differential arrangement of the measurement. The amount of heat that causes the detectable increase in the temperature of a liquid is larger at higher absolute temperatures. Consequently, the thermopulses are detected somewhat later and the measured apparent volume is larger when the temperature of a liquid increases. The Knauer II volumeter was found to be fairly insensitive to temperature variations.

The actual temperature in the vicinity of the orifice of a drop counter was found to be about 6°C higher than the ambient temperature. This is due to the heat emitted by the light source. An equilibrium temperature is reached in about 2 h after the start of the measurements. Thus, the difference between the volumes measured at the beginning and at the time of equilibrium may be about 1% for tetrahydrofuran.

Other operational parameters

The volume of both the siphon dumps and the drops in a drop counter are a functon of the spatial position of the volumeter. This is important for the repeatability of their responses. Microbubbles of gases or vapours released from the liquids may cause serious errors in the volume measured by drop counters and thermopulse volumeters. The effect of bubbles is very large in drop counters. The bubbles adhere to the walls of the polytetrafluoroethylene orifice, and both the bubbles and the drops continuously change in size. The effect of air-bubbles on drop-counter results is illustrated in Fig. 8.
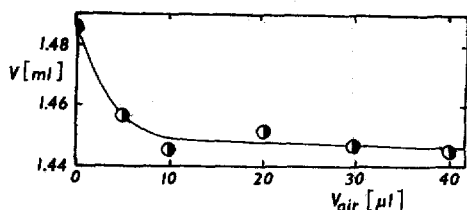

Fig. 8. Influence of the volume of air-bubbles, V_{air}, introduced into the drop counter orifice by a microsyringe, on the size of 40 drops of dimethylformamide at ambient temperature.

The reliability of siphon volumes depends chiefly on the wettability of the siphon walls by the liquid. Impurities, such as a film of grease on the walls of a siphon, may have dramatic effects on the measured volume, especially in the case of aqueous eluents. The size of the orifice of the drop counter has little influence on the precision of results.

Repeatability of measurements

The repeatability of volume measurements was tested with toluene and tetrahydrofuran at a flow-rate of *ca.* 1 ml/min at 20°C. The repeatability of ten independent measurements, expressed as a standard deviation, was ±1.16% for Knauer I, ±0.45% for the siphon, ±0.24% for the drop counter and ±0.14% for Knauer II.

CONCLUSIONS

(1) Because the volumeters tested yield only apparent volumes of liquids, calibration, *e.g.*, by weighing, is necessary if absolute values are to be obtained.

(2) All three types of volumeters tested are sensitive to the eluent composition and none of them can be used for volume measurements in gradient elution. Erroneous results must be expected also at higher sample concentrations in the column

effluent, especially in the case of siphons and drop counters and for surface-active or polymeric solutes.

(3) Particular volumeters are sensitive to different extents to variations in the operational parameters. The thermopulse capillary volumeter is the most sensitive to flow-rate changes and the least sensitive to changes in temperature and spacial position. The evaporation of liquid during operation is the most serious problem with drop counters.

(4) In their present state, the volumeters tested do not allow precise volume measurements at the extremely low flow-rates necessary for microbore HPLC columns.

(5) The Knauer II thermopulse volumeter showed the best repeatability under our experimental conditions and with the eluent usually employed in GPC, followed by the drop counter.

(6) Inevitably, further development is needed to construct highly precise and reliable volumeters with decreased sensitivity to changes in operational conditions.

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