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MULTI-COLUMN LIQUID CHROMATOGRAPHY

II. A MULTI-CUVETTE FOR MULTI-COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

A multi-cuvette for fast quantitation by spectrophotometry has been developed for use in liquid chromatography. Only glass and teflon is in contact with solvents and reagents.

INTRODUCTION

In our attempts to develop high-capacity multi-column systems for liquid chromatography we initially used methods in which quantitation by colorimetry was done by sucking samples into flow cells in a spectrophotometer or in colorimeters[1,2]. The limiting factor in obtaining high capacity was found to be the time necessary to move the sample from collection tube to spectrophotometer or colorimeter flow cell and to flush the previous sample out of the flow cell. A minimum of about 15 sec for sample transfer was found to limit capacity of the developed multi-column systems.

Seven years ago we started the development of cuvettes holding multiple samples as another approach to fast quantitation in multi-column chromatography. Since simple and fast transfer methods involving simultaneous transfer of many samples at a time to a multi-cuvette could fairly easily be developed such cuvettes held promise of greatly increasing capacity in multi-column chromatography. The current multi-cuvette design would seem of possible use to other chromatographers since it is adaptable to other approaches to collection and quantitation than the methodology developed by us.

DETAILS OF CONSTRUCTION

Our first designs were based on individual windows for each compartment of a multi-cuvette. Although feasible this proved a cumbersome design to work with. In particular the necessary occasional dismantling of the cuvette and cleaning of windows proved impractically time consuming. When the strong and flexible Chemcor™ glass from Corning Glass (Corning, N.Y., U.S.A.) became available we acquired some strips of this glass. Contrary to a number of other types of glass previously tried Chemcor was found to work without cracking in a simple design in which the glass was squeezed around a central cuvette section of teflon. Not only did the glass not crack initially but equally important the design was found to stand up in daily routine use.

The details of the construction are shown in Fig. 1. The central component is the forty-compartment cuvette section. This is machined from a piece of teflon™ (DuPont, Wilmington, Del., U.S.A.) 52 cm (20.5 in.) long, 25.4 mm (1 in.) wide and 31.75 mm (1.25 in.) in height. Each compartment is made by cutting a 25.4 mm (1 in.) deep by 4.8 mm (3/16 in.) wide groove almost the width of the teflon strip from the top and then cutting through with window openings of the same width from the sides. To prevent "creeping" between cuvette sections it is essential that the upper portion in each cuvette compartment has a solid teflon ridge across the window opening. Earlier designs with straight grooves in the teflon failed because of "creeping" of samples from one cuvette section to the adjoining compartments across the top of the cuvette compartments. The Chemcor glass strips used for windows were 52 cm (20.5 in.) long by 31.75 mm (1.25 in.) wide. The thickness of the glass varied. In earlier cuvettes we used the 6.4-mm (1/4-in.) thickness shown in Fig. 1. In cuvettes built recently the glass

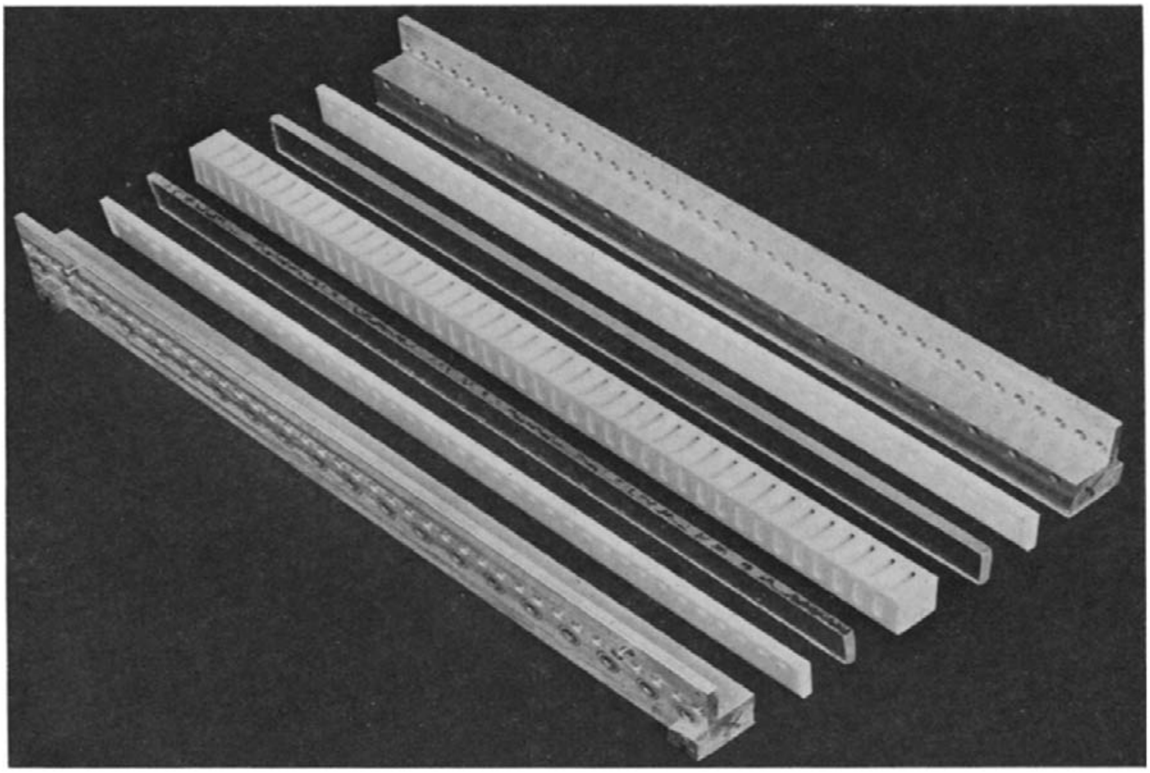

Fig. 1. The components of the multi-cuvette. A central grooved teflon strip serves as the cuvette proper and Chemcor glass windows are sandwiched between the central cuvette strip and outer teflon gaskets. The cuvette is bolted together in an outside aluminum frame.

has been the thinner 2.4-mm (3/32-in.) thick Chemcor currently the maximum thickness available from Corning. This thinner glass has been found of adequate strength in the design.

The glass strips are sandwiched between the central cuvette core and outside teflon strips with circular windows of somewhat larger diameter than the width of the groove drilled with the same intervals as the grooves. The central cuvette core, glass window strips and the teflon gaskets are bolted together in a outside holder machined from hard aluminum parts as shown in Fig. 2. The groove between the two halves of the aluminum holder allows the proper pressure to be exerted on the glass windows so that a non-leaking tight cuvette is obtained. The bolts in the cuvette are tightened using a constant torque wrench for the final adjustment so that even pressure is applied to all parts of the cuvette.

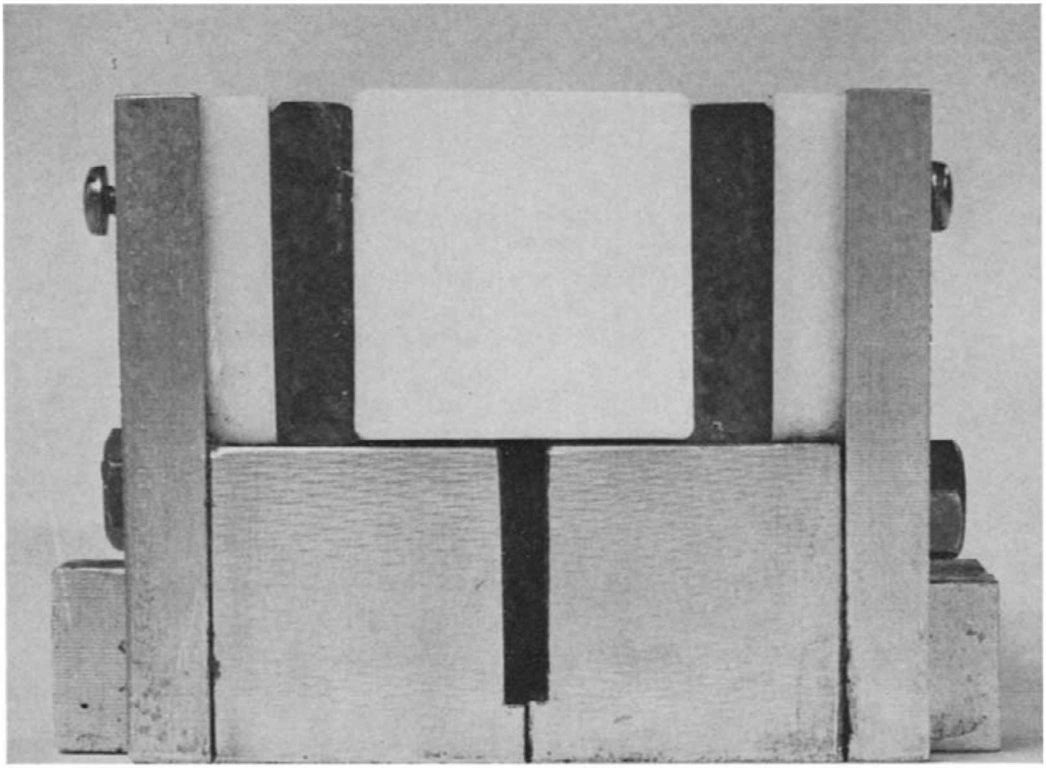

Fig. 2. End view of the cuvette assembly showing the sandwiching of the glass windows between central teflon cuvette strip and outer teflon gasket. A uniform and strong pressure is applied to the glass through the bolts slightly below the middle of the vertical aluminum plate.

PERFORMANCE

A number of multi-cuvettes have been in daily routine use in our laboratory for the last two years. They have stood up well to daily routine handling. No window has cracked which was our greatest worry when we started using this kind of cuvette.

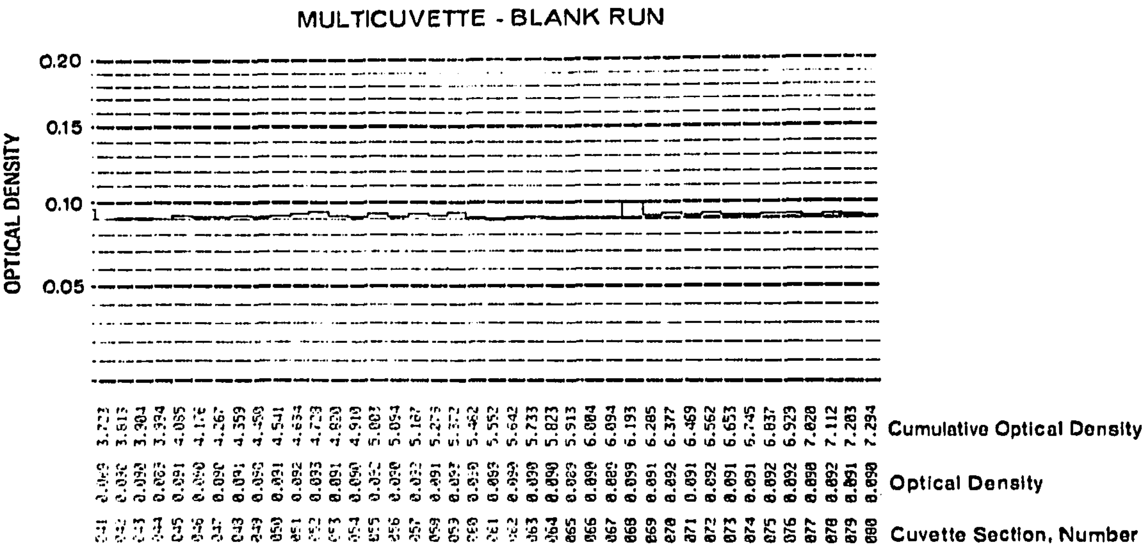

Fig. 3. "Blank" absorption in a multi-cuvette filled with distilled water.

The optical qualities of the cuvette have been investigated in a number of ways. Fig. 3 shows the absorption characteristics of a typical cuvette filled with distilled water in each section after more than one year of routine use. The absorption has been recorded in our quantitation system[3]. A calculation shows a standard deviation of the mean of 0.0017 optical density units for the absorbance readings for the forty sections of the cuvette as a measure of the variability from cuvette section to cuvette section. This is ordinarily quite satisfactory when the cuvette is used in routine chromatography. It is possible, however, when a minicomputer is part of the quantitation system to enter the absorbance of each section as an average value of for example five runs through the system with distilled water in the cuvette and program the computer to store and subtract such "blank" readings for each cuvette section when the cuvette is used in chromatography. It is also possible to increase reproducibility of readings by, in a similar way, doing repetitive runs and averaging for cuvettes when chromatograms are being quantitated in the system and subtracting "averaged blank" readings from "averaged sample" readings. As a check on the suitability of the cuvette for spectrophotometric assays the optical density of a number of cobalt ammonium sulfate solutions were run in the system. This chemical has been suggested by the National Bureau of Standards as a suitable standard in spectrophotometry to check on the linearity of absorbance in a spectrophotometric system[4]. The readings performed in a routine way without blank-cuvette correction are shown in Fig. 4. Complete adherance to Beer–Lambert's law over a wide concentration range is found. The sensitivity of the cuvette will depend on the absorptivity of the compounds under examination. The light path in the cuvette is 25.4 mm and the minimum volume necessary to fill each of the forty cuvette sections adequately for a reading is approximately 1 ml which will give a basis for a comparison with results obtained in other cuvette systems. For the steroids we have mostly been working with in routine applications of the cuvettes and using our modification of the Zimmermann color

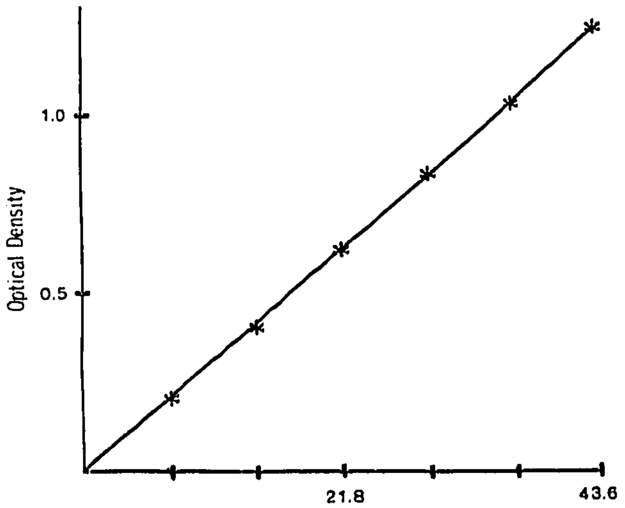

Fig. 4. Spectrophotometric evaluation of cuvette performance using cobalt ammonium sulfate in different concentrations recommended by the National Bureau of Standards to evaluate agreement with Beer–Lambert's law. Concentration *versus* absorbance at a wavelength of 512 nm.

reaction[5] we have found the lower level of detectability for individual steroids to be about 0.2 μg of steroid per cuvette section. It is quite possible that sensitivity can be improved in future designs of the cuvette using narrower and shallower grooves in the central cuvette section.

In daily use it is important that proper care be taken in the handling of the cuvette. After use and cleaning of the inside, a lid should be kept on the cuvette for storage between readings. We have constructed foam-rubber cushioned lids with teflon-film cover for this purpose. They hook easily onto the cuvette by attaching to the small screws at the ends of the vertical aluminum sections. The lids are also used generally to prevent dirt from getting into the cuvettes during readings and to prevent evaporation from the cuvette sections. The cuvettes should be stored in boxes between readings to protect them against outside dirt and dust getting to the windows. If left for longer periods of time a piece of adhesive paper is conveniently strapped temporarily over the row of holes in the outer aluminum plate. We have with intervals of approximately one month taken the cuvettes completely apart and thoroughly cleaned the glass windows with appropriate solvents. How frequently this has to be done depends on handling and procedures.

Although we have developed the multi-cuvette with the dimensions described as a part of a general system for multi-column chromatography it can be used to advantage also combined with other collection methods. We have for example poured samples after color reaction from our old test-tube collector-type collectors into the multi-cuvette for spectrophotometry. Transfer by fast-acting transfer pipettes commercially available from a number of firms makes it possible to fill a multi-cuvette in a few minutes from a row of tubes or other tube collectors. A multicuvette fitting in dimensions the snap-together tube collector available from Gilson (Middleton, Wisc., U.S.A.) could easily be constructed. We feel therefore that the multi-cuvette design may have wide applicability where spectrophotometric or colorimetric quantitation is planned in liquid chromatography.

ACKNOWLEDGEMENTS

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