vessels that can be demonstrated are 5 μm in diameter.

At present the equipment is being developed for in vivo dynamic recording of blood flow patterns through a variety of organs such as bone, kidney, intestine and skin. Previously this type of radiographic study has only been possible with high voltage X-ray generators and fine grained photographic emulsions[16]. The micro-focal X-ray unit allows fine structural detail to be recorded on relatively coarser grained emulsions and has the additional advantage that it produces less radiation damage to the tissues.

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## An Inexpensive Cuvet for Spectrophotometry of Samples in 5 μl Volumes

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*Summary.* A simple cuvet requiring about 5 μl of sample is described. Such a cuvet is easy to build and suitable for measurements in ordinary spectrophotometers. The measurements compare favourably in accuracy and reproducibility with those obtainable with standard macrocuvets.

The routine control of the quality and quantity of macromolecular preparations from biological samples quite often implies spectrophotometrical determinations.

In particular, this applies to ribonucleic acid, phenol extracted from tissue or cultured cells, which routinely is tested for purity by its UV-spectrum. Furthermore the RNA, if sufficiently pure, is evaluated quantitatively in terms of its optical density units at 260 nm. In those preparations where the RNA is further processed, e.g. fractionated by electrophoresis, these controls are usually performed by taking an aliquot of the final RNA solution and reading it after appropriate dilution in standard quartz cuvet usually requiring from 0.10 ml of sample (e.g. Zeiss MT 2).

In some cases, and particularly often in neurochemistry, one must deal with tissue samples in the milligram range resulting after extraction in a few microliters of RNA, at a concentration suitable for subsequent electrophoretic analysis[2,3]. In these cases, even with the use of commercially available but expensive micro-cuvets ranging down to working volumes of 20 μl (e.g. Zeiss MR 1 D), the UV-determinations mentioned above are hardly possible to perform.

In this report we describe how to build simply an inexpensive cuvet suitable for UV-spectrophotometry of samples in the range of 5 μl in the ordinary Zeiss PMQ II or PMQ 3 spectrophotometers.

The cuvet system consists of the cuvet proper and a brass casing, both being easily produced in the workshop of the laboratory (Figure 1). The cuvet proper is made from commercially available bored glass tubings (Chance Brothers Ltd Glassworks, Birmingham, England), which are cut at lengths slightly above the desired optical path-length. The cut ends are ground and polished to exact dimensions in a lathe on which a grinding disc is mounted to the chuck[4]. A small piece of quartz glass (manually cut) is glued onto one of the polished ends (low viscosity cyanoacrylate adhesive IS 03 Locktite, Dublin, Ireland). The brass casing consists of a socket in which the cuvet proper is placed and a screw on which another small quartz glass is fitted. This quartz glass forms the other window of the cuvet when socket and screw are joined. To compensate for possible errors in parallelism between the flat end of the screw and the polished end of the cuvet proper, a rubber packing ring is fitted between the screw and the quartz glass. Tightness of the cuvet is thus ensured.

Filling the cuvet is suitably made with the cuvet in vertical position, either within or outside the socket, by

Accuracy of readings at 260 nm in the MT 2 and in the microcuvet on repeated fillings (12 times) of the same RNA solution as in Figure 1, but diluted 1:1

| Cuvet | Mean absorbance | SD |
|---|---|---|
| MT 2 | 0.530 | 0.0016 |
| Microcuvet | 0.263 | 0.0026 |

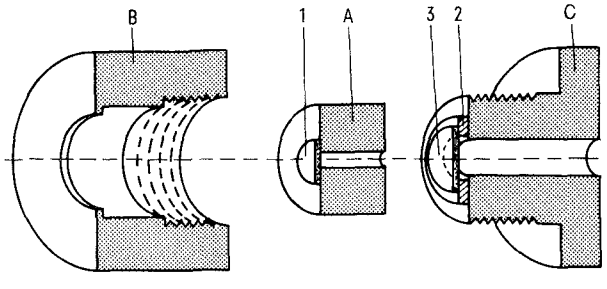


Fig. 1. Sectioned and exploded view of the cuvet and casing. A) Cuvet proper constituting a hollow conventional glass cylinder sealed at one end with a quartz glass [1]. External diameter 6.5 mm, internal diameter 1.0 mm, length 5.00 mm. B) Cuvet socket in brass with threading for knurled screw (C). External diameter 12 mm, internal diameter snuggly fitting the cuvet (A). Length 10 mm. The depth of the non-threaded part of the socket is smaller than the length of the cuvet proper so that the quartz glass of the knurled screw gets in touch with the cuvet when joined. C) Hollow knurled screw (bore appr. 2 mm in diameter) with glued rubber packing ring [2] onto which a quartz glass [3] is fitted.

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means of a capillary pipette brought to the bottom (glued quartz glass) to avoid trapping of air bubbles. The procedure is conveniently performed with the naked eye or under a low-power stereomicroscope. A small amount of excess fluid (~1 µl) above the rim of the cuvet prevents bubbletrapping when the screw is fastened to the socket. The risk of bubble formation is not serious even with detergent-containing media, e.g. SDS 0.5%.

The outer dimensions of the casing conform to the microcellhousing assessories (No. 507425) of the Zeiss PMQ II and PMQ 3 spectrophotometers, but may easily be adjusted for other instruments. The dimensions of the cuvet depicted in Figure 1 give a nominal volume of about 4 µl with an optical pathlength of 5 mm, but these dimensions can be arbitrarily changed by selecting different bored glass-tubings. Cleaning the cuvets is made by flushing the cavity with water by use of a hypodermic syringe.

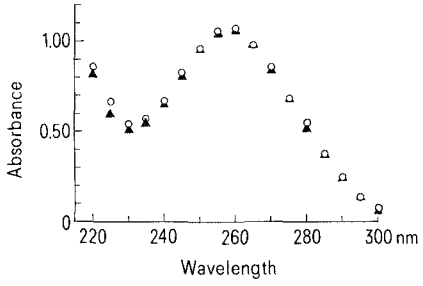


Fig. 2. UV-absorption spectrum of a preparation of rat brain microsomal RNA[5] as determined in a Zeiss quartz MT 2 cuvet (▲) and in the microcuvet described here (O). RNA was dissolved in 0.0036 *M* *Tris*-HCl, pH 7.6; 0.0034 *M* $NaH_2PO_4$; 0.0001 *M* EDTA; 10% sucrose. 150 and 5 µl of the solution were read respectively. The absorbance values for the microcuvet were corrected for the difference in pathlength by a factor 2.

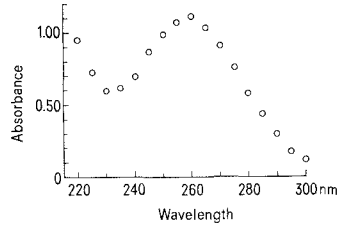


Fig. 3. UV-absorption spectrum of RNA extracted from a sample (about 5 mg) of the CA3 region of rat brain hippocampus as determined in the microcuvet. RNA was extracted according to a micromethod described previously[3] with the addition of a DNase treatment and a final ethanol precipitation. The RNA pellet was dissolved in 10 µl of sample buffer[2], 8 µl of the sample were taken for subsequent electrophoresis and a 2 µl aliquot was taken, diluted to 6 µl with the buffer and read in the microcuvet described.

We tested the accuracy of the measurements in this microcuvet by determining the UV-spectrum of microsomal RNA macroextracted from rat brain[5] both in an ordinary quartz cuvet (Zeiss MT 2) and in the cuvet described. In the first case we used 0.150 ml and in the second 5 µl of sample. The result, reported in Figure 2, shows an almost perfect agreement between the 2 sets of measurements with only slight differences at 220 and 225 nm. Moreover, readings after consecutive refillings indicate a high reproducibility of the measurements (Table).

As an example of application of the microcuvet, we report in Figure 3 the UV-spectrum of RNA extracted from a few milligrams of the CA3 region of rat brain hippocampus by a micro-method described previously[3]. This case is one of those where it would have been impossible to use standard cuvets for UV-absorbance measurements.

A microcuvet in the form of a capillary tube suitable for use in the Zeiss PMQ II microcuvet housing was described previously[6]. We think that the system described here presents two advantages, first the optical properties are accurate within a wider range of optical densities (Figure 1), second the mechanical build-up ensures a tight closure of the cuvet with no risk of bubble formation during prolonged measurements (e.g. determination of reaction kinetics or recording the spectrum of a substance).

The use of our cuvet may easily be extended to the visible range of the spectrum just by blackening its flat ends. An obvious utilization of such an extension appears to be the reduction to the microscale of the colour reactions for RNA, DNA and protein quantitation[7–9].

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## Directional Non-Uniformity of Pulsatile Intramyocardial Pressure

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*Summary.* A method using piezoelectric ceramics was newly devised which permitted measurements of intramyocardial pressure. In open-chest dogs, a directional non-uniformity of the intramyocardial pressure was observed, which may be attributable to the variation in the myocardial fibre orientation.

The musculature of the left ventricle consists of muscle layers gradually alter their orientation from the epicardial surface to the subendocardium. Myocardial fibres of each subsectional layer run in a specified direction. Therefore, the regional pulsatile intramyocardial pressure (IMP) caused by fibre contraction may have different force vectors according to the depth from the ventricular surface. To understand the physiological significance of such a non-uniform structure of the left ventricular myocardium, a small sensor was constructed, which permitted the measurement of local pulsatile intramyocardial pressure acting in a specified direction; it was used to measure