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Note

Assay of methyl methacrylate in blood samples by headspace capillary gas chromatography

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Methyl methacrylate polymerises easily to form a polymeric material with a wide range of industrial applications. It is also used frequently in medicine in orthopaedic reconstructive surgery. Many studies have shown that polymethyl methacrylate cementation can cause severe cardiopulmonary complications in patients [1—3]. As the mode of action of methyl methacrylate in these disorders is still debatable [4], however, it is important to find a precise and reliable analytical method for the determination of this compound in biological samples.

Established gas chromatographic (GC) methods of analysis of methyl methacrylate include elution with solvents [5], direct injection of blood [6] into the chromatograph, and headspace GC [2, 7]. In these methods, lack of sensitivity and/or a rapid hydrolysis of methyl methacrylate in the blood [1, 2, 6] limits detection and only allows measurement for a few minutes after the release of methyl methacrylate into the circulation [1, 3, 5].

We have developed a headspace capillary GC method for the assay of methyl methacrylate in blood samples, applying the method to samples taken from patients during orthopaedic surgery. In this method, the enzymatic degradation

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of the analyte was blocked. We have found methyl methacrylate to be easily measurable in the samples for as long as 60 min after the entry of the monomer into the blood circulation.

EXPERIMENTAL

Venous blood samples were collected via polyurethane cannulas inserted percutaneously in patients. The sampling was performed several times, consecutively, during each of a number of surgical operations. The drawn 2-ml samples were immediately placed in heparinised glass vials (20-ml volume) containing 0.6 g of sodium chloride and some crystals of hydroquinone (Union Chimique, Belgium). The vials were closed with PTFE-covered nitrile rubber septa and secured against leaks by crimping an aluminium cap onto the septum. The samples in the vials were frozen momentarily at the temperature of carbon dioxide ice (−78°C). The period between sampling and freezing was less than 15 s. The samples were kept deep-frozen (−70°C) until analysis by headspace GC.

In order to enrich the methyl methacrylate concentration in the gas phase, the samples were allowed to liquefy and thermostabilise at 70°C for 20 min prior to headspace analysis. The headspace equipment used was a custom-designed accessory in a Hewlett-Packard 5790A capillary gas chromatograph, comprising a dosing needle and a solenoid valve mounted in the carrier gas supply line of the gas chromatograph. In the assay, the gas sample under pressure was allowed to flow from the sample vial onto the inlet of a cooled (0°C) capillary column. After this sample transfer period (1.5 min), the column temperature was programmed to rise from 0 to 100°C at a rate of 30°C/min. The gas chromatograph was equipped with a phenyl silicone (SE 54) coated fused-silica capillary column (25 m × 0.23 mm I.D.) and a flame ionisation detector. Methyl methacrylate with hydroquinone as stabiliser (BDH, U.K.) was used for external calibration. Reference standards were prepared from methyl methacrylate by dilution with fresh human blood (0.59, 1.04 and 5.88 μg/ml), deep-frozen and handled in a similar manner. A Hewlett-Packard integrator 3390A was used for measurement and comparison of chromatogram peak areas.

RESULTS AND DISCUSSION

Foreign esters in the biological environment are hydrolysed rapidly by enzymes occurring in blood and liver [8]. The metabolic degradation of methyl methacrylate has been observed to be fast in vivo [1, 2]. Hydrolytic activity has been found to be intact in blood samples [2] in vitro, which was confirmed in our own calibration samples prepared in human blood without deep-freezing or the addition of sodium chloride. The enzyme activity is blocked when the samples are added on top of sodium chloride in the vials and immediately frozen. A high concentration (23%) of the strong electrolyte (sodium chloride) denatures the hydrolytic enzymes, and the lowered temperature stops the hydrolysis instantly before electrolyte denaturation is complete. The sodium chloride in the sample mixture also has a salting-out effect on the vaporisation

of methyl methacrylate in the sample. Sodium chloride lowers the solubility of methacrylate in the blood and thus increases the concentration in the gas phase utilised in the headspace analysis [9]. Sample storage for several weeks in a deep-freezer at −70°C causes no reduction in the concentration of the analyte of interest.

The detection limit of methyl methacrylate in this headspace analysis was 0.02 μg/ml of blood (0.2 μmol/l). The coefficient of variation (C.V.) calculated from six calibration blood samples was 2.9% at the concentration level of 0.44 μg/ml and 2.0% at 1.04 μg/ml.

The response of the detector was linear within the main concentration area of the analyses (from 0.05 to 50 μg/ml). The calibration data of the analyses fitted to a least-squares regression line with the equation $y = 25.4x + 0.59$ ($r = 0.999$). A typical chromatogram of a real blood sample is presented in Fig. 1.

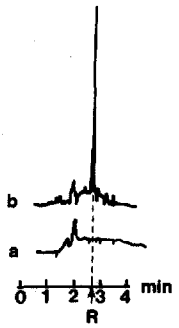

Fig. 1. Gas chromatogram of methyl methacrylate (R = retention time, 2.7 min) in a blood sample. (a) Blank blood; (b) blood with methyl methacrylate (0.56 μg/ml).

Application

As an application, methyl methacrylate was measured in seven patients during knee arthroplasty using tourniquet occlusion. After cementation of the prostheses, the tourniquet was released, at which time the methyl methacrylate

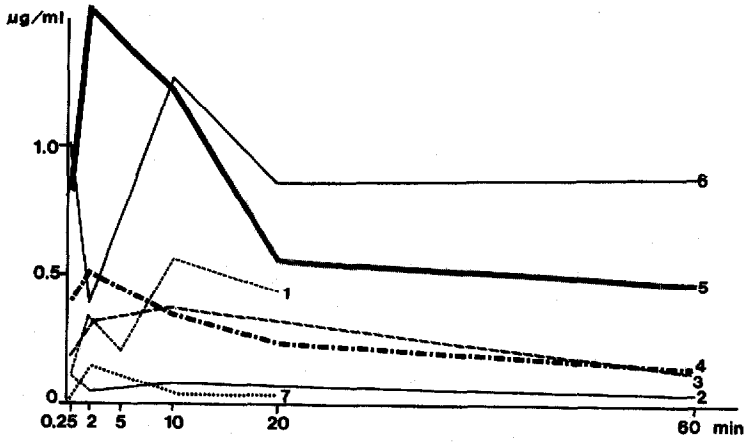

Fig. 2. Methyl methacrylate concentration in blood samples taken from seven patients following knee arthroplasticy.

reached the systemic circulation, and samples were collected after fixed time periods (0.25, 2.0, 10.0, 20.0 and 60.0 min). The results show individual variability (Fig. 2), but the trend is consistent: highest concentrations are encountered 2—10 min after the application of the half-cured bone cement (ca. 40 g) and the release of the tourniquet. The concentration of the monomer then decreased, often being still clearly measurable after 60 min. The results of these assays will be published and discussed in detail in a later article [10].

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