CHROM. 3651

Characterization of fiber parameters in agarose gels by light scattering

Gel chromatography has been proposed as a method for determining the fiber parameters of gels. Using this technique, the structures of dextran gels, a hyaluronic acid gel, agarose gels and an elastin gel have been postulated[1–5]. However, only in the case of hyaluronic acid has it been possible to confirm the results by an independent technique, namely, electron microscopy[6].

In this communication, light scattering data on agarose will be reported. They have been evaluated as proposed by CASASSA[7], and corroborate the parameters obtained by gel chromatography.

Experimental

Two different preparations of agarose were used. One was prepared according to HJERTÉN[8], and the other was a pearl-condensed agarose gel, kindly supplied by Dr. B. GELOTTE, Pharmacia, Uppsala. From these two batches, the following concentrations of agarose were prepared: 10×10^{-4}, 9×10^{-4}, 8×10^{-4}, 6×10^{-4}, 5×10^{-4}, 4×10^{-4}, 2×10^{-4} and 1×10^{-4} g/ml. All solutions were made up in 0.2 *M* NaCl.

The solutions were warmed to 90° for 18 h and then centrifuged in glass cells at 18,000 r.p.m. for 20 min in a Spinco Model L preparative ultracentrifuge using rotor SW 25, as described by DANDLIKER AND KRAUT[9]. The temperature of the rotor was 60–70°. This procedure cleared the solutions from dust.

After centrifugation, all solutions were allowed to stand at room temperature for 48 h, in order that the agarose can polymerize. The angular distribution of the scattered light at a wavelength of 4360 Å was then measured at 40°–130° in a Brice-Phoenix light scattering photometer using the same cells as those in which the centrifugations were performed. The photometer was calibrated with Ludox according to MARON AND LOU[10]. As agarose is a polygalactose, a value of the refractive increment

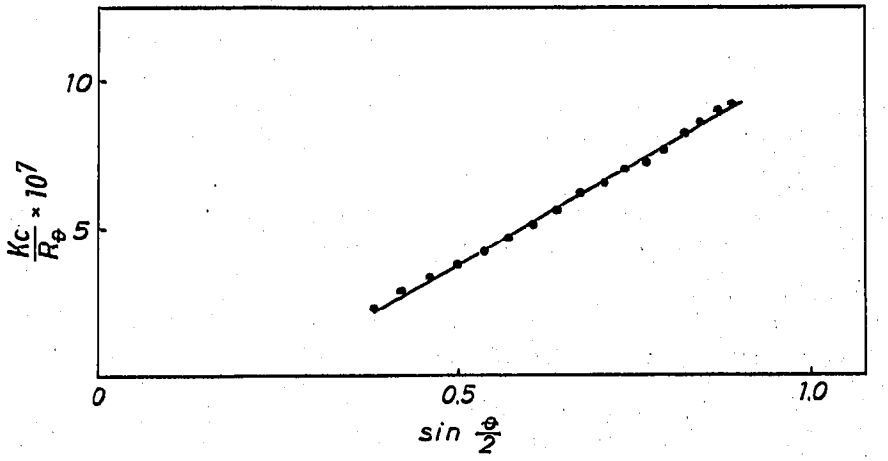

Fig. 1. Plot according to CASASSA[7] of light scattering data obtained on 6×10^{-4} g/ml agarose. ($K = 2\pi^2 n^2 (dn/dc)^2 / N\lambda^4$, where n is the refractive index of the solvent; dn/dc the refractive increment, 0.152 ml/g; N = Avogadros number and λ the wavelength of the light. c is the concentration of agarose in g/ml and R_0 is the reduced angular intensity measured at the angle θ). From the slope of the line, one can calculate a mass/length ratio of 1.72×10^{-13} g/cm.

of 0.152 ml/g was used. This corresponds to the refractive increment of dextran[11]. The refractive increments of other polyhexoses are in the same range[12].

The light scattering data were treated according to CASASSA (Fig. 1). A plot of $Kc/R\theta$ *vs.* sin $\theta/2$ should be linear for a system of randomly distributed fibers, and the slope of the line should be related to M/L, *i.e.* the mass per length unit of the fibers. The M/L values for agarose determined in this way for the concentrations 10×10^{-4}–4×10^{-4} g/ml are in the range 1.26–1.72×10^{-13} g/cm. Concentrations below 4×10^{-4} g/ml did not give straight lines, and hence no M/L-values could be calculated at these concentrations. This might depend on a less uniform fiber structure at these low concentrations. The corresponding values obtained from gel chromatography[4] are 1.19–1.69×10^{-13} g/cm and are in very good agreement with the light scattering results. It should be stressed that the concentrations of agarose in the gel chromatographic experiments, 4×10^{-2}, 6×10^{-2} and 8×10^{-2} g/ml respectively were much higher than the concentrations in the light scattering experiments. Light scattering could not be performed on more concentrated gels, due to the high turbidity.

Although CASASSA predicted that the line in Fig. 1 should pass through the origin, it is evident from the diagram that this is not the case. This can, however, be explained if it is assumed that a small number of very large aggregates are present in the gel. The slope of the line would not, on this assumption, be significantly changed. In experiments with fibrin, CASASSA found similar negative intercepts on the $Kc/R\theta$-axis.

Using two different methods, gel chromatography and light scattering, similar values for the fiber parameters of agarose gels were obtained thus providing additional evidence in favour of the theory for gel chromatography proposed by LAURENT AND KILLANDER[1], and justifying the use of gel chromatography for characterizing gels.

The investigation was supported by grants from the Swedish Medical Research Council (no. B68-13x-4-04C), the Swedish Cancer Society and the University of Uppsala.

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Received June 17th, 1968