corded. The reaction was followed for at least 3 half-life times. No 1-substituted indene could be detected at equilibrium. From the equilibrium solutions, the 3-substituted indenes were isolated and recrystallized from isopropanol. The distinct melting points of the indenes isolated and the NMR-spectra showed that the products were uniform in character. The equilibrium constants were determined by the NMR-technique with the aid of a computer of average transients. All equilibrium constants were larger than 50. The experimental technique did not allow a more precise determination.


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Isoelectric Focusing in Deuterium Oxide Density Gradient

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The present paper is a preliminary report on the use of deuterium oxide as a substitue for sucrose when preparing density gradients for small iso-electric focusing columns. Full details of the technique used and the results obtained will be given elsewhere.

The isoelectric focusing column utilized in the present work is a modification of the 1.5 ml column described by Jonsson et al. Inter alia, the bottom electrode of platinum wire has been exchanged for a sheet of palladium covering the bottom of the focusing chamber.

The D₂O density gradient has been created directly in the column by free interdiffusion of three D₂O solutions for 3 min. The resulting D₂O concentration curve, as obtained by measurement of the refractive index gradient, has a very high degree of linearity. The initial D₂O density gradient (0.018 g cm⁻³) is as strong as the initial sucrose density gradient used by Jonsson et al. As expected, the stability in time is less for the D₂O density gradient than for the sucrose density gradient. However, the D₂O density gradient remaining after the column has been standing in an upright position for two hours (0.018 g cm⁻³), is still more than twice as strong as the sucrose density gradient obtainable in an LKB 110 ml column with 500 g of sucrose per litre as initial bottom concentration. Thus, the D₂O density gradient described should normally be quite sufficient for stabilization of protein zones. This conclusion is supported by results obtained in this laboratory at isoelectric focusing of β-lactoglobulin (cf. Fig. 1) and sperm whale myoglobin.

Fig. 1. Scan of 1.5 ml column at 280 nm (solid curve) obtained after D₂O density gradient isoelectric focusing of 100 μg β-lactoglobulin in 1 % Ampholine pH 4 - 6 for 75 min. Average field strength 30 V cm⁻¹. Superimposed are pH values of 60 μl fractions of the column contents (circles). (A represents β-lactoglobulin A, and B β-lactoglobulin B.)

Isoelectric points evaluated from isoelectric focusing runs in D₂O density gradients were found to be about 0.1 pH unit higher than those obtained in sucrose density gradients. This increase seems to be the net result of two effects: the intrinsic pK_a values of the protolytic groups in proteins are higher in D₂O than in H₂O but simultaneously the pH meter reading in D₂O solution is lower than in H₂O solution for solutions of comparable acidity.


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