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 indenenes were isolated and recrystallized from isopropanol. The distinct melting points of the indenenes 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.

1. Bergson, G. and Ohlsson, L. *Acta Chem. Scand.* 23 (1969) 2175.
2. Meurling, L. *Acta Chem. Scand. B* 28 (1974) 295.
3. Ahlberg, P. *Chem. Scr.* 3 (1973) 183.
4. Hine, J. *Physical Organic Chemistry*, McGraw, New York 1962, p. 97.
5. Meurling, L. *Chem. Scr. In press.*

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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 substitute for sucrose when preparing density gradients for small isoelectric 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.*¹ *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.² The resulting D₂O concentration course, as obtained by measurement of the refractive index gradient,³ has a very high degree of linearity. The initial D₂O density gradient (0.018 g cm⁻⁴) 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 (0.016 g cm⁻⁴), 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 column¹ at 280 nm (solid curve) obtained after D₂O density gradient isoelectric focusing of 100 μ g β -lactoglobulin in 1 % Ampholine pH 4–6 for 75 min. Average field strength 30 V cm⁻¹. Superimposed are pH values of 60 μ l fractions of the column contents (circles).³ (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.

1. Jonsson, M., Pettersson, S. and Rilbe, H. *Anal. Biochem.* 51 (1973) 557.
2. Rilbe, H. and Pettersson, S. *Separation Sci.* 3 (1968) 535.
3. Fredriksson, S. *Anal. Biochem.* 50 (1972) 575.

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