

**NOTE**

**PREPARATION OF TRITIUM-LABELLED DEXTRAN AND INULIN**

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**SUMMARY**

Tritiated dextran and inulin were prepared by both a catalytic solid state and a liquid phase isotope exchange with gaseous tritium. The liquid phase procedure is convenient for preparation of the polysaccharides with specific activities up to 5 mCi/g, while the solid state procedure allows specific activities up to 700 mCi/g.

**Key Words:** [<sup>3</sup>H]dextran, [<sup>3</sup>H]inulin, catalytic isotope exchange

Among the large number of polysaccharides inulin and dextran are of special interest owing to their wide medical use: Inulin is used in bacteriology and for kidney functioning studies; a dextran fraction with molecular weight 60,000±10,000 is an effective plasma substituting antishock preparation; dextran fraction with molecular weight 35,000±5,000 is used for the treatment of capillary blood flow breaking.

Labelled analogues of these polysaccharides, for example [<sup>14</sup>C]carboxylated inulin and dextran, [methoxy-<sup>3</sup>H]dextran and [methoxy-<sup>3</sup>H]inulin, are commercially available [1,2]. The preparation of a partially oxidized [<sup>3</sup>H]dextran has also been described in literature [3]. Such labelled analogues might have different chemical, biochemical or pharmacological

properties compared to the original polysaccharides. Therefore, labelling methods which do not alter the structure of the original compounds are of great interest. Isotope exchange methods are undoubtedly the best for such purposes. However, an isotope exchange, carried out according to the Wilzbach method or using the traditional conditions of catalytic isotope exchange in a liquid phase does not provide compounds with specific activities higher than 1-2 mCi/g. In the present study, to increase the specific activity of dextran and inulin, we used catalytic isotope exchange methods with tritiated water or with gaseous tritium in the solid phase. These methods have not previously been used for the preparation of tritiated inulin and dextran.

An application of the isotope exchange with tritiated water for the preparation of reducing carbohydrates labelled in the 1<sup>st</sup> position has been described in literature, but there are no data on synthesis of tritiated polysaccharides. It has been shown, however, that a considerable amount of deuterium can be incorporated both into mono- and polysaccharides by heating carbohydrate solutions in deuterated water at 100 °C in the presence of Raney nickel [5].

We have shown that such conditions are suitable for the tritium-labelled polysaccharide preparations. The best results were obtained for isotope exchange with tritiated water (specific activity 0.5 - 0.6 Ci/ml) after heating at 100 °C for 10 h. in presence of Raney nickel. The specific activity of the labelled polysaccharides obtained was 3 - 5 mCi/g. An increase in the duration of isotope exchange leads to products with higher specific activities (Fig.1), but at the same time the yield decreases because of thermochemical and radiation destruction of the polymer chains. Use of water with higher specific activity leads to the same results. So, the catalytic isotope exchange

with tritiated water is a convenient way to produce the [<sup>3</sup>H]polysaccharides with relatively low specific activities.

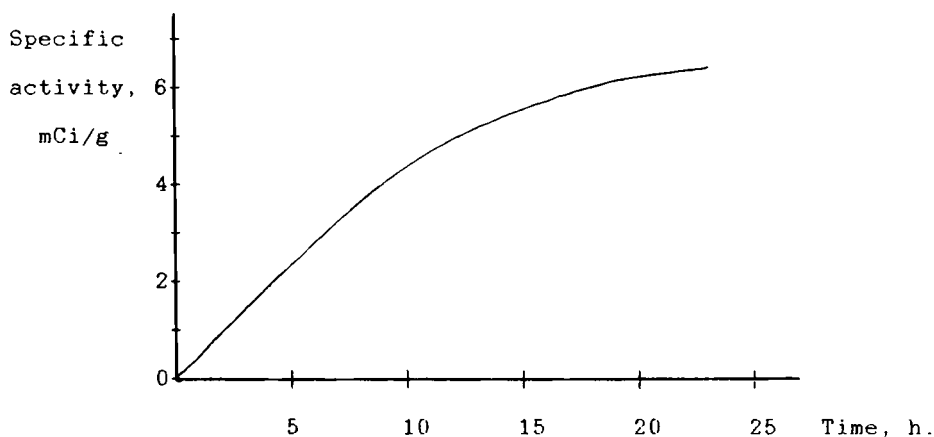


Fig.1. Effect of time on [<sup>3</sup>H]dextran specific activity for isotope exchange with tritiated water in presence of Raney nickel at 100 °C.

It was shown by Evans and coworkers [4] that liquid-phase catalytic isotope exchange with gaseous tritium does not allow to obtain high specific activities for nonreducing carbohydrates to which the inulin and dextran belong.

We use a solid state modification of isotope exchange with gaseous tritium (SSIE). It has been shown earlier [6,7] that using SSIE higher specific activities are obtained and the label is incorporated into non-traditional positions of the molecules.

Satisfactory results have been obtained by us in the preparation of tritium-labelled inulin and dextran fractions with 10% Pd/CaCO<sub>3</sub> as a catalyst. An optimum temperature is 100 °C, isotope exchange reaction duration is 1-2 h. The specific activity of tritiated polysaccharides obtained is 300-700 mCi/g, the yield is 25-40%.

## EXPERIMENTAL

Dextran fractions T-40 and T-70 were purchased from Pharmacia (Sweden), inulin from Laevosan-Gesellschaft (FRG). The labelled compounds were purified by column chromatography on Sephadex G-200 for the labelled dextran fractions, and on Sephadex G-100 for [G-<sup>3</sup>H]inulin.

The molecular weights were determined by using a gel column TSK-G 3000SW (7.5x300 mm) and LKB HPLC equipment. Elution was performed by water with 0.05% of sodium azide at 25 °C.

The concentrations of the labelled polysaccharides for the specific activity calculations were determined by color reactions with anthrone (for dextran) or resorcinol (for inulin) followed by UV-spectrophotometry at 625 nm. Radioactivity was measured by liquid scintillation counting.

## REFERENCES

1. Live Science Products, Amersham, 1990.
2. NEN Research Products, Catalogue, 1990.
3. Augustine, J., Pardee, A.B., Castellot, J.J. - Radiochem. Radioanal. Letters. 32: 261 (1978).
4. Evans, E.A., Scheppard, H.C., Turner, J.C., Warrel, D.C. - J. Labelled Compounds. 10: 569 (1974).
5. Koch, H.J., Stuart, R.S. - Carbohydr. Res. 59: C1-C6 (1977).
6. Zolotarev Ju.A., Kozik, V.S., Zaitsev, D.A., Dorokhova, E.M., Myasoedov, N.F. - Dokl. AN SSSR. 308: 1146 (1989).
7. Akulov, G.P., Snetkova, E.V., Kaminski, Ju.L., Kudelin, B.K., Efimova, V.L. - Radiochimia. 33: 74 (1991).