[Chem. Pharm. Bull. 18(9) 1935 (1970)] UDC 547.962.3.03 : 543.426

Ultra-micro Analysis of Serum Protein in a Few Nanoliter of Samples

1-Dimethylaminonaphthalene-5-sulfonyl (dansyl) chloride (DNS-Cl) reacts readily with amino group of organic compounds in slightly alkaline medium to produce strongly fluorescent N-substituted amides. The reagent has been widely used in protein and peptide chemistry for labelling of antibodies,[1] sequential analysis of proteins,[2] micro analysis of bioactive peptides,[3] *etc.*

We have utilized this reagent for fluorometric assay of serum protein in a few nanoliter of samples by adsorbing and reacting the protein on a silica gel.

Established procedure is as follows: Ten μl of 0.5% DNS-Cl solution in *n*-hexane is charged on a thin-layer plate of silicagel H and the solvent is removed for 2 min in the air. Five to 20 nl of sample containing *ca.* 0.1 to 1% serum protein is spotted on the same area with a calculated micropipette by the aid of micromanipulator under a view of microscope. The complete dansylation is achieved by setting the plate for 10 hr in a chamber saturated with the vapor of 0.1M triethylamine bicarbonate buffer (pH 8.5). Excess of DNS-Cl and byproducts such as DNSOH and $DNSNH_2$, together with labelled amines, amino acids and peptides of small molecular weight, are removed from the origin by successive development with the solvent system of *n*-butanol–acetic acid–water (4:1:5) and isopropanol–methyl acetate–28% ammonia (9:7:4). The plate is dried in the air for 1 hr in a dark place and fluorescence intensity of labelled protein remaining at the origin is measured with Yamato Kagaku scanning fluorometer Model SFR II using the bovine albumin (albumin stock standard Daiichi Pure Chem. Co.) as the standard. Working curves for bovine albumin are shown in Fig. 1.

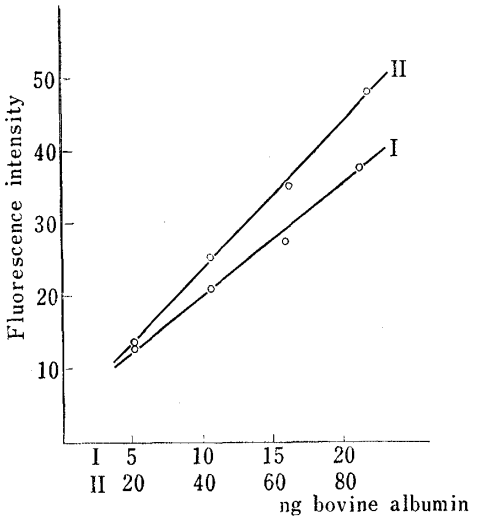

Fig. 1. Working Curves for Bovine Albumin in 5.3 and 20.7 nl of Samples

sample volume I: 5.3 nl (measured at range 0.2)
II: 20.7 nl (measured at range 0.5)
thickness of glass plate 1 mm
silicagel layer 0.25 mm

Application of the method is in progress combining with the micropuncture techniques. The details will be discussed in elsewhere.

Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo — Terumi Nakajima

Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo — Hitoshi Endou, Fuminori Sakai

Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo — Zenzo Tamura

Received July 2, 1970

1) R.M. Clayton, *Nature*, **174**, 1059 (1954).
2) W.R. Gray and B.S. Hartley, *Biochem. J.*, **89**, 59 (1963).
3) H. Sato, T. Tanimura, T. Nakajima and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 2188 (1969).