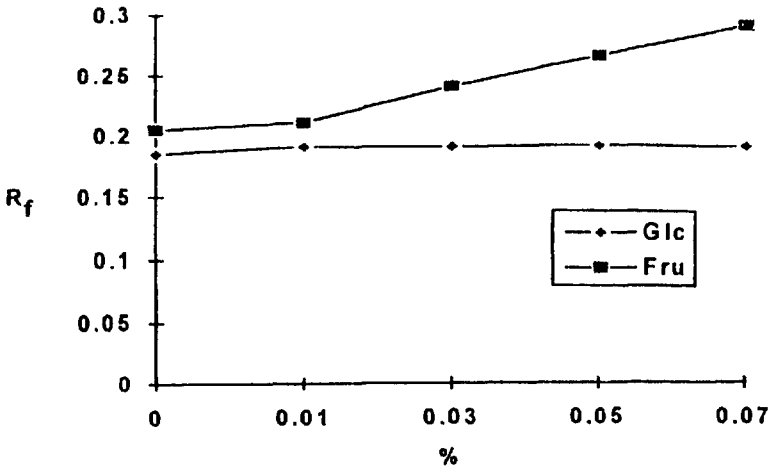

Figure 4 The influence of NST concentration on migration of glucose and fructose. Conditions: Silica gel TLC plates; acetonitrile–phosphate buffer pH 5.9 (85:15) solvent system; single development; detection by ADP, transmission, 560 nm, computer-controlled scanner (Camag); Ifc-AMMP software.

In contrast, derivatization of reducing saccharides with the highly fluorescent fluorophore 2-aminoacridone or with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) and separation of the derivatives by polycrylamide gel electrophoresis enable detection of subpicomolar quantities of the fluorescent saccharides using a cooled charge-coupled device (CCD) imaging system (10b,11a). Undoubtedly, the development and introduction of new fluorescent labeling reagents to carbohydrate analysis are challenges for thin-layer chromatographers.

B. Postchromatographic Derivatization

The visualization of sugars on TLC plates is often performed with postchromatographic derivatization reagents. Differentiation has to be made between reducing and nonreducing sugars. Detection of nonreducing sugars is usually based on their oxidation with strong mineral acids. Ethanolic solutions of sulfuric acid, sulfuric acid alone or mixed with nitric acid or permanganate are suitable for detecting sugars at the microgram level. These reagents, although suitable for silica, should not be used on organic layers such as cellulose or polyamide.

Derivatization procedures in quantitative thin-layer chromatography include instrumental dipping of the developed and dried plate into the respective derivatization solution and activation by heating. Manual dipping or spraying of the plate with the derivatization reagent, followed by activation, is rarely used in quantitative TLC but is popular in qualitative and semiquantitative analysis. The zones usually become colored and show intense fluorescence. The fluorescence intensity can be stabilized, or enhanced, by dipping the plate into a mixture of paraffin and *n*-hexane (1:3 to 1:1) for 2 s (20,65). Some of the most frequently used reagents for routine postchromatographic derivatization of common sugars are presented in Table 7.

Anisaldehyde and α-naphthol are additional carbohydrate spray reagents in common use (24,77a). Amino sugars are usually detected on ammonia-free layers with ninhydrin or with other reagents specific for amino groups such as fluorescamine, NBD chloride, or OPA-mercaptoethanol (21b). The reducing amino sugars can also be detected with silver nitrate (43) or with other reagents used for common sugars. Sugar alcohols can be detected with reagents suitable for nonreducing sugars such as 2,6-dichlorofluorescein–lead tetraacetate reagent.

Detection of glycolipids on thin-layer chromatograms is usually done using a spray reagent of orcinol in 75% sulfuric acid (31c), because this reagent does not give false-positive reactions with other lipid components. In making the reagent, the source of orcinol is important (Fisher Scientific is a recommended source). Gangliosides, negatively charged glycosphingolipids that