contain sialic acid, can specifically be visualized using a resorcinol-based spray reagent. (Be sure to clamp a glass plate over the sprayed surface of the chromatogram for optimal development.)

C. Visualization by Heating

Most of the common simple sugars are visible on TLC plates under ultraviolet light or in visible light when the developed plate is heated for a few minutes. All aldoses and ketoses from the Merck sugar reference standard kit show intense fluorescence after heating at 160°C for 10 min. The fluorescing spots are visible under UV light at 366 nm. In this condition, no fluorescence is obtained with nonreducing sugars such as alcohols (e.g., mannitol, sorbitol, *meso*-erythritol, *meso*-inositol) and C_1–C_1 linked di- and oligosaccharides (e.g., sucrose, raffinose, and trehalose) (75).

By varying the temperature, it is possible to distinguish different sugars. Di- and trisaccharides are less sensitive and require higher temperatures for activation. Figure 1 shows that detection under visible light is also possible without any derivatization except heating. For each type of sugar, there is a characteristic detection temperature (76,77b).

The application of a thermal in situ reaction is particularly useful on amino-bonded silica gel layers. Heating the developed plate to approximately 150°C for 3–4 min converts the separated sugars into stable, fluorescing derivatives with a practically unlimited life (56). Longer activation time is necessary for nonreducing di- and trisaccharides. The derivatization expires more quickly but the sensitivity increases when the developed plate is conditioned in the presence of concentrated hydrochloric acid (16). In the presence of acidic moisture, nonreducing di- and trisaccharides are most likely cleaved into more reactive monosaccharide components that undergo a Maillard reaction (16,56).

D. Nondestructive Detection Methods

Nondestructive detection methods have been used primarily for qualitative determination of sugars, but recent developments in near-infrared (NIR) imaging detector arrays should enable the development of NIR video densitometers (78–80). Compared to derivatization reagents, detection limits achieved by this hyphenated technique are relatively high and exceed 1 μg per spot of selected sugar (80).

Visualization of sugars by iodine vapor has been popular in paper chromatography. Although this method is not very sensitive, it is nondestructive because of the short exposure time required and because the adsorbed iodine evaporates when the plate is exposed to the air (19).

Separated zones of sugars can be detected on silica gel TLC plates by immersing the developed and dried aluminum-backed silica gel plates into hexane (81). After 5 min, the silica layer appears slightly transparent whereas the sugar spots remain opaque.

More sensitive nondestructive methods for determination of selected sugars after separation include biological tests such as enzymatic reactions (82) and immunochemical detection (83). Immunostaining is particularly popular in molecular biochemistry and clinical chemistry research because of the possibility of direct detection of conjugated carbohydrates such as glycolipids (84). TLC-overlay procedures allow for the specific detection of carbohydrate moieties of glycosphingolipids using antibody binding, lectin binding, or binding to toxins such as cholera toxin or Shiga toxin (verotoxin) (85a,85b,85c). This detection method is analogous to Western blotting of proteins except that glycolipids are not typically blotted to a membrane but remain on the TLC plate during the entire procedure. An example of TLC overlay used in detection of glycolipid receptors (globotriaosyl ceramide) for Shiga toxin (verotoxin) in murine tissue lipid extracts is illustrated in Fig. 5. Such glycolipid immunostaining is highly specific for carbohydrate conformations; for example, Shiga toxin recognizes only glycosphingolipids that contain terminal galactose α1-4 galactose residues (104). Neoglycolipids synthesized by using carbohydrates isolated from more complex glycoconjugates (105) have also been used in overlay procedures in circumstances where carbohydrate moieties are specifically investigated as potential receptors for bacteria and toxins (106).

Silica-based TLC plates must be chosen that can withstand prolonged exposure to aqueous solution. Spraying typical silica-based plates with polyisobutylmethacrylate has been reported to