p-Hydrazinobenzenesulfonic Acid Derivatives of Carbohydrates and Their Capillary Zone Electrophoresis

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Abstract: *p*-Hydrazinobenzenesulfonic acid is explored as a novel ultraviolet labeling reagent for capillary electrophoresis (CE) of mono- and disaccharides. The labeling reaction takes less than 10 minutes and introduces both of absorption and charge groups into the sugars.

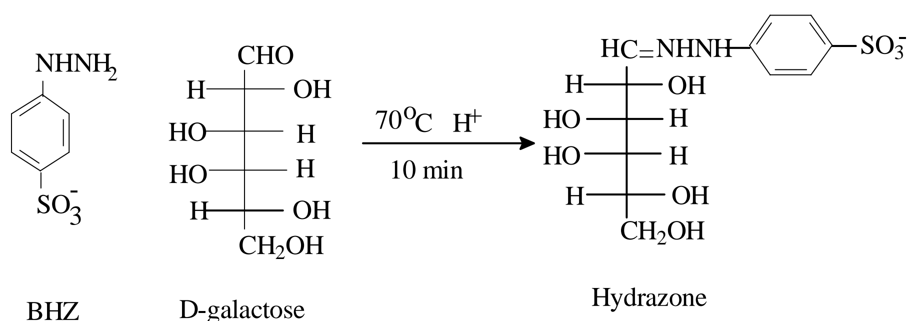
Keywords: *p*-Hydrazinobenzenesulfonic acid, carbohydrate, derivatization, capillary zone electrophoresis.

Precolumn derivatization is the most widely used means to overcome the detection problem in capillary electrophoresis. However most of the adopted reactions in carbohydrate labeling are time-consuming and not very selective, leading to lots of byproducts produced. We have thus tried to explored hydrazino-containing reagents which have been applied to TLC[1,2] and HPLC[2~5] with quite successfulness. Perez and Colón[6] have also reported that dansylhydrazine can be used in CE-LIF of sugars. The most appealing feature of using such kind of labeling reagents is the short reaction time needed, meanwhile they can specifically react with aldehyde and ketone groups without reductive step. This fact gives possibility to be directly used in complex biological sample analysis[6]. The problem left is that the reported hydrazino reagents such as dansylhydrazine do not possess any charge group. Its derivatives can not be separated over a wide pH range with different buffer system. It is favorable to have the labeling reagents with both of UV absorption and charge groups. *p*-Hydrazinobenzenesulfonic acid (BHZ) was expected as such reagent. In this paper, we will briefly show BHZ's reaction properties and its usefulness in CE of sugar.

The labeling reaction is schematically shown in **Figure 1**. Precolumn labeling conditions were checked by using N-acetylglucosamine, fucose, galactose as testing samples. The results were evaluated by capillary zone electrophoresis performed on LKB TACHOPHOR 2127 which was modified to make it suitable for performing CE. Separations were carried out in a fused silica capillary (Yongnian Optical Fiber Factory, Hebei, China) of 50μm i.d.×55cm (efficiency length), applied with 300 V/cm electrical field strength. Samples were introduced into the positive tip of the capillary by hydrodynamic injection for 20s at 10cm height. Detection was achieved by on-column

UV absorption at 206nm with a slit of 100×800μm. The running buffer was composed of 100mmol.L^{-1}boric acid, adjusted pH10.4 with KOH pellet.

Figure 1. Reaction scheme for the derivatization of galactose with BHZ.



Because the reaction needs H^+ as catalyst, pH should be well controlled. Normally the labeling pH should be below 6. Unexpectedly, too low pH decreases the reaction products, possibly due to the protonization of hydrazino group. pH has thus to be optimized between 3~5. In addition, the molar ratio of reagent to sugar, temperature and reaction time are found to influence on derivatization. The optimum labeling reaction happens at 70°C and the molar ratio between 10:1~20:1 (BHZ to sugar). In this case the labeling took less than 10 minutes. The products can be stored in dark at -20°C for 15 days without significant decomposition. Further study showed that some sugars such as N-acetylglucosamine may produce a major and a minor peaks, which also experienced in some other hydrazino-containing reagents labeling[3,7]. The reasons are still not clear. The minor component could be reduced significantly if the labeling temperature was above 40°C and pH > 1. At the optimum conditions, the minor peak could hardly be detected. Unfortunately, such way could not successfully be applied to some disaccharides (see **Figure 2**).

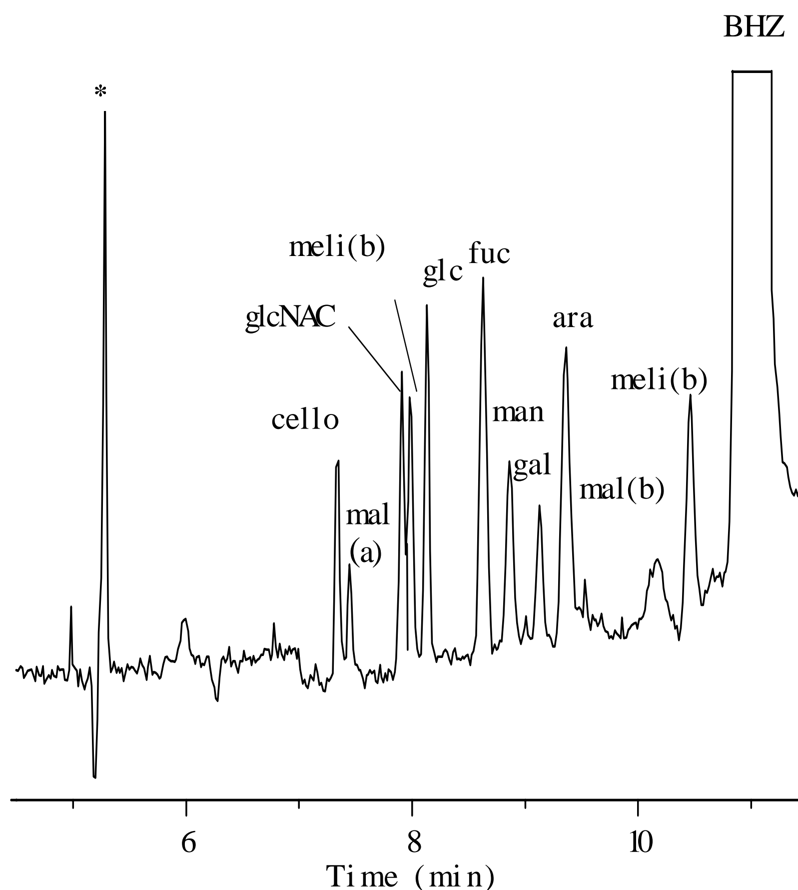
Capillary electrophoresis separation of BHZ derivatives could easily be achieved in borate buffers. Normally, they can be resolved well with 100mmol.L^{-1} borate acid at pH 10.2~10.8. **Figure 2** shows a separation example composed of 9 sugars. The detection limit of glucose is 17 fmol and concentration sensitivity reaches 3.2μM.

The new labeling method is also suitable for quantitative analysis. A linear relationship between peak area and sugar concentration is obtained over the range of 0.057~0.91mmol.L^{-1} with linear correlative cofficient above 0.992. The reproducibility of peak area is highly acceptable with the relative standard deviations (n=5) below 4.9%.

The applicability of the method has been demonstrated by analyzing the monosaccharide components of laminarin, yielding a content ratio of galactose: mannose: fucose: glucose obtained is 1.08:0.099:1:0.018 (molar ratio), for which is comparable with other methods.

Figure 2. Separation of nine sugar-BHZ derivative

Separation condition: Beckman P/ACE 2050, 200nm detection; fused silica capillary, 55/62 cm×50



μm i.d.; voltage, 18.6 KV; carrier, 100mmol.L^{-1}boric acid (pH 10.24); press injection, 3.44 KPa , 3s.

In conclusion, the new method is rapid, simple, reproducible and quite reliable in quantitative analysis. Although some sugars may produce two peaks, it is still applicable

because we can largely reduce the minor component by controlling the reaction conditions. In addition, the two-peak phenomenon can also be used for peak identification. More discussions will be made in detail elsewhere in short time.

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