ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 2, 2021

Capillary Electrophoresis for the Separation and Quantification of D-Allose in the Presence of Process-Related Impurities

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Abstract:

Due to its scarcity, researchers are synthesizing it through chemical, enzymatic, and microbial routes. Enzymatic methods are preferred to avoid unwanted impurities and harsh chemicals. However, large-scale production of rare sugars requires optimization of several parameters to increase yield and quality. A quick and efficient monitoring method is essential to optimize the enzymatic conversion process. Various analytical methods such as HPLC, GC-MS, LC-MS, NMR, SEC, and HPAEC-PAD are available for sugar analysis, but each has its limitations and may not be suitable for rare sugar analysis. Capillary electrophoresis (CE) has emerged as a promising technique for the separation and quantification of traditional sugars in food and plant materials. CE offers advantages like micro-volume samples, less reagent consumption, high resolution, sensitivity, and reproducibility, making it a potential tool for rare sugar analysis. The objective of this study is to develop a sensitive, simple, rapid, and cost-effective CE method for quantifying D-allose in the presence of five other sugars, including two more rare sugars (D-altrose and D-psicose). The method aims to overcome the limitations of existing analytical methods and provide accurate quantification and validation of D-allose during large-scale production from D-psicose in a continuous bioreactor. Through this investigation, we intend to establish a robust analytical method that enables efficient monitoring of D-allose production and facilitates the development of rare sugars as potential food additives or supplements for managing weight gain effectively.

Materials and methods:

Reagents and Solutions:

Analytical grade chemicals and reagents, including D-glucose, D-fructose, sucrose, D-psicose, D-allose, D-altrose, D-trehalose, disodium hydrogen phosphate (Na2HPO4), and sodium hydroxide (NaOH)[1], were procured from Sigma-Aldrich (India). HPLC grade water was

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obtained from the Milli-Q system (Millipore, Bedford, MA, USA) [2]. pH calibration buffers of 1.68 and 12.00 were procured from Reagecon [3], and other buffers of pH 4.00, 7.00, and 9.20 were procured from Merck [4]. Hydrochloric acid and acetic acid were procured from Merck [5]. Fused silica capillaries with a length of 60 cm and an internal diameter of 50 µm (50.2 cm effective length) were obtained from Sciex, USA [6].

Preparation of Standard Solution:

Standard stock solutions of each sugar with a concentration of 20 mM were prepared using HPLC grade water and then suitably diluted as needed [7]. To establish the linear relationship between concentration and electrophoretic response (peak area), standard solutions of sucrose, D-glucose, D-fructose, D-psicose, D-allose, and D-altrose at concentrations of 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 mM were prepared [8]. The internal standard D-trehalose (1.0 mM) was spiked with the standards.

Preparation of Background Electrolyte:

Various conventional buffers were investigated for their effectiveness, and the buffer composition of 130 mM NaOH and 36 mM Na2HPO4 was found to be effective[9]. The buffer at pH 12.6, voltage 18.0 kV, and ionic strength 0.217 M provided good resolution of the investigating sugars. D-allose showed strong UV absorption in the range of 100 to 130 mM, and to detect the sugars with a UV detector [10], the concentration of NaOH had to be in this range. At 130 mM NaOH, the resolution of the peaks was optimum. The addition of Na2HPO4 in the buffer improved the baseline and separation quality by increasing the viscosity of the electrolyte solutions and decreasing the mobility of analytes [11]. The optimum Na2HPO4 concentration was found to be 36 mM [12].

The background electrolyte (BGE) buffer solution containing 130 mM NaOH and 36 mM Na2HPO4.2H2O was freshly prepared to minimize the carbon dioxide effect and improve reproducibility [13]. The Mettler Toledo (GMBH, model #8603) pH meter was calibrated with standard buffers before adjusting the BGE solution to a pH ranging from 12.0 to 12.8 and an ionic strength of 0.217 M [14].

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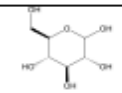
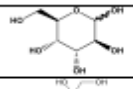
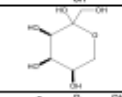
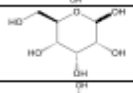
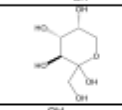
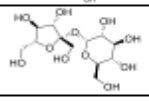
Table 1 Carbohydrates studied: name, molecular formulas, molar masses, pKa values, and chemical structures

	Compound Name	Molecular Formula	M/ W	pKa	Structure
1.	D-Glucose	$C_6H_{12}O_6$	180.156g/mol	11.8	
2	D-Altrose	$C_6H_{12}O_6$	180.16	12.45	
3	D-Psicose	$C_6H_{12}O_6$	180.156	11.80	
4	D-allose	$C_6H_{12}O_6$	180.155	11.3	
5	D-Fructose	$C_6H_{12}O_6$	180.16	12.06	
6	Sucrose	$C_{12}H_{22}O_{11}$	342.3	12.62	

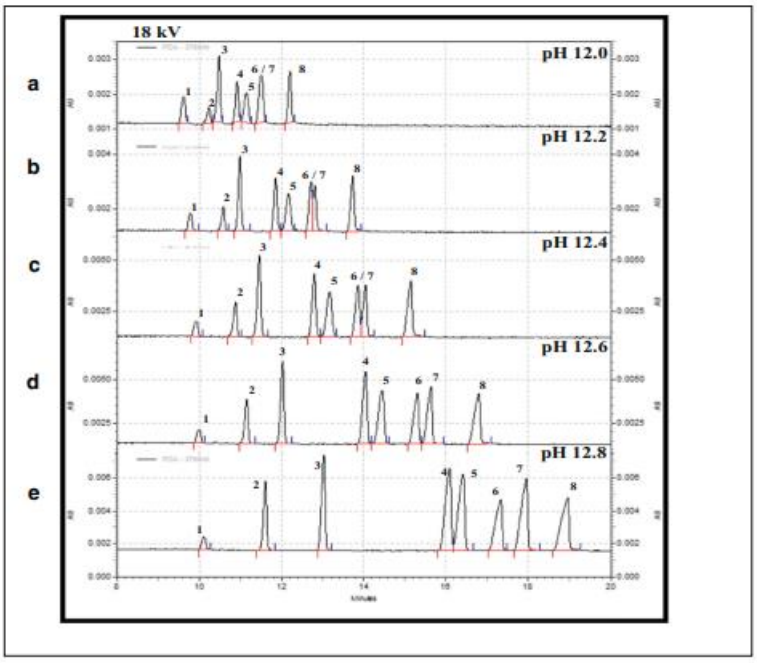


Fig. 1 Separation of seven sugar mixtures at constant separation voltage 18 kV with different pHs: (A) pH 12.0; (B) pH 12.2; (C) pH 12.4; (D) pH 12.6; (E) pH 12.8. Peak identities: (1) EOF; (2) trehalose; (3) sucrose; (4) D-allose; (5) glucose; (6) fructose; (7) altrose; (8) psicose. Background electrolyte solution: 130 mM NaOH and 36 mM $Na_2HPO_4.2H_2O$

ISSN PRINT 2319 1775 Online 2320 7876

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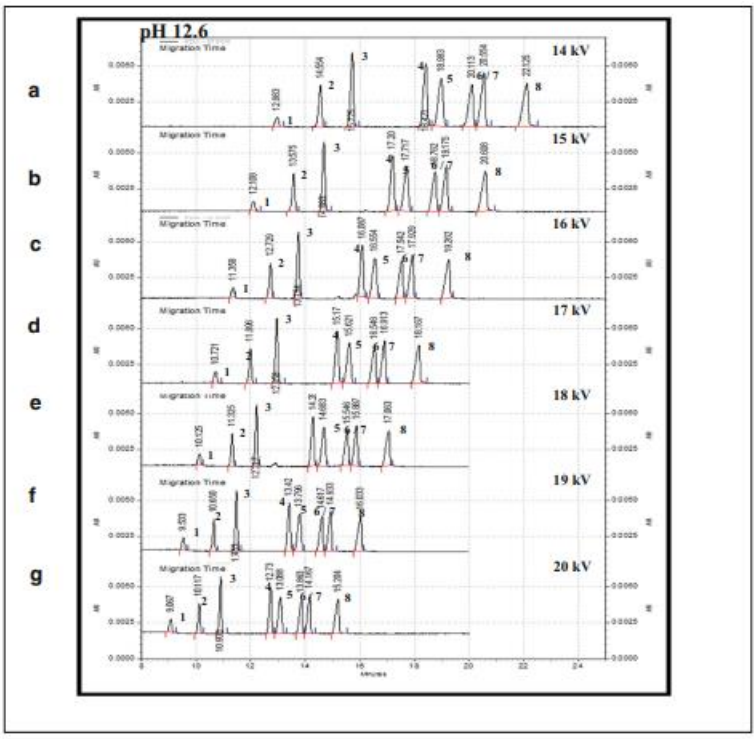

Fig. 2 Separation of seven sugar mixtures in an electrolyte solution of pH 12.6 at different separation voltages. (A) 14 kV; (B) 15 kV; (C) 16 kV; (D) 17 kV; (E) 18 kV; (F) 19 kV; (G) 20 kV. Peak ids: (1) EOF, (2) D-trehalose, (3) sucrose, (4) D-allose, (5) D-glucose, (6) D-fructose, (7) D-altrose; (8) D-psicose entities. Background electrolyte solution: 130 mM NaOH and 36 mM $Na_2HPO_4.2H_2O$

Results and discussion:

Capillary electrophoresis (CE) separates ions based on their electrophoretic mobility under the influence of voltage [15]. The electrophoretic mobility depends on the charge of the molecule, the viscosity of the electrolyte solution [16], and the size of the moving analyte. The rate of movement is directly proportional [17] to the applied electric field, and charged species move faster than neutral species [18]. The success of separation and reproducibility in CE depends on factors such as voltage and pH [19]. To optimize the conditions for the successful separation and quantification of D-allose in the presence of other sugars [20], the electrophoretic migration of sugars with varying pH and voltage was investigated [21]. The pH of the electrolyte solution was maintained above 12.0 to impart fast electrophoretic mobility to the sugars [22]. At pH 12.6 and voltage 18 kV, the separation of sugars was satisfactory with sufficient buffer capacity and low electromigration dispersion, resulting in symmetric peak shapes [23].

The separation order of the sugars was found to be trehalose (IS) < sucrose < allose < glucose < fructose < altrose < psicose [24]. The migration time of the sugars increased with an increase in pH [25]. At pH 12.0, D-fructose and D-altrose peaks were merged, and as the pH increased,

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these peaks resolved into two distinct peaks [26]. At pH 12.6 or above, the peaks of all sugars were well-separated [27], and pH 12.6 was considered the [28]optimum pH for the buffer solution [29].

The influence of voltage on the separation[30] of sugar mixtures was investigated by varying voltages from 14.0 to 20.0 kV, while keeping the pH constant at 12.6 [31]. With an increase in voltage, the migration time decreased. At 14 kV,[32] D-psicose had peak tailing, but at 18.0 kV, the tailing was reduced, [33]and all sugars were well-resolved. However, at higher voltages (19 kV and 20 kV), the resolution between D-allose and D-glucose decreased, leading to the formation of a valley[34].

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