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Separation and direct UV detection of sugars by capillary electrophoresis using chelation of copper(II)

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Abstract

A new method for the capillary electrophoretic separation of neutral sugars with direct UV detection based on the chelation reaction with copper (II) is demonstrated. Using an electrolyte consisting of copper(II) sulphate and ammonia, UV absorption at 245 nm results from the chelation of Cu(II) by the sugars under alkaline conditions. In addition, the sugar mobility increases with increasing Cu(II) concentration in the electrolyte. Thus, the chelation with Cu(II) provides both separation and direct UV detection of neutral sugars. Detection limits for sugars in the range of 50–100 μM are achieved. Linearity over two orders of magnitude is observed. Furthermore, using the same electrolyte, the simultaneous detection of amino acids due to complexation with Cu(II) and the indirect detection of inorganic cations is possible. © 1998 Elsevier Science B.V.

Keywords: Detection, electrophoresis; Complexation; Sugars; Copper; Amino acids; Inorganic cations

1. Introduction

In recent years, capillary electrophoresis (CE) has been demonstrated to be a powerful tool in carbohydrate analysis. As most carbohydrates do not contain easily chargeable and chromophoric moieties, CE separation and sensitive detection is most challenging. Despite these intrinsic difficulties due to the lack of outstanding structural features of most carbohydrates, many strategies have been developed in order to use the high efficiency and speed of CE [1,2].

Borate complexation [3] of underivatized sugars provides satisfactory separations particularly at higher temperatures. However despite a 2- to 20-fold increased absorbance at 195 nm, sensitivity is insufficient for many problems.

As an universal detection scheme in CE for non-UV-absorbing compounds, indirect detection of sugars was performed using background electrolytes either for indirect fluorescence [4] or indirect UV detection [5,6]. However, the weak-acid behaviour of sugars requires highly-alkaline conditions for their separation. According to Garner and Yeung [4], high hydroxide concentrations lead to a decreased transfer ratio and therefore to rather limited sensitivity compared to other indirect systems.

Amperometric detection, pulsed [7,8] or at constant potential [9] was shown to be a sensitive and selective method for carbohydrate detection. Unfortunately, no amperometric detection system for CE is commercially available yet.

The most common detection scheme for carbohydrates is based on precolumn derivatization with a suitable chromophore or fluorophore. A wide variety

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of derivatization reagents is used for this purpose [2]. In most cases, reductive amination of the sugar carbonyl group is employed. Extremely sensitive determinations of sugars are possible particularly in combination with laser-induced fluorescence detection (LIF) [10,11]. If charged derivatization reagents are employed, charged carbohydrate derivatives are formed, which can be separated in CZE, if the hydrodynamic radii of the species are different [11]. In most cases separation by borate complexation [12,13] or by micellar electrokinetic chromatography (MEKC) [14,15] is required.

However, derivatization procedures are elaborate and time-consuming and derivatization of compounds which lack a free carbonyl group like sucrose is not possible in this way.

Direct UV or fluorescence detection by dynamic labelling of carbohydrates is an alternative to these common detection strategies. In this approach, dynamic complexation of the carbohydrates with a complex partner yields UV-absorbing or fluorescent products. Only a few CE applications based on dynamic labelling of carbohydrates have been described until now. The separation and direct LIF detection of mixtures of cyclodextrins (CD) was demonstrated using fluorescence enhancement of 2-anilinonaphthalene-6-sulphonic acid due to inclusion complexation in the hydrophobic CD cavity [16].

The complexation of starch and starch compounds with iodine provided charges to obtain mobility as well as the possibility for direct Vis detection at 560 nm [17].

Very recently Toida et al. reported the detection of glycosaminoglycans (GAGs) at 240 nm as their copper(II) complexes [18]. Absorbance was suggested to result from a charge-transfer complexation between Cu(II) and the GAG carboxylate groups. Therefore, this method is not applicable to carbohydrates which do not have such structural preferences.

In this paper, we report on a new method for the capillary electrophoretic separation of monosaccharides and sucrose with direct UV detection based on the chelation reaction with copper(II) under alkaline conditions. It is not a new fact that polyhydroxy compounds can act as chelating ligands in the formation of coordination complexes of several metal cations [19–21].

Honda et al. [22] demonstrated the strong enhancement of resolution of 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of reducing carbohydrates in CZE using complexation with alkaline-earth metal cations. Bourne et al. [23] achieved resolution of some polyhydroxy compounds by paper electrophoresis in copper(II) acetate solutions and by chromatography on the Cu(II) form of a cation-exchange resin. Briggs et al. [21] compared the stabilities of carbohydrate complexes of several metal cations by thin-layer ligand-exchange chromatography. They found copper(II) to complex carbohydrates to the greatest extent. Reeves [24] observed a strongly increased absorption in the near UV region after complex formation between cuprammonia and D-mannosan.

Based on these observations, we developed a capillary electrophoresis approach for sugar determination. Using an electrolyte consisting of copper(II) sulphate and ammonia, both the separation and the direct UV detection of neutral sugars were achieved. In addition to sugar detection, the simultaneous detection of amino acids and inorganic cations was possible.

2. Experimental

2.1. Apparatus

The experiments were carried out on a laboratory-built CE system, equipped with a Lambda 1000 UV detector (Bischoff, Leonberg, Germany) and a high voltage power supply type HCN 6 *M*–30000 from FUG (Rosenheim, Germany).

Untreated fused-silica capillaries (CS – Chromatographie Service, Langerwehe, Germany) with an inner diameter of 50 μm were employed.

The UV spectra were obtained with a spectrophotometer Omega 10 (Bruins-Instruments, Puchheim, Germany).

2.2. Chemicals

Copper(II) sulphate was purchased from Fluka (Buchs, Switzerland). Ammonia solution (25%) was obtained from Merck (Darmstadt, Germany). All standard substances were purchased in the highest quality available, inorganic cations (as their chloride

salts), sugars from Merck and amino acids from Fluka. All electrolytes and standard chemicals were dissolved in Milli-Q water and filtered through a 0.45-μm filter. The electrolytes used in this work were prepared by dissolving copper(II) sulphate in 500 m*M* of ammonia solution and a further addition of ammonia solution (25%) until the right pH was reached.

2.3. Conditioning of capillaries

To achieve maximum reproducibility of migration times, the capillary was conditioned every morning using the following rinsing procedure: 2 min with water, 10 min 0.1 *M* HCl, 2 min water, 10 min 0.1 *M* NaOH, 2 min water and 20 min with the copper(II) electrolyte to reach an equilibrium between the buffer solution and the capillary wall. Between two runs, the capillary was rinsed with electrolyte for 5 min. For capillary storage overnight, the capillary was rinsed with 0.5 *M* ammonia solution for 5 min followed by 5-min rinsing with water and finally dried with air.

3. Results and discussion

3.1. Direct UV detection of sugars

Fig. 1a shows UV spectra of a copper(II) sulphate solution at pH 5.1 with and without the addition of glucose. It is obvious that either no chelation occurs or a chelate is formed but no change in UV absorption results. However, a marked increase in absorptivity between 230 nm and 320 nm was observed when copper(II) in ammonia solution at pH 11.3 was used instead of the aqueous copper(II) sulphate solution as shown in Fig. 1b. In ammonia solutions, Cu(II) is coordinated by four ammonia and two water ligands. Chelation with glucose leads to displacement of water or ammonia ligands by hydroxy groups of the polyhydroxy compound. Under these alkaline conditions the new ligand-metal bonds give rise to increased absorption in the UV range, which is considerably bathochromically shifted compared to the $Cu(NH_3)_4^{2+}$ complex. A similar absorptivity change results on addition of other sugars. Obviously the chelation of Cu(II) leads to formation of a new

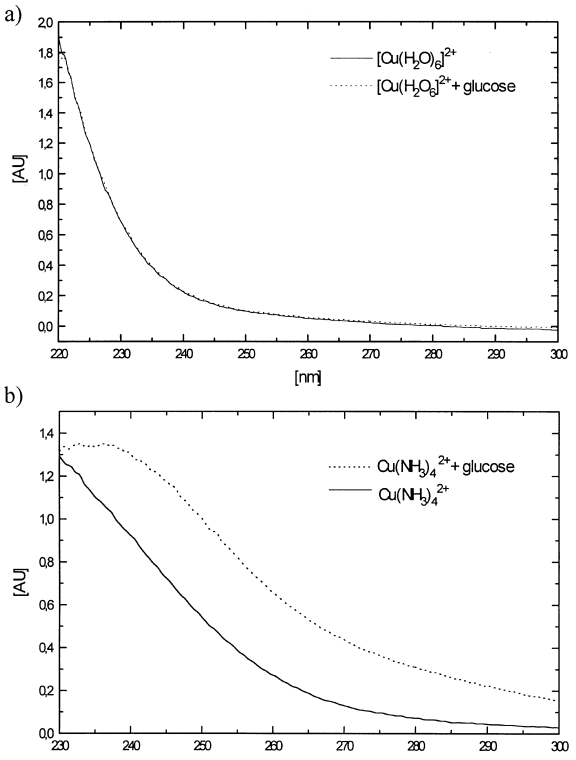

Fig. 1. UV spectra with and without the addition of glucose of (a) 200 μ*M* copper(II) sulphate solution at pH 5.1; (b) 200 μ*M* copper(II) sulphate in ammonia solution at pH 11.3.

chromophore in this alkaline solution. Presumably at this high pH, the polyhydroxy compound is bonded to Cu(II) via alcoholate groups, causing an inner-ligand band in the UV region.

The assumption of alcoholate formation agrees with early investigations of Traube [25] and Bourne et al. [26], who observed that copper(II) hydroxide redissolves in alkaline solutions of polyhydroxy compounds but not in neutral or acid-aqueous solutions.

3.2. Separation and detection of sugars by CE

Fig. 2 shows the CE separation of three sugars with direct UV detection using a Cu(II) electrolyte consisting of 6 m*M* copper(II) sulphate and 500 m*M* ammonia at pH 11.6. Detection was performed at 245 nm, corresponding to the maximum difference between absorptivity of the sugar chelates and the $Cu(NH_3)_4^{2+}$ complex, and also, in the CE system

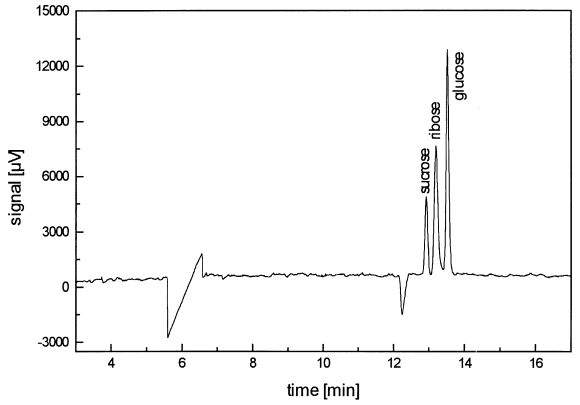

Fig. 2. CE separation and direct UV detection of sucrose, ribose and glucose with Cu(II)-electrolyte; capillary: length 73 cm, 50 cm to detector, 50 μm I.D.; electrolyte: 6 m*M* $CuSO_4$, 500 m*M* NH_3, pH 11.6; conditions: voltage 25 kV, current 13 μA; injection hydrostatically 5 cm, 50 s; sample: each 500 μ*M*; detection: direct UV, 245 nm.

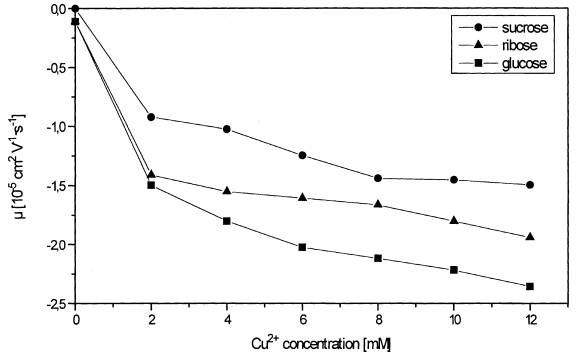

Fig. 3. Dependence of the sugar mobilities on the concentration of Cu(II) in the electrolyte; electrolytes: varying Cu(II) concentrations, pH adjusted to 11.6 with ammonia solution.

developed, the best signal-to-noise ratio was observed at this wavelength. The function of ammonia in the CE electrolyte solution was to prevent a precipitation of copper(II) hydroxide at the high pH required.

Baseline separation was achieved within 14 min. The negative signal at 12.2 min corresponds to the electroosmotic flow. The large signal at 6 min is a system peak which occurs due to the absence of Cu(II) and ammonia in the sample zone. It disappeared when the sample was prepared in the electrolyte.

Most remarkable is the fact that the sugars migrated after the EOF as anionic species despite the two positive charges of Cu(II) in the sugar–copper(II) chelate. Moreover the anionic mobility of the sugars increased with increasing Cu(II) concentration in the electrolyte, as shown in Fig. 3, whereas rapidly decreased mobility was observed for very low copper concentrations. These results confirm the assumption of alcoholate bonds between the sugars and Cu(II). In the chelate formed, the two charges of Cu(II) must be overcompensated by the negative charges of complexing alcoholate groups to result in the observed anionic mobility.

Additionally, the EOF was largely influenced by Cu(II). Increased Cu(II) concentration leads to reduced EOF mobility, as this highly-charged transition metal affects the electrical double-layer at the capillary wall and thus the ζ-potential. This is similar to an observation by Honda et al. [22] who found a strongly decreased or even reversed EOF in electrolytes containing high concentrations of alkaline-earth metals.

However, in contrast to 'free' copper(II), the $Cu(NH_3)_4^{2+}$ present in the electrolyte solutions employed, did not reverse the EOF at any concentration.

The optimum electrolyte concentration was found to be 6 m*M* Cu(II). Higher concentrations lead to higher baseline noise due to the UV absorptivity of $Cu(NH_3)_4^{2+}$ and therefore to decreased signal-to-noise ratios. Resolution of the compounds increased with increasing Cu(II) concentration (according to increasing chelation) and decreased rapidly below 2 m*M* Cu(II). The theoretical plate numbers for the 3 compounds (about 200 000/m) were not affected much by the electrolyte concentration.

These results show that chelation with Cu(II) on the one hand facilitates the mobilization and thus resolution of sugars due to the anionic character of the Cu(II)-sugar species formed and on the other hand allows direct UV detection resulting from the UV absorptivity of this chelate.

3.3. Calibration, reproducibility and detection limits

Calibration curves showed excellent linearity between a concentration of 200–400 μ*M* and 40 m*M* with correlation coefficients between 0.9997 and

0.9999. Of course, the sensitivity of the proposed system is limited by the baseline noise caused by the UV absorptivity of the Cu(II) electrolyte. The limits of detection (3σ) were found to be 50 μM for glucose, 70 μM for ribose and 90 μM for sucrose. Reproducibility was 3% R.S.D. for the migration times and 4.5% R.S.D. for the peak areas of 1 mM standards for eight consecutive runs.

3.4. Simultaneous determination of sugars, inorganic cations and anions

Fig. 4 shows the simultaneous determination of inorganic cations, sugars and amino acids using a 7.5 mM Cu(II) electrolyte. Similar to the sugars, direct detection of amino acids was possible due to complexation of Cu(II), which gave rise to a positive signal in this system. Detection of inorganic cations was achieved by an indirect detection mechanism. $Cu(NH_3)_4^{2+}$ occurs as a cationic species with absorptivity at the wavelength employed and therefore acts as detector-active co-ion for the indirect detection of cations. Sensitivity for cations is of course rather low, because the UV absorption of $Cu(NH_3)_4^{2+}$ is not very high at 245 nm and mobility does not match very well to inorganic cations. Nevertheless millimolar concentrations could be determined, when the Cu(II) concentration in the electrolyte was increased to 7.5 mM. It has to be admitted that the increased Cu(II) concentration leads to reduced EOF velocity and therefore to a longer analysis time, as seen in Fig. 4.

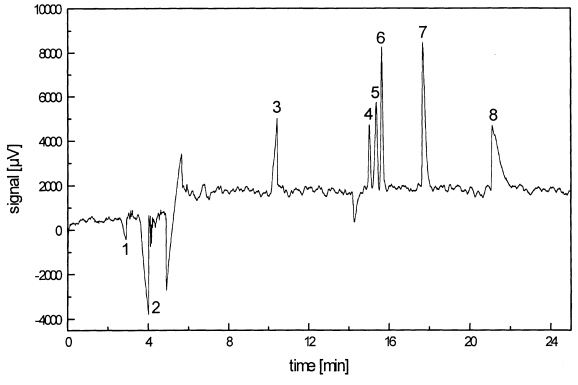

Fig. 4. Simultaneous determination of sugars, inorganic cations and amino acids; electrolyte contains 7.5 mM $CuSO_4$, other conditions as in Fig. 2. Peak identities: 1=K^+, 2=Na^+, 3=glutamine, 4=sucrose, 5=ribose, 6=glucose, 7=aspartate, 8=glutamate.

Nevertheless the $Cu(NH_3)_4^{2+}$ electrolyte may be useful for the simultaneous determinations of sugars, amino acids and inorganic cations in biological samples, where inorganic ions occur in very high concentrations.

4. Conclusion

A new electrolyte system for the CE separation of sugars and direct UV detection without derivatization was developed. Chelation of Cu(II) by sugars under alkaline conditions gave rise to absorption in the UV range and provided for the separation of sugars. Additionally, simultaneous detection of inorganic cations and amino acids was possible. Thus, the new electrolyte allows the determination of chemically very different solutes, and thus much information can be obtained from a single run. Of course, on the other hand, with this approach an inevitable loss in selectivity results, compared to the sugar determinations often achieved using pre-column derivatization, where simultaneous detection of such species is not provided.

In future papers, the applicability of the Cu(II) electrolyte for sugar determinations in biological samples of very low volumes down to single plant cells will be investigated. In such low volume biological samples (pl–nl range), derivatization of sugars is nearly impossible and simultaneous determinations of different compounds are essential, as division of pl-samples for different determinations is rather difficult.

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