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Simultaneous Quantitative Analysis of Maillard Reaction Precursors and Products by High-Performance Anion Exchange Chromatography[||]

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A new analytical setup allowing the simultaneous analysis of precursors and products of the Maillard reaction is described. It is based on high-performance anion exchange chromatography with electrochemical (ECD) and diode array detectors (DAD) coupled in series. Chromatography and detection were optimized to permit simultaneous monitoring of compounds relevant to the Maillard reaction, such as the sugar, the amino acid, and the corresponding Amadori compound as well as the cyclic intermediates 5-(hydroxymethyl)-2-furaldehyde, maltol, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one. Separation was achieved on a CarboPac PA-1 column using a gradient of sodium acetate in aqueous sodium hydroxide. The Amadori compound, glucose, and glycine were monitored by an ECD operating in the integrated amperometry mode. The number of analyzed compounds was further increased by coupling the ECD with a DAD for the analysis of ultraviolet-active constituents. This method was successfully applied to model Maillard reaction mixtures based on glucose and glycine.

KEYWORDS: Maillard reaction; Amadori compounds; quantitative analysis; anion exchange chromatography; integrated amperometry; simultaneous analysis

The Maillard reaction, also called nonenzymatic browning, is a complex network of reactions involving carbonyl and amino compounds, such as reducing sugars and amino acids (*1*). It is the main reaction responsible for the transformation of precursors into colorants and flavor compounds during food processing. In general, similar mechanisms also occur under physiological conditions, and a considerable part of Maillard research has been devoted to medical chemistry focusing on health and disease (*2*, *3*). In the course of the Maillard reaction, the sugar molecule is broken down into reactive intermediates that enter further reactions, thus increasing not only the complexity but also the diversity of the reaction mixture.

The initial phase of the Maillard reaction leads to the formation of 1-amino-1-deoxyketoses of amino acids, known as Amadori compounds. For example, glucose and glycine give rise to *N*-(1-deoxy-D-fructos-1-yl)glycine (**1**) (**Figure 1**) that undergoes further reactions, basically following two main decomposition pathways: 1,2-enolization (A) and 2,3-enolization (B). 5-(Hydroxymethyl)-2-furaldehyde (**4**) is formed from hexoses via 3-deoxyhexosones (**2**, pathway A), whereas 1-deoxy-2,3-hexosuloses (**3**) generate 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**5**) as a typical marker of pathway B. As the early stage of the Maillard reaction influences the course of the whole reaction, understanding of these phenomena is crucial. This requires quantitative data of compounds representing the various reaction stages, preferably obtained using methods allowing simultaneous analysis of various types of Maillard compounds. Even though this is usually a challenging task for the analytical chemist, such quantitative data are the basis for optimization of flavor formation, control of browning reaction, or reaction modeling. This approach reduces significantly the number of analytical runs required for data collection.

Separation and quantitative determination of individual classes of Maillard compounds have been achieved using various chromatographic techniques (**Table 1**). In general, high-performance liquid chromatography (HPLC) offers neither sufficient resolution nor satisfactory sensitivity for simultaneous analysis of Maillard compounds. An efficient HPLC method for analyzing Amadori compounds, which offers both good separation and sensitivity, has been reported by Eichner and co-workers (*8*, *9*). However, whereas parent sugars and Amadori compounds can be analyzed in the same run, parent amino acids cannot. Although GC shows better separation efficiency as compared to HPLC, the Amadori compounds need to be converted into volatile compounds prior to analysis. The necessity to derivatize and the ability of gas chromatography

[||] Dedicated to Professor Dr. Werner Grosch on the occasion of his 70th birthday.

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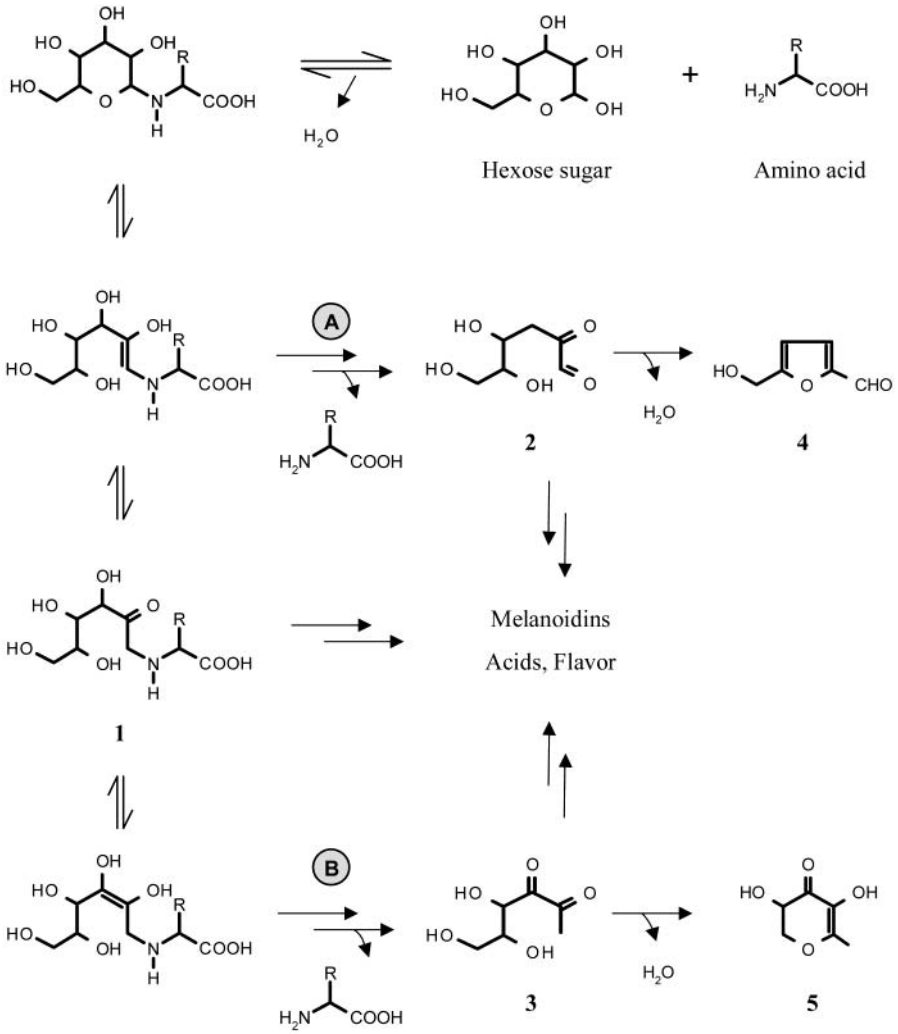

Figure 1. Simplified scheme showing parts of the Maillard reaction (for explanation see text): **1**, *N*-(1-deoxy-D-fructos-1-yl)glycine (R = H); **2**, 3-deoxyhexosone; **3**, 1-deoxy-2,3-hexosulose; **4**, 5-(hydroxymethyl)-2-furaldehyde; **5**, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one.

(GC) to separate tautomeric forms of Amadori compounds are the major drawbacks of this method (*10*).

Chromatographic analysis of sugars can be substantially improved by high-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). This technique takes advantage of the affinity between the ionized group of sugars at alkaline pH and a pellicular quaternary amine stationary phase (*15*), thus offering excellent resolution, and also the highly selective and sensitive detection of nonderivatized sugars at picomole levels with minimal sample cleanup. HPAEC should also offer good resolution for Amadori compounds as they contain the sugar moiety (**Table 1**). Indeed, phenylalanine, glucose, and the corresponding Amadori compound were analyzed by HPAEC using a CarboPac PA-1 column with a PAD to determine the sugar and an ultraviolet (UV) detector to monitor the amino acid and Amadori product (*12*). However, this method was applicable only to aromatic amino acids and their Amadori compounds. Amino acids and Amadori compounds were determined quantitatively by PAD using a gradient of acetonitrile and phosphate buffer on an aminopropyl column (*11*).

The aims of this work were (i) to define both chromatographic and electrochemical conditions for achieving separation and determination of Amadori compounds and the parent precursors and (ii) to extend the method to UV-active cyclic Maillard products.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: glycine, D-mannose, D-fructose, sodium acetate (NaOAc), diethyl ether, 2-propanol, acetic acid, and sodium chloride (Merck, Darmstadt, Germany); D-glucose, 5-(hydroxymethyl)-2-furaldehyde (**4**), maltol, disodium hydrogenphosphate, and deuterium oxide (^2H_2O, Sigma-Aldrich, Steinheim, Germany); piperidine and ethanol (Fluka, Buchs, Switzerland); sodium hydroxide (NaOH) 46/48% solution (Fisher Scientific, Pittsburgh, PA); and sodium hydrogen carbonate (Prolabo, Paris, France). The solutions and eluents were prepared using ultrapure deionized water (specific resistivity ≥ 18.2 MΩ·cm) from a Milli-Q-system (Millipore, Bedford, MA). NaOH solutions used as eluents were prepared by diluting a carbonate-free 46/48% (w/w) NaOH solution in water previously degassed with helium gas. The poly(vinylidene fluoride) (PVDF) filter (0.22 μm/25 mm) was from Supelco (Bellefonte, PA). The Amadori compound *N*-(1-deoxy-D-fructos-1-yl)glycine (**1**) was prepared from D-glucose and glycine as reported by Staempfli and co-workers (*16*).

Synthesis. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**5**) was prepared following the procedure described by Kim and Baltes (*17*) using some modifications. After glucose (0.2 mol) and piperidine (0.2 mol) had been refluxed in ethanol (150 mL) for 90 min, acetic acid (0.2 mol in 30 mL ethanol) was added, and the mixture was further refluxed for 22 h. Ethanol was evaporated under reduced pressure to one-third of its original volume, then filtered, and washed with 2-propanol (30 mL). After evaporation of the solvent, water (200 mL) and sodium chloride (60 g) were added. The pH was adjusted to 4.0 with HCl (0.1 mol/L), and the neutral compounds were extracted

Table 1. Analytical Methods for Separation and Quantification of Nonvolatile Maillard Reaction Products

method	column	detection[a]	compounds	comments	ref
HPLC	Aminex A-9 (H^+)	refractometer	5 Amadori compounds amino acids	limited detection sensitivity and separation efficiency	*4*
HPLC	μBondapak CH	UV (254 nm), as *p*-nitro-benzoyloximes	Amadori compounds	separated from aromatic amino acids using μBondapak-NH_2	*5*
HPLC	μBondapak NH_2	refractometer	4 Amadori compounds and parent precursors	semipreparative isolation and purification of Amadori comp.	*6*
HPLC	DEAE-Si	bis (480 nm), as triphenyl formazans	16 Amadori compounds	postcolumn derivatization with triphenyl tetrazolium chloride	*7*, *8*
HPLC	RP-C_{18}	refractometer	1 Amadori compound and parent precursors	Amadori compound separated from glucose and proline	*9*
GC	capillary, OV-101	FID, MS, as trimethylsilyl-oxime derivatives	11 Amadori compounds	double peaks due to oximation (syn and anti isomers separated)	*8*, *10*
HPAEC	aminopropyl	PAD, fluorescence detector, DAD	3 Amadori compounds and parent precursors	fluorescence detector for tryptophan and derivatives	*11*
HPAEC	CarboPac PA-1	PAD (Amadori, sugar), UV (amino acid)	3 Amadori compounds and parent precursors	only aromatic compounds, interference from NH_4OH	*12*
HPAEC	CarboPac PA-1	PAD	5 Amadori compounds and glucose	amino acids not detected	*13*
HPAEC	CarboPac PA-1	ECD, integrated amperometry	1 Amadori compound and parent precursors	applied to kinetic studies in Maillard model systems	*14*

[a] DAD, diode array detector; ECD, electrochemical detector; FID, flame ionization detector; GC, gas chromatography; HPAEC, high-performance anion exchange chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometer; PAD, pulse amperometric detector; RP, reversed phase; UV, ultraviolet detector; vis, visual.

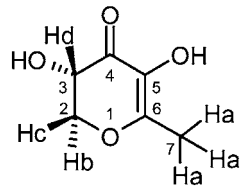

Figure 2. Chemical structure of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**5**).

overnight with diethyl ether (150 mL) at 45 °C using a rotation perforator (300 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure. Distillation of the residue at 0.03 mbar and 170 °C gave a yellow oil containing the target compound as evidenced by GC. Diethyl ether (1 mL) was added to the distillate leading to the target compound by precipitation in the refrigerator. The pale yellow solid material was filtered. The precipitation was repeated twice until the target compound was obtained as a white solid (440 mg, 3.1 mmol, 1.5% yield, 95% purity by GC): GC, RI(DB-Wax) = 2302; MS (*m*/*z*, rel %) 144 (M^+, 30), 101 (25), 73 (15), 72 (15), 55 (15), 45 (20), 44 (60), 43 (100); ^{1}H NMR (360 MHz, ^2H_2O) δ 2.10 (s, $3H_a$, CH_3), 4.29 (dd, $1H_b$, CH_2, ^3J = 10.3 Hz, ^2J = 8.1 Hz), 4.30 (dd, $1H_c$, CH_2, ^3J = 2.8 Hz, ^2J = 8.1 Hz), 4.43 (dd, $1H_d$, CH, ^3J = 2.8 Hz, ^3J = 10.3 Hz); ^{13}C NMR (90 MHz, ^2H_2O) δ 18.2 (CH_3, C_7), 70.3 (CH_2, C_2), 74.2 (CH, C_3), 144.0 (C_q, C_6), 168.2 (C_q, C_5), 191.3 (C=O, C_4). For signal assignment and numbering, see **Figure 2**.

Reference Solutions. Stock solutions A, B, and C were prepared by dissolving 0.5 mmol of each pure compound in 100 mL of deionized water, that is, solution A contained D-glucose (90 mg), D-mannose (90 mg), D-fructose (90 mg), and glycine (37.5 mg); solution B contained Amadori compound **1** (118.5 mg); solution C contained compound **4** (63 mg), compound **5** (64 mg), and maltol (63 mg). The solutions were distributed in small tubes (5 mL) and stored at −20 °C. When needed, the stock solutions were defrosted and diluted to establish calibration curves. Dilutions ranged from 0.013 to 1000 μmol/L.

Model Reactions. Aliquots (2 mL) of solution of Amadori compound **1** (5 mmol) in phosphate buffer (50 mL, 100 mmol/L, pH 8) were placed in Pyrex tubes and heated at 120 °C for a defined period of time. Alternatively, a solution of glucose (150 mmol) and glycine (150 mmol) in water (150 mL), adjusted to pH 9 with NaOH, was heated at 90 °C. After cooling, an aliquot of the reaction mixture (1 mL) was diluted 1000 times with deionized water, passed through a PVDF filter (0.22 μm/25 mm), and analyzed by HPAEC.

Table 2. Gradient Program for the Simultaneous Analysis of Sugars, Amadori Compounds, Amino Acids, and Cyclic Maillard Intermediates[a]

		gradient (% by vol)		
time (min)	modification	water	NaOH	NaOAc
0	initial conditions	96	4	0
1	increase NaOAc	93	4	3
29	isocratic conditions	93	4	3
34	increase NaOAc	16	4	80
39	isocratic conditions	16	4	80
54	cleaning, regeneration	0	100	0
64	conditioning of column	96	4	0

[a] The concentration of the eluents was 300 mmol/L for both sodium acetate (NaOAc) and sodium hydroxide (NaOH). The flow rate was kept constant at 1 mL/min throughout the program.

HPAEC. D-Glucose, D-mannose, D-fructose, glycine, maltol, and compounds **1**, **4**, and **5** were analyzed on a DX 500 Dionex system (Dionex Corp., Sunnyvale, CA) composed of an autosampler (model AS 50 with a 50 μL sample loop), a gradient pump (model EG40) with on-line degassing, a diode array detector (DAD, model UVD 340S), and an electrochemical detector (ECD, model ED40). Separation was accomplished on a CarboPac PA1 anion exchange column (250 × 4 mm, Dionex) and a CarboPac PA1 guard column (50 × 4 mm, Dionex) using the gradient as shown in **Table 2** with a constant flow rate of 1 mL/min throughout the program. Compound **4** (RT = 5.4 min, λ = 285 nm), maltol (RT = 33.4 min, λ = 320 nm), and pyranone **5** (RT = 34.9 min, λ = 350 nm) were quantified using a DAD. D-Glucose (RT = 18.1 min), D-mannose (RT = 19.5 min), glycine (RT = 27.4 min), and Amadori compound **1** (RT = 36.5 min) were quantified with an ECD working in the integrated amperometry mode and equipped with a gold working electrode. The waveform was used as previously described (*14*). All compounds were quantified using calibration curves by comparing the peak areas with those of standard solutions containing known amounts of pure compounds.

Gas chromatography—mass spectrometry (GC-MS) was carried out on an HP-6890A GC coupled to an HP-5973N mass selective detector (Hewlett-Packard). Samples were injected splitless (1 μL). MS-EI spectra were generated at 70 eV. Analyses were performed on a DB-Wax, 60 m × 0.25 mm, 0.25 μm film thickness (J&W Scientific, Folsom, CA) or an HP-PONA, 60 m × 0.25 mm, 0.25 μm film thickness (Hewlett-Packard) using the following temperature program: 20 °C (1 min), 70 °C/min to 60 °C, 4 °C/min to 240 °C.

Nuclear Magnetic Resonance (NMR) Spectroscopy. Samples were prepared in Wilmad 528-PP 5 mm Pyrex NMR tubes, using deuterated water as solvent. NMR spectra were acquired on a Bruker AM-360 spectrometer, equipped with a quadrinuclear 5 mm probe head, at 360.13 MHz (^{1}H) and 75.56 MHz (^{13}C) under standard conditions (*18*).

RESULTS AND DISCUSSION

In this work, HPAEC was evaluated for the simultaneous determination of glucose, glycine, and the corresponding Amadori compound *N*-(1-deoxy-D-fructos-1-yl)glycine (**1**), as well as the cyclic Maillard intermediates 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**5**), 5-(hydroxymethyl)-2-furaldehyde (**4**), and maltol using a nondestructive DAD coupled in series with an ECD. These compounds represent some of the major reaction pathways and intermediates of the Maillard reaction (**Figure 1**).

Analytical Conditions. As Amadori compounds contain both the sugar and amino acid moiety, they possess positive or negative charges depending on the pH of the solution. Therefore, Amadori compounds can be retained not only on an anion exchange resin but also on a cation exchanger. Retention of Amadori compounds on a cation exchange resin is often used as a cleanup step in the synthesis or prior to the analysis of Amadori compounds. However, these columns are not capable of retaining sugars and, thus, are not suitable for simultaneous analysis of sugars and corresponding Amadori compounds. As anion exchange columns can retain not only the Amadori compounds and amino acids but also sugars, the anion exchange column CarboPac PA-1 containing a pellicular quaternary amine stationary phase was selected in this work.

The gradient of sodium acetate in aqueous sodium hydroxide was evaluated to find chromatographic conditions enabling (i) separation of glucose, glycine, and Amadori compound **1** and (ii) quantification of all three compounds by electrochemical detection. No interferences with the ECD were observed under these conditions. Best results were obtained with a gradient composed of water, sodium hydroxide (300 mmol/L), and sodium acetate (300 mmol/L) as shown in **Table 2**. The gradient starts with a mixture of water and NaOH (4%), followed by a first rapid increase of sodium acetate to 3% (1 min), isocratic for 28 min, and a second increase of sodium acetate to 80% (5 min), then isocratic for 5 min. Each analytical cycle was followed by cleaning and regeneration of the column with NaOH (300 mmol/L) and equilibration of the column using the initial gradient conditions.

Glucose, glycine, and Amadori compound **1** were detected using an ECD working in integrated amperometry mode. This detection permits direct and simultaneous analysis of all three compounds without any derivatization (**Figure 3A**). In addition, mannose and fructose can be detected in the same run. Using the conditions described above, three hexose sugars, glycine, and the Amadori compound **1** were separated in one chromatographic run within 40 min.

The spectrum of the compounds of interest was further increased by coupling the ECD with a DAD. The analysis of compound **4** and cyclic enolones is of special interest as these compounds represent markers of specific degradation pathways (1,2-enolization or 2,3-enolization) in the Maillard reaction. Under the chromatographic conditions described above, a DAD was successfully used to analyze compound **4** ($\lambda = 285$ nm), maltol ($\lambda = 320$ nm), and pyranone **5** ($\lambda = 350$ nm) in solutions prepared from reference compounds (**Figure 3B−D**). Depending on the specific interest, the wavelength can be adapted to increase detection sensitivity. For example, compound **4** is preferably detected at 285 nm (**Figure 3B**), whereas compound **5** responds best at 350 nm (**Figure 3D**).

Table 3. Detection Limit and Linear Range for the Analysis of Some Hexose Sugars, the Amadori Compound (**1**), Glycine, 5-Hydroxy-2-furaldehyde (**4**), Maltol, and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**5**)

compound	method of detection	detection limit (μmol/L)	linear range (μmol/L)	R^2 value
glucose	integrated amperometry	1.0	1.0–100	0.9998
				0.9988
				0.9996
mannose	integrated amperometry	1.0	1.0–100	0.9998
				0.9990
				0.9996
fructose	integrated amperometry	1.2	1.2–100	0.9992
				0.9996
				0.9999
glycine	integrated amperometry	2.0	2–50	0.9966
				0.9941
				0.9978
Amadori compound (**1**)	integrated amperometry	0.5	0.5–100	0.9988
				0.9999
				0.9941
4	UV ($\lambda = 285$ nm)	0.01	0.01–500	1.000
				0.9984
				0.9983
maltol	UV ($\lambda = 320$ nm)	0.06	0.06–1000	1.000
				0.9999
				0.9999
pyranone **5**	UV ($\lambda = 350$ nm)	0.2	0.2–450	0.9977
				0.9993
				0.9990

Detection Limits and Linearity. The detection limits, defined as the amount of a compound producing a signal-to-noise ratio of ≥ 3, were determined using standard compounds (**Table 3**). They were in the range of 0.01−2.0 μmol/L, depending on the substance. Compound **4** showed the lowest detection limit of 0.01 μmol/L and glycine the highest detection limit of 2.0 μmol/L. The detector response for individual compounds was linear over a broad concentration range and went up to ~100 μmol/L for compounds detected by ECD and to ~500−1000 μmol/L for compounds detected by DAD. Parts A and B of **Figure 4** show the calibration curves of Amadori compound **1** and pyranone **5** based on electrochemical and UV detection, respectively.

Analysis of Maillard Samples. Although Maillard reaction samples are known to be very complex, containing a broad range of small and polymeric substances, no sample cleanup was required for the analysis of Maillard reaction mixtures due to the sensitivity and selectivity of the electrochemical detection. Thus, sample preparation is very convenient and rapid, as it consists only in dilution of the sample with water. An example of a typical chromatogram obtained after glucose and glycine had been heated in a phosphate-buffered solution of pH 9 at 90 °C for 3 h is shown in **Figure 5**. Elution of the compounds was accelerated, compared to **Figure 4**, by increasing the NaOAc concentration. The data indicate isomerization of glucose to mannose and fructose under alkaline conditions and also the formation of the Amadori compound **1**. It should be noted that this type of result can hardly be obtained by any other analytical method in a single run.

Similarly, the degradation of the Amadori compound **1** can be studied using the method described in this work. As shown in **Figure 6**, electrochemical detection permitted quantification of Amadori compound **1**, glycine, glucose, and mannose. In addition, compound **4** and pyranone **5** were quantified in the

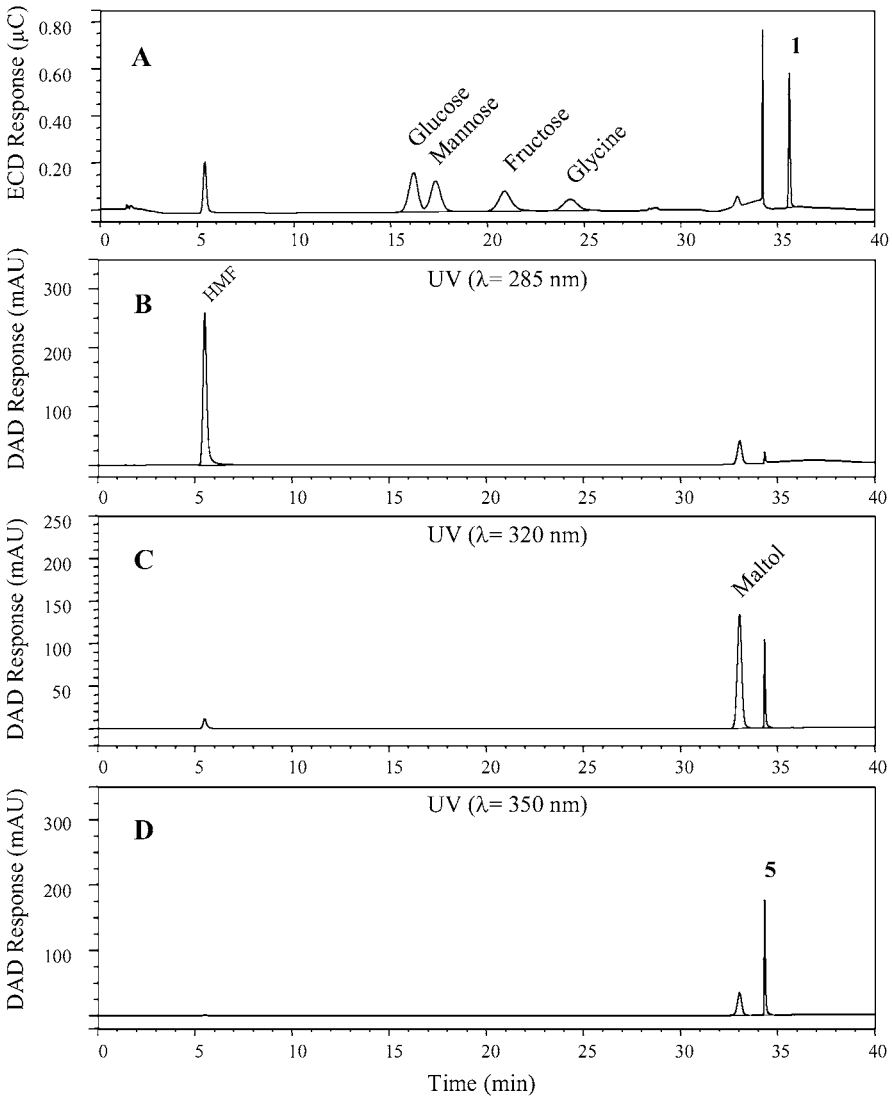

Figure 3. Chromatogram of reference compounds obtained on a CarboPac-PA1 column by (**A**) HPAEC-integrated amperometry for sugars, glycine, and the Amadori compound **1**; (**B**) HPAEC-DAD (λ = 285 nm) for compound **4**; (**C**) HPAEC-DAD (λ = 320 nm) for maltol; and (**D**) HPAEC-DAD (λ = 350 nm) for pyranone **5**.

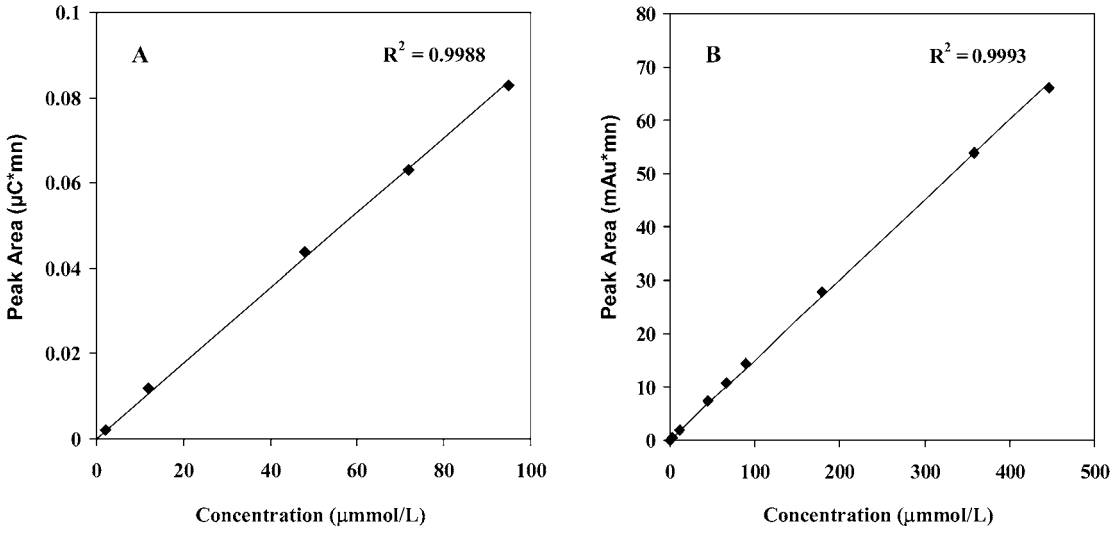

Figure 4. Calibration curves of selected Maillard compounds showing the long linear range for quantification: (**A**) Amadori compound **1**; (**B**) 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**5**).

same run using UV detection at 285 and 350 nm, respectively. Maltol was not detected as it is formed only in trace quantities from glucose or Amadori compound **1**. The simultaneous analysis of these Maillard compounds represents a breakthrough

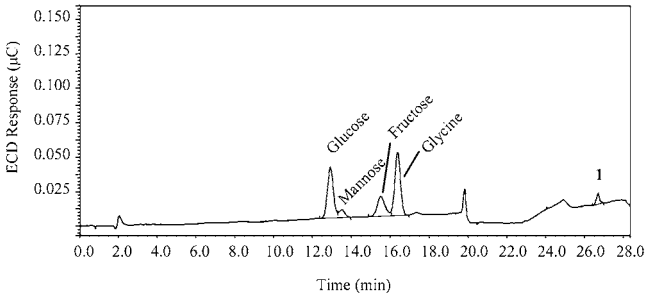

Figure 5. Chromatogram of a Maillard reaction sample obtained after the heating of glucose and glycine in a phosphate-buffered solution of pH 9 at 90 °C for 3 h.

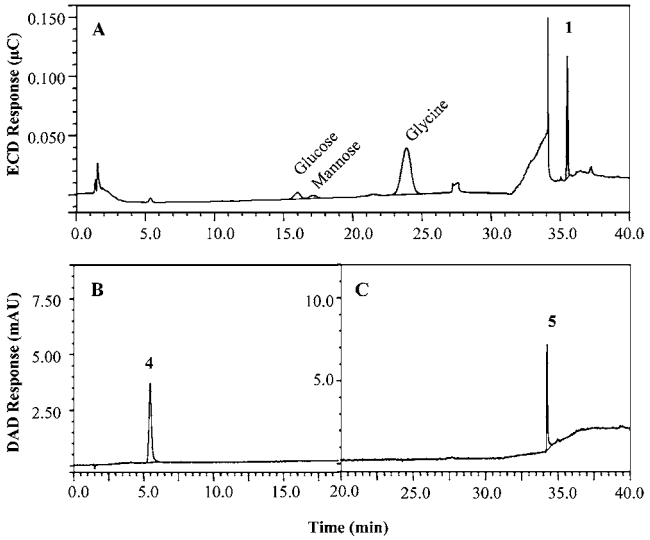

Figure 6. Chromatogram of a Maillard reaction sample containing the Amadori compound **1** heated in a phosphate-buffered solution of pH 8 at 120 °C for 1 h; (**A**) integrated amperometry; (**B**) UV detection at 285 nm; (**C**) UV detection at 350 nm; **1**, *N*-(1-deoxy-D-fructos-1-yl)glycine; **4**, 5-(hydroxymethyl)-2-furaldehyde; **5**, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one.

for studying mechanistic aspects of the Maillard reaction and has recently been used to substantiate the hypothesis of the reversibility of the Amadori rearrangement (*14*). This has become possible as mannose, the epimer of glucose, could be analyzed in the same run along with the Amadori compound, without any isolation step prior to analysis.

In principle, the method described here is also applicable to food and biological samples, provided an appropriate cleanup or concentration step is established. As this depends very much on the sample to be analyzed, optimization would be required to adapt sample cleanup to food and biological systems. For example, in food samples containing high levels of free amino acids, the determination of compound **1** or other glucose-derived Amadori compounds may be complicated by interferences from amino acids. In this case, an ECD can be used in pulsed amperometric mode, instead of integrated amperometry, leading to higher selectivity of the detection due to application of potentials normally used for the determination of sugars ($E_1 = 0.05$ V; $E_2 = 0.75$ V; $E_3 = -0.15$ V). Under these conditions, only sugars and Amadori compounds would be detected (*13*).

In conclusion, a new analytical method based on HPAEC was developed and demonstrated in this work to be an excellent analytical tool to simultaneously monitor the early and intermediate phases of the Maillard reaction. The method permits one, in a single analytical run, to follow not only the Maillard precursors (e.g., glucose and glycine) but also key intermediates such as Amadori compounds and cyclic intermediates (e.g., compound **4** and pyranone **5**), the latter being markers of the 1,2- and 2,3-enolization degradation pathways, respectively. Simple and rapid sample preparation in combination with high sensitivity of detection are the principal advantages of this method as compared to HPLC or GC. It is especially suited to generate kinetic data in a medium/high-throughput manner for multiresponse modeling of the Maillard reaction and to estimate Maillard reaction efficiency as a function of processing conditions. This in turn provides major guidance in the understanding of reaction mechanisms, which is in progress and will be published elsewhere.

ABBREVIATIONS USED

DAD, diode array detector; DEAE-Si, *N*,*N*-diethylaminoethyl-modified silica gel; ECD, electrochemical detector; EI, electron impact; FID, flame ionization detector; GC, gas chromatography; HPAEC, high-performance anion exchange chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; PAD, pulsed amperometric detector; PVDF, poly(vinylidene fluoride); RI, retention index; RP, reversed phase; UV, ultraviolet; vis, visual.

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