Journal of Chromatography B, 878 (2010) 877–882

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Identification and quantification of the glucose degradation product glucosone in peritoneal dialysis fluids by HPLC/DAD/MSMS

Stefan Mittelmaier[a], Michael Fünfrocken[b], Dominik Fenn[b], Thomas Fichert[b], Monika Pischetsrieder[a,*]

[a] Department of Chemistry and Pharmacy, Food Chemistry, Emil Fischer Center, University of Erlangen-Nuremberg, Schuhstr. 19, 91052 Erlangen, Germany
[b] Fresenius Medical Care Deutschland GmbH, Frankfurter Str. 6-8, 66606 St. Wendel, Germany

ARTICLE INFO

Article history:
Received 11 December 2009
Accepted 3 February 2010
Available online 10 February 2010

Keywords:
[alpha]-Dicarbonyl compounds
Glucose degradation products (GDPs)
Glucosone
HPLC
Peritoneal dialysis fluid
Quinoxaline derivatives

ABSTRACT

Glucose degradation products (GDPs) formed during heat sterilization of peritoneal dialysis (PD) fluids exert cytotoxic effects and promote the formation of advanced glycation end-products in the peritoneal cavity. As a result, long-term application of continuous ambulatory peritoneal dialysis is limited. The composition and concentration of GDPs in PD fluids must be known to evaluate their biological effects. The present study describes a targeted screening for novel GDPs in PD fluids. For this purpose, dicarbonyl compounds were converted with o-phenylenediamine to give the respective quinoxaline derivatives, which were selectively monitored by HPLC/diode array detector. Glucosone was thereby identified as a novel major GDP in PD fluids. Product identity was confirmed by LC/MSMS analysis using independently synthesized glucosone as a reference compound. Furthermore, a method was developed to quantify glucosone in PD fluids by HPLC/UV after derivatization with o-phenylenediamine. The method's limit of detection was 0.6 μM and the limit of quantitation 1.1 μM. A linear calibration curve was obtained between 1.1 and 113.9 μM (R^2 = 0.9999). Analyzed at three different concentration levels, recovery varied between 95.6% and 102.0%. The coefficient of variation ranged between 0.4% and 4.7%. The method was then applied to the measurement of glucosone in typical PD fluids. Glucosone levels in double chamber bag PD fluids varied between not detectable and 6.7 μM. In single chamber bag fluids, glucosone levels ranged between 28.7 and 40.7 μM.

1. Introduction

Peritoneal dialysis (PD) has become an adequate alternative to hemodialysis. In PD, the dialysis fluid is administered into the peritoneal cavity using the peritoneum as a natural dialysis membrane. Insufficient biocompatibility of conventional PD fluids, however, limits the long-term application and may lead to fibrosis and vascular sclerosis in the peritoneum. As a consequence, severe peritonitis with impaired remesothelialization occurs, leading to the development of a cellular desert and loss of ultrafiltration capacity [1]. Heat sterilization of PD fluids has been shown to induce formation of glucose degradation products (GDPs). There is growing evidence that GDPs are a main factor in the toxic effects that PD fluids exert on the peritoneum. It has also been shown that GDPs in PD fluids are cytotoxic against peritoneal mesothelium cells and retard remesothelialization *in vitro* [2,3]. Furthermore, GDPs interfere with cell signaling related to functional and morphological alterations in peritoneal cells [4,5]. In patients undergoing PD, peritoneal lesions and increased membrane permeability correlate with the levels of advanced glycation end-products (AGEs), which are non-enzymatic protein modifications caused by sugars or other reactive carbonyl compounds [6,7]. Although GDPs are present at much lower concentration than glucose, those highly reactive compounds are the predominant cause for AGE formation by PD fluids [8]. Finally, apart from the local effects, there is also evidence that GDPs are absorbed during PD, resulting in detrimental systemic effects and AGE formation [9,10]. The important role of GDPs in the adverse effects of PD fluids is further underscored by several clinical or animal studies showing higher biocompatibility of PD fluids with low GDPs, but otherwise analogous composition to conventional PD fluids [11–13].

Detailed knowledge of the composition and concentration of GDPs in PD fluids is crucially important for the investigation of biological and toxicological consequences. To date, glyoxal, methylglyoxal, 3-deoxyglucosone, 3,4-dideoxyglucosone-3-ene, formaldehyde, 5-hydroxymethylfurfural, and 2-furaldehyde have been identified as degradation products of glucose and

Abbreviations: PD, peritoneal dialysis; GDPs, glucose degradation products; AGEs, advanced glycation end-products; DAD, diode array detector; ESI, electrospray ionization; HR-EI, high resolution electron ionization; OPD, o-phenylenediamine; diacetyl$_{qx}$, diacetylquinoxaline; glucosone$_{qx}$, glucosonequinoxaline; LOQ, limit of quantitation; LOD, limit of detection.

* Corresponding author. Tel.: +49 9131 8524102; fax: +49 9131 8522587.
E-mail address: monika.pischetsrieder@lmchemie.uni-erlangen.de (M. Pischetsrieder).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.02.004

quantified in PD fluids [8,14–17]. Additionally, acetaldehyde, which is formed by a glucose dependent mechanism from lactate, has been detected in PD fluids [18]. With knowledge of the chemical composition of GDPs in PD fluids, toxicologically relevant components can be identified and included in clinical studies, quality control, and the development of mitigation strategies.

2. Material and methods

2.1. Reagents and samples

For all experiments, water was taken from a Synergi-185 labwater-system (Millipore, Schwalbach, Germany). HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). All other chemicals and enzymes were purchased from Sigma (Taufkirchen, Germany), Fluka (Steinheim, Germany), or Acros (Geel, Belgium) and were at least of analytical grade, unless noted otherwise.

For the study, typical 1- or 2-chamber PD fluids were used, each type with three different glucose concentrations. Unsterilized PD models were prepared containing either the salts of a conventional PD fluid alone (PD model free of glucose and glucosone), or the salts together with glucose (4%, PD model theoretically free of glucosone).

2.2. Instrumentation

The HPLC/diode array detector (DAD) system consisted of a Jasco (Gross-Umstadt, Germany) 1580 degasser, 1555 autosampler, and 1510 multiple wavelength detector as well as an Agilent (Palo Alto, CA, USA) 1100 series quaternary pump with control module. The system was controlled by Chrompass 1.8. A C18 column (Supelco LC-18-DB, 150 mm × 3 mm, Sigma) was used with the following gradient: A, ammonium formate buffer (10 mM, pH 3.7), B, acetonitrile:ammonium formate buffer 50:50; flow rate: 1 mL/min; [time (min)/% A] 0/94, 20/54, 20.1/100, 23/100, 23.1/94, 28/94. The injection volume was 50 μL.

For HPLC/DAD/MSMS analysis, a Sciex API 2000 quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Applied Biosystems, Foster City, CA, USA) was coupled to an Agilent Series 1100 degasser, binary pump, and diode array detector and a Perkin Elmer (Boston, MA, USA) Series 200 autosampler. System control, data acquisition and processing were performed by Analyst 1.4.1 software.

LC conditions were the same as described for HPLC/DAD, but the flow of the LC was reduced to 0.4 mL/min prior to MS-injection by a three-way-splitter. The ESI-MS was run in the positive ion mode (ion spray voltage: 1500 V). Nitrogen was used as drying gas for solvent evaporation. Source temperature was kept at 500 °C.

High resolution electron ionization (HR-EI-)MS data was acquired using a JMS-GC mate 2 GC/MS system (Jeol, Eching, Germany) operated in direct-inlet mode with electron impact ionization (positive mode, 70 eV). The NMR spectra were recorded with an Avance 600 system (600 MHz, Bruker Daltonics, Bremen, Germany).

2.3. Synthesis of glucosone, glucosonequinoxaline, and diacetylquinoxaline

For the synthesis of glucosone, 5 g D-glucose, 3 mg pyranose oxidase (9.4 u/mg, Sigma, Taufkirchen, Germany), and 3 mg catalase (1927 u/mg, Sigma, Taufkirchen, Germany) were dissolved in 20 mL water and incubated at 25 °C on a roller mixer for about 48 h. Every hour, the solution was aerated with compressed air for 3 min. After removing the enzymes by ultrafiltration (Macrosep Omega, MW cutoff 10,000 Da, Pall, Dreieich, Germany) and lyophilization, a palish yellow powder was obtained.

NMR analysis showed, aside from smaller peaks from other cyclic forms, the α-pyranose form of glucosone as main isomer with the following chemical shifts (signal assignment according to *Freimund and Koepper* [19]; atom numbering refers to Fig. 1, formula 1b): ^{1}H-NMR (D_6-DMSO): δ 7.48 (d, 1H, OH-1); 5.53 (d, 1H, OH-4); 5.33 (d, 1H, OH-3); 4.89 (d, 1H, H-1); 4.58 (t, 1H, OH-6); 4.31 (m, 1H, H-3); 3.93 (m, 1H, H-5); 3.68 (m, 1H, H-6a); 3.55 (m, 1H, H-6b), 3.35 (m, 1H, H-4); ^{13}C-NMR (D_6-DMSO): δ 202.2 (C-2); 93.5 (C-1); 76.1 (C-3); 74.0 (C-4); 72.5 (C-5); 60.4 (C-6). Anal. calcd. for $C_6H_{10}O_6 \times 1H_2O$: 37.03% C, 5.99% H. Found: 36.74% C, 6.17% H.

For the synthesis of (1R,2S,3R)-1-(quinoxalin-2-yl)butane-1,2,3,4-tetraole, the quinoxaline derivative of glucosone, 50 mg glucosone were dissolved in 2 mL aqueous solution of 20 mg/mL o-phenylenediamine (OPD). The mixture was allowed to stand light protected for 2 h at room temperature. The resulting crystals were removed by centrifugation, washed three times with 1 mL water and lyophilized.

The dried product was analyzed by NMR (signal assignment based on H/D-exchange, heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum correlation (HMQC); atom numbering refers to Fig. 1, formula 2) and HR-EI-MS.

^{1}H-NMR (D_6-DMSO): δ 9.10 (s, 1H, H-1); 8.06 (m, 2H, Ar-H); 7.82 (m, 2H, Ar-H); 5.58 (d, 1H, OH-3); 5.15 (d, 1H, H-3); 4.68 (d, 1H, OH-5); 4.60 (d, 1H, OH-4); 4.37 (t, 1H, OH-6); 3.68 (m, 2H, H-5, H-4); 3.65 (m, 1H, H-6a); 3.47 (m, 1H, H-6b); ^{13}C-NMR (D_6-DMSO): δ 159.4 (C-2); 145.2 (C-1); 141.0; 140.8; 129.9; 129.2; 128.8; 128.5; 74.3 (C-4); 72.4 (C-3); 71.2 (C-5); 63.5 (C-6); HR-EI-MS: 250.0954 Da measured; 250.0954 Da calculated.

Diacetylquinoxaline was synthesized analogously to the quinoxaline derivative of glucosone starting with 200 μL diacetyl. In HPLC/DAD/MS analysis, the product appeared as a peak at 18.1 min with a *m/z* of 159 corresponding to [diacetylquinoxaline + H]$^+$; HR-EI-MS: 158.0844 Da measured; 158.0844 Da calculated.

2.4. Verification of peak identity by LC/MSMS

Aliquots of 325 μL each of PD fluid, PD fluid spiked with 51 μM glucosone, or water spiked with 51 μM glucosone were mixed with 75 μL OPD (0.8% in water) and allowed to stand light protected

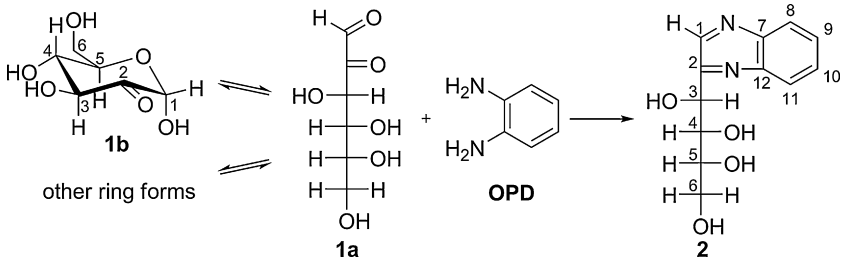

Fig. 1. Structure of glucosone in its open chain form (**1a**), in its α-pyranose form (**1b**) and the corresponding quinoxaline (**2**), which is formed after derivatization with OPD.

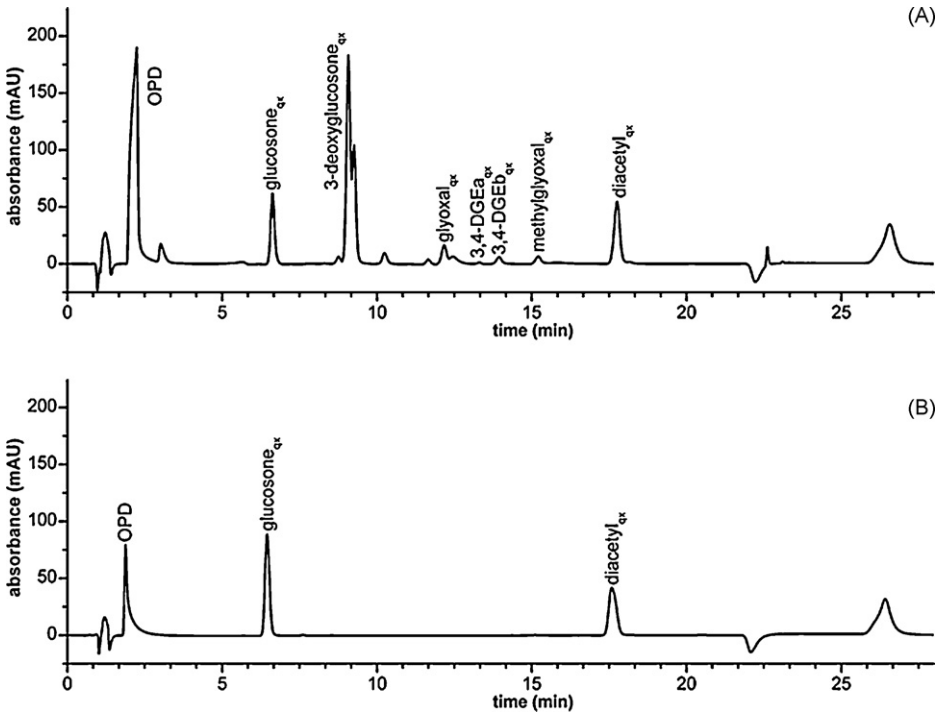

Fig. 2. HPLC/DAD chromatograms of a typical PD fluid (A) and synthesized glucosone in water (B) after adding diacetylquinoxaline and derivatization with OPD, recorded at 316 nm.

for approximately 2 h. HPLC/DAD/MSMS was then used to compare retention times, UV spectra, Q1 spectra, and product ion-spectra of *m*/*z* 251.

2.5. Development of a method to derivatize glucosone with OPD

Aliquots of 400 μL sample, 50 μL diacetylquinoxaline (diacetyl$_{qx}$) (50 μg/mL in water) and 50 μL of different OPD solutions were mixed in amber vials. HPLC/DAD analysis was performed after incubation at room temperature for different periods of time up to 24 h after mixing as indicated. The reaction conditions were optimized using 1%, 2% or 4% OPD in water as reagent and solutions of 51 μM glucosone in unheated PD-fluid or water as sample.

De novo formation of glucosone during derivatization in a PD model initially free of glucosone was determined for water, ammonium formate buffer (1 M, pH 3.8), sodium phosphate buffer (1 M, pH 7.0), and HEPES buffer (1 M, pH 7.0, 6.5, and 7.5) as solvent for a 4% OPD solution. Results were calculated as area of glucosonequinoxaline (glucosone$_{qx}$) peak/area of diacetyl$_{qx}$ peak and plotted against the derivatization time.

2.6. Quantification of glucosone in PD fluids and validation of the method

Aliquots of 400 μL of each sample were mixed with 50 μL diacetyl$_{qx}$ (50 μg/mL in water) and 50 μL OPD (4% in 1 M HEPES buffer, pH 7.0), allowed to stand light protected for at least 5 h and used for HPLC/DAD analysis within 24 h after addition of the derivatization reagent. Quantification was carried out with a calibration curve using the quotient of the areas of glucosone$_{qx}$ and diacetyl$_{qx}$. For the calibration curve, a stock solution (1.014 mg/mL glucosone in water) was prepared and diluted with water to give standard solutions of 1.1, 2.8, 5.7, 11.4, 28.5, 56.9, and 113.9 μM.

For validation, the following parameters were determined: precision, recovery, linearity, limit of quantitation (LOQ), and limit of detection (LOD).

To determine recovery rates, an unheated PD fluid was spiked with 113.9, 11.4, or 1.1 μM of glucosone. These samples and an unspiked fluid were analyzed as described above after four different derivatization times (5, 11, 17, 24 h). The mean recovery of four experiments for each concentration level and derivatization time was determined and expressed as: (glucosone concentration–glucosone concentration of the unspiked sample)/added glucosone concentration × 100%.

Precision was calculated by four independent replicate measurements and was expressed as standard deviation and coefficients of variation.

LOD was calculated as $y_{blank} + 3.3 \times SD_{blank}$ and LOQ as $y_{blank} + 10 \times SD_{blank}$ from 10 independent replicate acquisitions using a PD model mixture without glucose as blank.

The seven-point linearity curve was prepared in four replicates (1.1–113.9 μM glucosone in water). The calibration curve was obtained by plotting the quotient of the peak areas of glucosone$_{qx}$ and diacetyl$_{qx}$ against the glucosone concentration. The linearity of the calibration curve was evaluated by linear regression analysis with a minimally acceptable correlation coefficient of 0.990. Furthermore, linearity and homogeneous distribution of variances was confirmed according to Mandel.

3. Results

3.1. Targeted screening for α-dicarbonyl compounds in PD fluids

The purpose of the present study was to screen PD fluids for the presence of unidentified GDPs with α-dicarbonyl structure. Samples of PD fluids were derivatized with OPD and analyzed by HPLC/DAD. OPD efficiently converts α-dicarbonyl compounds into the corresponding quinoxaline derivative (Fig. 1) that shows a characteristic maximum of UV absorbance at 316 nm. This approach is very effective for a targeted screening for α-dicarbonyl compounds in PD fluids. At a retention time of 6.7 min, a major signal with the characteristic UV spectrum of a quinoxaline appeared, which

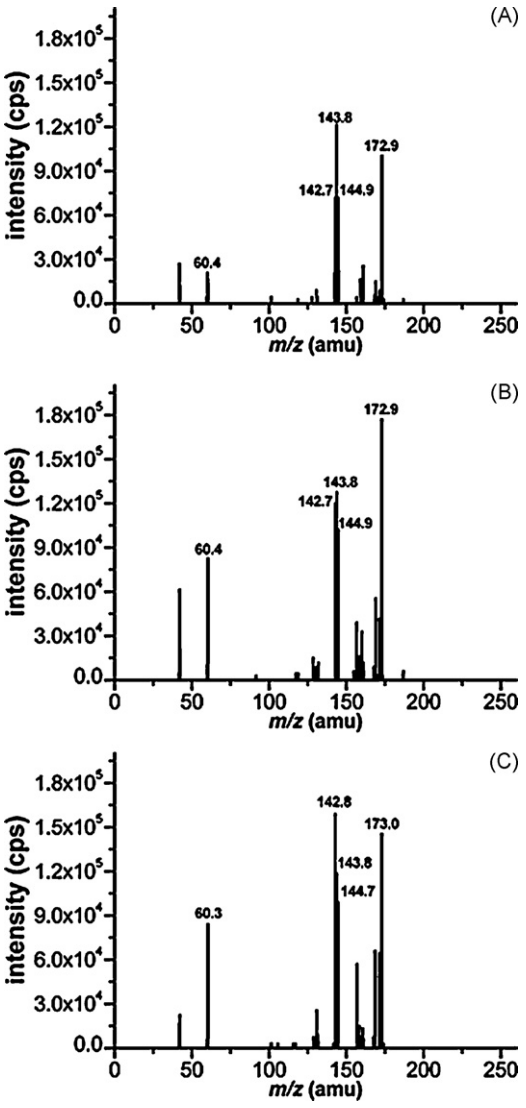

Fig. 3. Product ion scans of the peak at 6.7 min in PD fluid (A), PD fluid spiked with glucosone (B) and water spiked with glucosone (C) recorded by LC/MSMS; *m*/*z* 251 was used as parent ion.

did not correspond to any of the previously identified α-dicarbonyl compounds in PD fluids [16] (Fig. 2A).

In order to identify the unknown putative α-dicarbonyl compound, the sample was analyzed by LC/MSMS revealing a signal for *m*/*z* 251 in the Q1 spectrum at the corresponding retention time. Fig. 3A shows the product ion spectrum of the parent ion *m*/*z* 251. The mass of 251 Da indicates the presence of the quinoxaline derivative of glucosone, an oxidation product of glucose $[glucosone_{qx} + H]^+$.

3.2. Synthesis of a glucosone standard

To verify the identity of the unknown GDP, glucosone was synthesized as a reference compound. Huwig et al. reported the preparation of glucosone by oxidation of D-glucose using pyranose oxidase and catalase to remove the by-product H_2O_2 [20]. After some modifications, this method yielded a practically complete conversion of glucose, at the same time avoiding supplements such as buffer salts. Thus, pure glucosone could be obtained as a palish yellow powder without major purification steps. The NMR data were in good accordance with literature confirming the coexistence of several ring forms with the α-pyranose form as the main isomer in D_6-DMSO [19]. Purity was confirmed by elemental analysis. Furthermore, the reference compound was converted to the corresponding quinoxaline by derivatization with OPD. Different NMR experiments as well as HR-EI-MS confirmed that the synthesized product indeed was glucosone.

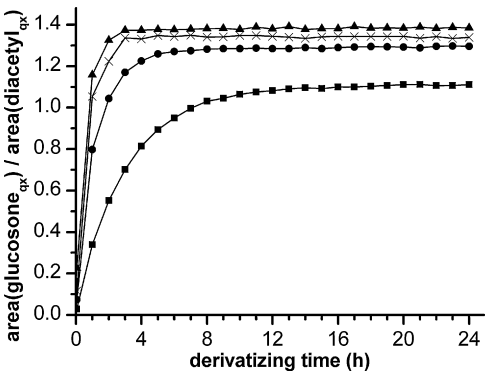

Fig. 4. Effect of derivatization time on measured glucosone amount in water (×) or unsterilized PD fluid (■, ●, ▲) spiked with 50 μM using different OPD concentrations in water (×, ■ 1% OPD; ● 2% OPD; ▲ 4% OPD). Representative time courses of at least two independent experiments are shown. Note, the curve obtained for the PD fluid with 1% OPD (■) did not reach a plateau, but a slight slope was recorded between 8 and 24 h derivatization time.

3.3. Identification of the unknown GDP as glucosone

To assure that the unknown GDP in the PD fluid was identical with the synthesized glucosone, equal amounts of glucosone were added to a PD fluid and water. Furthermore, the PD fluid was analyzed without any addition of glucosone. After derivatization with OPD, samples were analyzed by HPLC/DAD/MSMS: All the samples showed a peak at 6.7 min with identical UV-, Q1- and product ion-spectra (Fig. 2B, Fig. 3). The peak area of the spiked PD fluid at 316 nm amounted approximately to the sum of the peak areas of the unspiked fluid and the aqueous glucosone solution. The UV spectra showed maxima at 238 and 316 nm, which are characteristic for quinoxalines. Under the described MSMS conditions, *m*/*z* 173, 145, 144, 143, and 60 were identified as the main fragments of the parent molecule ion (*m*/*z* 251).

3.4. Development of a derivatization procedure for quantification of glucosone

The derivatization conditions were further optimized for a maximum derivatization yield while minimizing *de novo* formation. Thus, an aqueous solution of glucosone and an unsterilized PD fluid spiked with glucosone (51 μM) were derivatized with 1% OPD in water (the final dilution of OPD was 0.1%) and analyzed by HPLC/DAD. The peak area of the glucosone-derived quinoxaline was related to the peak area of the internal standard, the diacetyl-derived quinoxaline. In order to avoid bias due to different reaction rates of diacetyl and glucosone with OPD, diacetylquinoxaline was used instead of diacetyl [21]. Thus, a curve was obtained for the aqueous solution of glucosone that reached a plateau after approximately 4 h, indicating that derivatization had been completed (Fig. 4). In contrast, the curve obtained for glucosone in PD fluid did not show a plateau. Under these conditions, the reaction yielded less derivatization product than the reaction in water. This finding can be explained by other substances in the PD fluid that react with amine functions of OPD, thus lowering the reaction rate.

To increase reaction rate and yield, the PD fluid was reacted with higher concentrated OPD. When using 2% OPD, $glucosone_{qx}/diacetyl_{qx}$ ratios were still lower than for the reaction in water, although a plateau could be observed after 8 h. The appli-

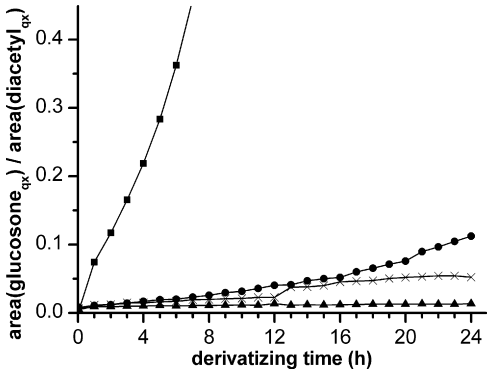

Fig. 5. Effect of derivatization time on the measured glucosone amount in a PD model mixture without glucosone (4% glucose) using OPD in different buffers (x water; ■ ammonium formate pH 3.7; ● sodium phosphate pH 7.0; ▲ HEPES pH 7.0).

cation of 4% OPD, however, led to glucosone$_{qx}$/diacetyl$_{qx}$ ratios that were even slightly higher than for the water model. As the unsterilized PD fluid itself contains a small quantity of glucosone, this result was to be expected. A stable plateau was reached after 5 h. Since 5 h is an applicable reaction time and the limited solubility of OPD in water hampers the use of higher concentrations, we opted for 4% as the optimal concentration of the derivatization reagent.

To determine a possible *de novo* formation of glucosone during derivatization, the behavior of PD fluids without glucosone was tested under different derivatization conditions. For this purpose, unheated model PD fluids were prepared that contained the same salts as commercial PD fluids and 4% glucose of high purity.

When water was used as solvent for OPD without any additives, the quotients of glucosone$_{qx}$ and diacetyl$_{qx}$ areas slightly increased with prolonged reaction time (Fig. 5), which may lead to inaccurate quantification. To avoid this effect and to improve the robustness of the process, several buffers were tested as solvent for OPD. Whereas sodium phosphate buffer (pH 7.0) showed a slope similar to that of pure water, buffering with ammonium formate (pH 3.7) led to a dramatic increase of the glucosone$_{qx}$/diacetyl$_{qx}$-ratio up to 2.8 after 24 h (out of scale in Fig. 5), indicating considerable *de novo* formation. In contrast, no incline of the graph, and thus no *de novo* formation, was observed when HEPES was used at pH 7.0. An increase or decrease of the pH by 0.5 did not result in any noticeable change of the derivatization behavior (data not shown). A slight basal level of glucosone-derived quinoxaline was still detected in these samples. This level, however, was dependent on the purity of the used glucose (data not shown). Besides, the maximum peak area was already reached after a derivatization time of 1 h without further increase during the 24 h of derivatization process. Therefore, it was concluded that the signal is caused by inevitable glucosone impurities in glucose rather than by artificial formation during derivatization. Furthermore, the measured concentrations were lower than the calculated LOD of the method.

Table 1
Validation parameters of the HPLC/DAD method for quantification of glucosone in PD fluids after derivatization with OPD.

Glucosone concentration	Derivatizing time [h]			
	5	11	17	24
113.9 μM glucosone				
Glucosone measured (mean, $n=4$) [μM]	115.0	115.3	116.1	115.4
Standard deviation [μM]	1.0	0.4	0.5	0.8
Variation coefficient [%]	0.8	0.4	0.4	0.7
Recovery (mean, $n=4$) [%]	101.0	101.2	102.0	101.3
11.4 μM glucosone				
Glucosone measured (mean, $n=4$) [μM]	11.5	11.4	11.5	11.6
Standard deviation [μM]	0.3	0.3	0.3	0.3
Variation coefficient [%]	2.3	2.3	2.2	2.7
Recovery (mean, $n=4$) [%]	100.6	100.3	100.9	101.7
1.1 μM glucosone				
Glucosone measured (mean, $n=4$) [μM]	1.1	1.1	1.1	1.1
Standard deviation [μM]	0.0	0.1	0.1	0.0
Variation coefficient [%]	2.7	4.4	4.7	2.7
Recovery (mean, $n=4$) [%]	96.2	97.6	95.6	96.5

Since the reaction rate of a spiked PD fluid was not slower than the reaction of the aqueous glucosone solution when using 4% OPD in HEPES (pH 7.0) and a derivatization time of 5–24 h, these parameters were defined as optimal reaction conditions.

3.5. Validation of the method and quantification of glucosone in PD fluids

For validation, the parameters precision, recovery, linearity, LOD, and LOQ were determined. LOD and LOQ were calculated as 0.6 μM (LOD) and 1.1 μM (LOQ) by using a blank matrix containing the salts and buffers of a conventional PD fluid. The seven-point calibration curve showed a very good linearity over the concentration range of 1.1–113.9 μM ($y = 0.026x + 0.0034$; $R^2 = 0.9999$). Recovery and precision were determined for four different derivatization times at three different concentration levels (Table 1). In all cases, the variation coefficient was less than 5% and recovery did not deviate more than 5% from the actual concentration. The results verify that the described procedure is a very reliable and precise method to quantify glucosone in PD fluids in the specified ranges.

As a last step, glucosone was measured in typical PD fluids with different glucose concentration (1- and 2-chamber bag fluids) using the validated method. The results are summarized in Table 2.

4. Discussion

GDPs, which are formed during heat sterilization of PD fluids containing glucose, have been identified as a primary cause for the low biocompatibility of PD fluids, considerably limiting the long-term application of this blood purification technique. The

Table 2
Concentrations of glucosone in 1- and 2-chamber PD fluids. All values are expressed as μM glucosone in ready-to-use PD-fluid ± standard deviation.

Type of PD fluid	Lot 1[a]	Lot 2[a]	Lot 3[a]	Mean[b]
Double chamber, lactate buffer, 1.5% glucose	2.2 ± 0.1	3.2 ± 0.1	n.q.	2.0 ± 1.3
Double chamber, lactate buffer, 2.3% glucose	n.d.	1.4 ± 0.1	n.d.	0.9 ± 0.5
Double chamber, lactate buffer, 4.25% glucose	6.7 ± 0.1	1.5 ± 0.1	2.3 ± 0.1	3.5 ± 2.8
Single chamber, lactate buffer, 1.5% glucose	31.4 ± 0.8	28.7 ± 0.3	30.3 ± 1.2	30.1 ± 1.4
Single chamber, lactate buffer, 2.3% glucose	26.6 ± 0.5	28.2 ± 0.9	26.6 ± 0.3	27.1 ± 0.9
Single chamber, lactate buffer, 4.25% glucose	35.2 ± 1.0	40.7 ± 0.8	40.2 ± 0.6	38.7 ± 3.0

n.d. below LOD (0.6 μM/L); n.q. below LOQ (1.1 μM/L).
[a] Standard deviation calculated from 4 replicate experiments.
[b] Standard deviation calculated from the different lots.

most common form of PD fluids are single chamber bag fluids, in which glucose is sterilized in a lactate buffered solution at a pH of about 5. In order to reduce GDP formation, double chamber bag PD fluids have been developed, in which the glucose solution is sterilized at a lower pH of about 3. The physiological pH of the PD fluid is subsequently obtained by mixing the glucose solution with a buffer from a separate compartment immediately prior to use [11,16,22].

The present study was the first to identify glucosone as an important GDP in PD fluids. Glucosone has been previously described as a product formed by autoxidative or enzymatic degradation of the Amadori product, which is a primary adduct of glucose and amines [23,24]. In the absence of amines, glucosone is derived from glucose, for example by γ-radiolysis or thermal treatment [25,26].

The different GDP-derived quinoxalines in PD fluids were separated by a method similar to one described in literature [16]. However, changes of the packaging material, the dimension of the HPLC column and the gradient considerably reduced analysis time from 45 to 28 min, without any loss of chromatographic resolution. Prior to quantification, the kinetics of the derivatization reaction with OPD were examined in detail to find the best conditions for PD fluid as matrix. Derivatization with chromophoric agents like OPD or 2,3-diaminonaphthalene is a convenient method that allows separation and detection of dicarbonyl compounds by HPLC/DAD. However, the derivatization conditions must be controlled carefully, because incomplete derivatization or *de novo* formation of the analyte during the process may lead to incorrect quantification [21]. GDPs with large carbon backbone like glucosone show high sterical complexity. Their hemiacetal ring must be opened prior to condensation. As a result, the reaction rates are slow and thus, a strong derivatization agent like OPD is required in high concentration. On the other hand, OPD promotes oxidative reactions during the derivatization process. This may lead to a *de novo* formation of glucosone from glucose, which is abundant in PD fluids. Very good validation results proved the reliability of the optimized derivatization procedure.

The highest glucosone concentrations were detected in heat sterilized single chamber bag PD fluids. Double chamber bag fluids contained considerably lower glucosone concentrations. A similar situation has been observed for other GDPs in PD fluids [16]. The low GDP concentration in double chamber bag fluids has been attributed to the lower pH value in the respective glucose compartment (≤pH 3) in contrast to single chamber bag fluids (≈pH 5) [18]. Obviously, the thermally induced autoxidation of glucose to give glucosone is also significantly inhibited at the low pH present in double chamber bag fluids. In contrast to other GDPs, however, the glucosone concentration did not increase with increasing glucose concentration in the single chamber bag fluids. This result indicates that other factors, for example the quantity of dissolved oxygen, may limit glucose oxidation in PD fluids. Further investigations are now required to identify mechanisms and factors that influence glucosone formation.

In several studies, the biological effects of glucosone have been studied. Thus, it has been shown that glucosone generates superoxide anion at pH 7 and 37 °C [27,28]. Superoxide anion is a reactive oxygen species that can further react to hydrogen peroxide and hydroxyl radicals inducing oxidative stress to a wide range of biomolecules [29,30]. Furthermore, antiproliferating and cytotoxic effects [31,32], which were mediated by the formation of reactive oxygen species [33], were reported as well as mutagenic activity of glucosone *in vitro* [34]. Further studies are now necessary to determine the contribution of glucosone to the toxic effects of GDPs in PD fluids on the peritoneum.

5. Conclusion

Because of the relatively high concentration in PD fluids and potential physiological implications, glucosone is an important, novel glucose degradation product that must be monitored. The newly developed and validated method for the analysis of glucosone in PD fluids proved to be reliable and sensitive and can be applied to product control and the development of new PD fluids with higher biocompatibility.

Acknowledgement

The study was supported by Fresenius Medical Care Deutschland GmbH, Germany.

References

[1] J.W. Dobbie, Perit. Dial. Int. 12 (1992) 14.
[2] J. Witowski, J. Wisniewska, K. Korybalska, T.O. Bender, A. Breborowicz, G.M. Gahl, U. Frei, J. Passlick-Deetjen, A. Jorres, J. Am. Soc. Nephrol. 12 (2001) 2434.
[3] L.W. Morgan, A. Wieslander, M. Davies, T. Horiuchi, Y. Ohta, M.J. Beavis, K.J. Craig, J.D. Williams, N. Topley, Kidney Int. 64 (2003) 1854.
[4] J.C. Leung, L.Y. Chan, F.F. Li, S.C. Tang, K.W. Chan, T.M. Chan, M.F. Lam, A. Wieslander, K.N. Lai, Nephrol. Dial. Transplant. 20 (2005) 1336.
[5] K.N. Lai, J.C. Leung, L.Y. Chan, F.F. Li, S.C. Tang, M.F. Lam, K.C. Tse, T.P. Yip, T.M. Chan, A. Wieslander, H. Vlassara, Clin. Exp. Immunol. 138 (2004) 466.
[6] M. Nakayama, Y. Kawaguchi, K. Yamada, T. Hasegawa, K. Takazoe, N. Katoh, H. Hayakawa, N. Osaka, H. Yamamoto, A. Ogawa, H. Kubo, T. Shigematsu, O. Sakai, S. Horiuchi, Kidney Int. 51 (1997) 182.
[7] K. Honda, K. Nitta, S. Horita, W. Yumura, H. Nihei, R. Nagai, K. Ikeda, S. Horiuchi, Nephrol. Dial. Transplant. 14 (1999) 1541.
[8] A. Tauer, T. Knerr, T. Niwa, T.P. Schaub, C. Lage, J. Passlick-Deetjen, M. Pischetsrieder, Biochem. Biophys. Res. Commun. 280 (2001) 1408.
[9] A. Tauer, T.O. Bender, E.H. Fleischmann, T. Niwa, A. Jorres, M. Pischetsrieder, Mol. Nutr. Food Res. 49 (2005) 710.
[10] M. Zeier, V. Schwenger, R. Deppisch, U. Haug, K. Weigel, U. Bahner, C. Wanner, H. Schneider, T. Henle, E. Ritz, Kidney Int. 63 (2003) 298.
[11] J.D. Williams, N. Topley, K.J. Craig, R.K. Mackenzie, M. Pischetsrieder, C. Lage, J. Passlick-Deetjen, Kidney Int. 66 (2004) 408.
[12] B. Rippe, O. Simonsen, O. Heimburger, A. Christensson, B. Haraldsson, G. Stelin, L. Weiss, F.D. Nielsen, S. Bro, M. Friedberg, A. Wieslander, Kidney Int. 59 (2001) 348.
[13] S. Mortier, D. Faict, N.H. Lameire, A.S. De Vriese, Kidney Int. 67 (2005) 1559.
[14] T. Linden, G. Forsback, R. Deppisch, T. Henle, A. Wieslander, Perit. Dial. Int. 18 (1998) 290.
[15] T. Linden, A. Cohen, R. Deppisch, P. Kjellstrand, A. Wieslander, Kidney Int. 62 (2002) 697.
[16] M. Frischmann, J. Spitzer, M. Funfrocken, S. Mittelmaier, M. Deckert, T. Fichert, M. Pischetsrieder, Biomed. Chromatogr. 23 (2009) 843.
[17] C.B. Nilsson-Thorell, N. Muscalu, A.H. Andren, P.T. Kjellstrand, A.P. Wieslander, Perit. Dial. Int. 13 (1993) 208.
[18] T. Zimmeck, A. Tauer, M. Fuenfrocken, M. Pischetsrieder, Perit. Dial. Int. 22 (2002) 350.
[19] S. Freimund, S. Kopper, Carbohydr. Res. 339 (2004) 217.
[20] A. Huwig, H.-J. Danneel, F. Giffhorn, J. Biotechnol. 32 (1994) 309.
[21] M.A. Glomb, R. Tschirnich, J. Agric. Food Chem. 49 (2001) 5543.
[22] J. Passlick-Deetjen, M. Pischetsrieder, J. Witowski, T.O. Bender, A. Jorres, C. Lage, Perit. Dial. Int. 21 (Suppl. 3) (2001) S96.
[23] S. Kawakishi, J. Tsunehiro, K. Uchida, Carbohydr. Res. 211 (1991) 167.
[24] M. Takahashi, M. Pischetsrieder, V.M. Monnier, J. Biol. Chem. 272 (1997) 3437.
[25] S. Kawakishi, Y. Kito, M. Namiki, Agric. Biol. Chem. 41 (1977) 951.
[26] T. Usui, S. Yanagisawa, M. Ohguchi, M. Yoshino, R. Kawabata, J. Kishimoto, Y. Arai, K. Aida, H. Watanabe, F. Hayase, Biosci. Biotechnol. Biochem. 71 (2007) 2465.
[27] B.J. Ortwerth, H. James, G. Simpson, M. Linetsky, Biochem. Biophys. Res. Commun. 245 (1998) 161.
[28] S. Kawakishi, S. Nasu, R.Z. Cheng, T. Osawa, in: T.-C. Lee, H.-J. Kim (Eds.), Chemical Markers for Processed and Stored Foods, American Chemical Society, Washington, DC, 1996, p. 77.
[29] T. Nakayama, K. Terazawa, S. Kawakishi, J. Nutr. Sci. Vitaminol. (Tokyo) 38 (1992) 593.
[30] R.Z. Cheng, K. Uchida, S. Kawakishi, Biochem. J. 285 (Pt 2) (1992) 667.
[31] K.A. Reiffen, F. Schneider, J. Cancer Res. Clin. Oncol. 107 (1984) 206.
[32] T. Nakayama, K. Terazawa, S. Kawakishi, J. Agric. Food Chem. 40 (1992) 830.
[33] T. Nakayama, M. Yamada, T. Osawa, S. Kawakishi, J. Nutr. Sci. Vitaminol. (Tokyo) 38 (1992) 381.
[34] J.G. Niemand, L. den Drijver, C.J. Pretorius, C.W. Holzapfel, H.J. van der Linde, J. Agric. Food Chem. 31 (1983) 1016.