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Quantitation of dextran 70 in peritoneal dialysate from patients administered 7.5% polyglucose

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Abstract

A method using gel permeation chromatography was evaluated for the quantitation of dextran 70 in dialysate samples containing polyglucose. Dialysate samples containing dextran 70 and polyglucose were pretreated using the enzyme α-amylase to selectively hydrolyze the α(1–4)-linked polyglucose, while leaving the α(1–6)-linked dextran 70 intact. Following sample deproteinization with trichloroacetic acid, dextran 70 was quantitated using gel permeation chromatography with refractive index detection. This method was evaluated for accuracy, precision, specificity, linearity, range, and analyte stability. Adequate method linearity with a correlation of >0.999 was established over the range of dextran 70 concentration from 1 to 0.025 mg/ml. Method precision was approximately 2% R.S.D. and accuracy (% recovery) was approximately 98–100% in the typical sample concentration range (1–0.5 mg/ml). This method was applied to the determination of intraperitoneal fluid kinetics in continuous ambulatory peritoneal dialysis (CAPD) patients administered daily night-time intraperitoneal exchanges with either 7.5% polyglucose or 4.25% dextrose. Dextran 70 was added to the dialysis solutions to yield an initial concentration of 1 mg/ml. Dialysate samples were collected at various times over a 10-h dwell-time and assayed for dextran 70. Intraperitoneal volume profiles based on dextran 70 concentrations and drain volumes were then calculated for each dialysis solution.

Keywords: Polyglucose; Dextrans

1. Introduction

Dextran 70 has been successfully used as a marker for the measurement of intraperitoneal fluid kinetics in peritoneal dialysis patients [1,2]. This procedure requires the addition of dextran 70 (1 g/l) to approximately 2 l of fresh dialysis solution, instillation into a patient, and dialysate sample collection at various times during and at the end of the dwell. After draining at the end of the dwell period, the peritoneal cavity is rinsed with 500 ml of fresh dialysis solution and a sample collected (rinse bag) to calculate residual volume.

Polyglucose (PG), a soluble polymeric form of glucose with an average molecular weight of approximately 14 000–20 000 daltons, has been demonstrated to be an effective osmotic agent for peritoneal dialysis in end-stage renal disease (ESRD) patients providing sustained positive net ultrafiltration [3–5] for long dwells (8–16 h). In order to study intraperitoneal fluid kinetics for exchanges performed with PG-containing dialysis fluid, a method to quantitate dextran 70 in the presence of PG is required. Although a method for determining dextran

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70 in the presence of high glucose concentrations has been described [6], this method is not applicable to samples containing PG. Size separation between dextran 70 and PG is complicated because of overlapping molecular weight distributions and the disproportionately large concentration of PG relative to dextran 70.

This paper fully describes a method that uses the enzyme α-amylase to selectively hydrolyze the α(1–4)-linked polyglucose, while leaving the α(1–6)-linked dextran 70 intact. Following sample deproteinization with trichloroacetic acid, the dextran 70 is quantitated using gel permeation chromatography with refractive index detection. The validation of this method and its application to clinical patient samples for the measurement of intraperitoneal fluid kinetics is presented in this report. A procedure describing the need for enzymatic hydrolysis of the α(1–4)-linked polyglucose (icodextrin) prior to dextran 70 analysis has been previously presented in abstract form [7].

2. Experimental

2.1. Materials

A solution of 6% Gentran 70 (dextran 70) in 0.9% sodium chloride for injection was obtained from Baxter Healthcare (Deerfield, IL, USA). Dulbecco's phosphate buffered saline (PBS), α-amylase (Type II-A) and trichloroacetic acid (TCA) were purchased from Sigma (St. Louis, MO, USA). Sodium hydroxide (10 N) solution (J.T. Baker, Phillipsburg, NJ, USA) and MilliQ water (Millipore, Bedford, MA, USA) were used. An Eppendorf centrifuge Model 5415C (Brinkmann Instruments, Westbury, NY, USA) was used for sample deproteinization following TCA precipitation.

2.2. Apparatus and chromatographic conditions

The chromatography system (Waters, Milford, MA, USA) consisted of a Model 600E gradient pump, a Model 410 refractive index detector, and a Model 712 injector. The analytical columns, TSK gel G4000PWXL (30×0.78 cm, 10μ Phenogel) and a TSK gel G3000PWXL (30×0.78 cm, 10μ Phenogel) Phenomenex (Torrance, CA, USA) were used in series and were maintained at a temperature of 35°C. The pump flow-rate was 1.0 ml/min. Detection was performed using a refractive index detector with a sensitivity of 32 and an internal temperature of 35°C. The injection volume was 140 μl. For rinse-bag samples, the injection volume was increased to 200 μl and the detector sensitivity changed to 64. Data was acquired and integrated using a Waters 860 Data system running ExpertEase v3.1 chromatography software for quantitation by peak height. The dextran 70 peak elutes at approximately 14 min with a total run-time of 50 min.

2.3. Preparation of standard and samples

The 6% Gentran (dextran 70) solution was diluted with PBS to yield a 1 mg/ml stock standard solution. The 1 mg/ml stock standard was diluted with PBS to yield working standards at concentrations of 0.75, 0.50, 0.25, 0.10, 0.050, and 0.025 mg/ml.

Dialysate samples were prepared by transferring 0.5 ml of the sample to a 1.5-ml eppendorf tube. A volume of 0.025 ml of 1 mg/ml α-amylase in water was added and the samples were incubated at 37°C for 20 min. Samples were then cooled on ice for at least 5 min, 0.1 ml of cold 1-g/ml TCA was added, and the samples were mixed by vortexing. Precipitated protein was separated by centrifuging at 14 000×g for 10 min. A volume of 0.25 ml of supernatant was transferred into a 0.3-ml limited-volume insert and 0.025 ml of 10 N sodium hydroxide added to neutralize the pH. Samples were maintained at approximately 4°C until injection. Spike samples were prepared by the addition of dextran 70 to spent dialysate containing PG to yield concentrations of 0.75, 0.25, 0.1, 0.05, and 0.025 mg/ml.

2.4. Clinical sample analysis

Clinical dialysate samples were collected at 5 min, 1, 2, 4, 6 and 10 h during the exchange and from the rinse-bag following administration of either 7.5% PG or 4.25% dextrose [8]. Dextran 70 was added to the dialysis solutions prior to instillation to yield an initial concentration of 1 mg/ml. Samples were

stored at approximately −70°C until assayed for dextran 70.

Calibration based on the 1, 0.75, 0.5, and 0.25 mg/ml standards was used for analysis of dextran 70 samples collected during the dwell. The 0.25, 0.1, 0.05 and 0.025 mg/ml standards were used for analysis of dextran 70 samples collected from the rinse-bag. The 0.75 and 0.1 mg/ml spike samples (as prepared in Section 2.3) served as QC samples to monitor assay performance.

2.5. Calculation of volume profiles

Intraperitoneal volume profiles based on dextran 70 concentrations and drain volumes were calculated for 7.5% PG and 4.25% dextrose dialysis solutions. Intraperitoneal volume (V_t) at any time during the dialysis exchange was calculated using Eq. (1).

$$V_t = \frac{A_{in} - [(A_{in} - A_{out})^* t/10]}{C_t} \quad (1)$$

where: A_{in} = [Volume infused (ml) + V_r (ml)]* dextran 70 concentration (mg/ml) at t = 5 min.; A_{out} = [Volume drained (ml) + V_r (ml)]* dextran 70 concentration (mg/ml) at 10 h; C_t = dextran 70 concentration (mg/ml) at time t; t = Time at which volume is estimated.

Residual volume (V_r) was determined using Eq. (2).

$$V_r = \frac{(C_r^* 500)}{(C_{t=10\ h} - C_r)} \quad (2)$$

where: C_r = dextran 70 concentration (mg/ml) in the rinse bag; 500 = volume of the rinse solution (ml); $C_{t=10\ h}$ = dextran 70 concentration (mg/ml) at t = 10 h.

3. Results and discussion

The primary focus of method development was the separation of dextran 70 at an initial concentration of 1 mg/ml from polyglucose (PG) at a concentration of 75 mg/ml. Chromatographic conditions during these initial experiments were equivalent to those described in Section 2.2 except that a single analytical column (TSK gel G3000PWXL) and an injection volume of 100 μl were used. Following system equilibration, dextran 70 at a concentration of 1 mg/ml was injected (Fig. 1a). Initial sample preparation used a modified procedure previously described for the measurement of dextran 70 in the presence of inulin [6]. A 0.5-ml aliquot of dialysate containing PG was mixed with 0.1 ml of TCA, incubated for 1 h at 45°C to hydrolyze the PG, and injected. The resulting chromatogram (Fig. 1b) indicated that the dextran 70 peak was completely obscured by the PG peak. An alternative procedure was assessed using α-amylase to selectively hydrolyze the linked PG, while leaving the α(1–6)-linked dextran 70 intact. Following optimization of sample/enzyme incubation conditions, separation between the dextran 70 peak and hydrolyzed PG peak was feasible as shown in Fig. 1(c). Although approximately 7% of the PG is linked α(1–6), hydrolysis of only the α(1–4) linkages was sufficient to effectively remove this interfering peak. Other modifications included the addition of a second analytical column (G4000PWXL) to improve resolution between the dextran 70 peak and the hydrolyzed PG peak and an increased injection volume to compensate for sample dilution. Following validation as described below

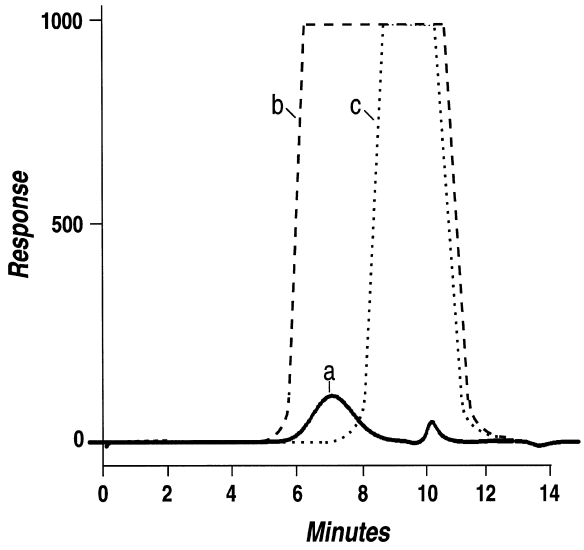

Fig. 1. Overlaid chromatograms of dextran 70 alone (a); dialysate containing 7.5% PG following sample preparation as described by Koomen et al. [6] (b); and dialysate containing 7.5% PG following sample preparation as described in this paper (c).

this method was applied to the analysis of clinical samples.

3.1. Standard response range

A linear model was evaluated for lack of fit testing and residual plots over the standard concentrations of 1 to 0.025 mg/ml. Results demonstrated adequate linearity with a correlation of >0.999 and no significant lack of fit. All subsequent standard calibrations were performed using four standard concentrations bracketing sample injections.

3.2. Precision and accuracy

A summary of the method precision and accuracy based on samples spiked at 0.75, 0.25, and 0.05 mg/ml and analyzed in triplicate on three different days is presented in Table 1. These results were assessed based on acceptance criteria of not more than 10% R.S.D. for precision and accuracy. Acceptance criteria were met at all levels except 0.05 mg/ml. Therefore, the method was modified by increasing the injection volume to 200 μl and changing the detector sensitivity to 64. Method precision and accuracy were then repeated in samples spiked at 0.1, 0.05, and 0.025 mg/ml. Results are presented in the second half of Table 1 verifying adequate precision and accuracy above dextran 70 concentrations of 0.05 mg/ml. Decreased method precision and accuracy was only observed in the range of rinse-bag samples (0.2–0.05 mg/ml).

Inter-day method performance was also assessed based on the results of QC samples (0.75 and 0.1 mg/ml dextran 70 in dialysate containing 7.5% polyglucose) prepared and assayed with the clinical samples. Assayed values averaged 0.745 mg/ml ($n=23$), %R.S.D. = 1.7 and 0.103 mg/ml ($n=7$), %R.S.D. = 4.1 demonstrating acceptable method precision and accuracy.

3.3. Specificity

Polyglucose was dissolved in spent dialysate samples from six different peritoneal dialysis patients to yield a concentration of 75 mg/ml. Dextran 70 was then added to these samples to yield a spike concentration of 0.5 mg/ml and the samples were assayed as previously described. Results demonstrated no interference from endogenous compounds based on percent recoveries of dextran 70 ranging from 99–103.

3.4. Stability

No significant change in dextran 70 concentration was detected in dialysate samples stored at −70°C for up to four months nor after two freeze–thaw cycles. Following sample preparation, dextran 70 proved to be stable when stored at 4°C for up to 72 h.

3.5. Clinical sample analysis

The disappearance of dextran 70 from dialysate was used to generate intraperitoneal volume profiles in patients administered dialysis solutions containing either 7.5% PG or 4.25% dextrose. Dialysate samples collected at 5 min, 1, 2, 4, 6, and 10 h during the exchange and from the rinse bag were assayed for dextran 70 [8]. A typical chromatogram from a patient at 10 h collected at the end of a PG exchange

Table 1
Inter-day precision and accuracy of dextran 70 in dialysate

Concentration added (mg/ml)	*n*	Mean concentration found (mg/ml)	Precision (% R.S.D.)	Accuracy (% Recovery)
0.750		0.740	0.9	98–100
0.250	9	0.250	2.5	99–103
0.050	9	0.050	21.5	93–115
0.100[a]	8	0.102	2.1	99–105
0.050[a]	9	0.051	8.6	93–111
0.025[a]	9	0.025	21.2	81–123

[a] Reassessed following method modification.

is shown in Fig. 2A. Intraperitoneal volumes based on dextran 70 concentrations were determined for three patients administered both 7.5% PG and 4.25%

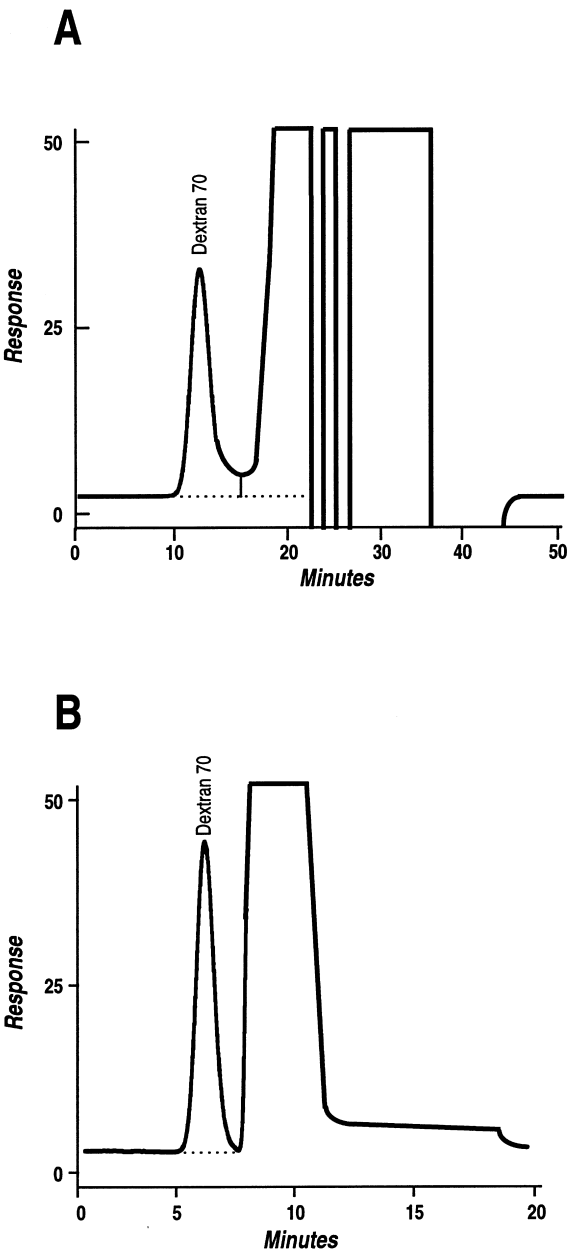

Fig. 2. (A) A representative chromatogram of dextran 70 in patient dialysate sample following a 10-h dwell with 7.5% PG solution. (B) A representative chromatogram of dextran 70 in patient dialysate sample following a 10-h dwell with 4.25% dextrose solution using a modified procedure.

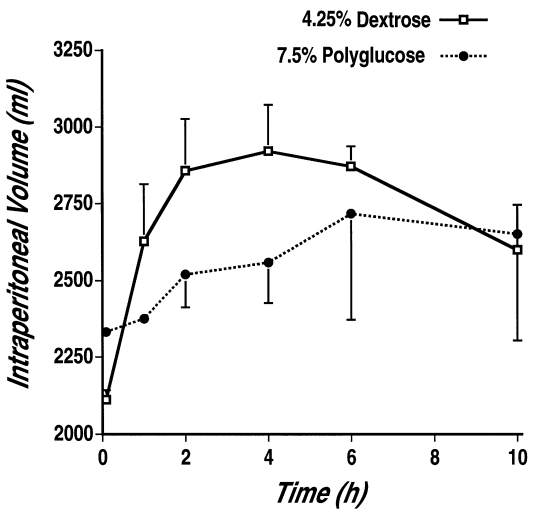

Fig. 3. Intraperitoneal volume profiles, based on dextran 70 dilution, from patients ($n=3$) administered either 4.25% glucose or 7.5% PG dialysis solutions.

dextrose over a 10-h dwell on different days. Intraperitoneal volume profiles averaged by solution type are presented in Fig. 3. Results indicate PG-containing dialysis solutions yield a more sustainable rate of ultrafiltration with lower peak intraperitoneal volumes relative to high glucose-containing dialysis solutions.

Intraperitoneal volume kinetics using 7.5% PG-containing dialysis solutions have been previously described [8,9]. However, these studies have either not evaluated ultrafiltration kinetics during exchanges of >10 h or have failed to use a stable volume marker like dextran 70 [10]. New studies investigating ultrafiltration kinetics using PG-containing dialysis solutions will benefit from the availability of this validated method for quantitation of dextran 70.

3.6. Modified procedure

Although not the focus of this paper, a modified method to determine dextran 70 in the presence of only glucose-containing dialysis solutions has also been evaluated. The analytical conditions were identical to those described in Section 2.2 except that a single analytical column (TSK gel G4000PWXL) was used and the pump flow-rate was increased to

1.5 ml/min. Sample preparation was performed as described in Section 2.3 except that incubation with α-amylase was not required. Using this modified method, dextran 70 elutes at approximately 6 min with a total run-time of 20 min. A typical chromatogram from a patient at 10 h collected at the end of a 4.25% dextrose exchange is shown in Fig. 2B. Comparable precision, accuracy, and specificity for dextran 70 using this modified method would be expected relative to the method presented in this paper. The advantages of this modified procedure over the previously reported method [6] include the elimination of sample pretreatment columns and improved method selectivity for dextran 70 quantitation by peak area instead of peak height. A more comprehensive validation of this modified method would be required prior to use.

4. Conclusions

The method as presented provides a simple, accurate, and precise technique for determining dextran 70 in the presence of polyglucose-containing dialysis solutions. This analytical method can be applied to analysis of clinical samples to examine polyglucose ultrafiltration kinetics. A modified method for the quantitation of dextran 70 in glucose-containing dialysis solutions may offer significant advantages over previously reported methods.

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