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Short communication

A multicommuted flow system for the determination of dextrose in parenteral and hemodialysis concentrate solutions

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Abstract

A new method is presented for the automation of the determination of dextrose in parenteral and hemodialysis solutions. The method is based on multicommutation flow analysis (MCFA) and exploits enzymatic reactions providing a colored derivative that is detected spectrophotometrically (Trinder's method). The reagent, comprising glucose oxidase, peroxidase, 4-hydroxybenzoate and a buffer was obtained from a commercial kit for the determination of glycemia.

The flow system used three 3-way solenoid valves operating under computer control. The necessary software was developed for the purpose and compiled in QuickBASIC 4.0

The influence of some operating variables (segment size, number of segments and reactor length) was studied.

Calibration curves in the range 0–1 g/L presented a slight curvature and were fitted with a second-degree polynomial ($h = -0.0632C^2 + 0.6039C + 0.166$, $r^2 = 0.9973$, h being the peak-height (absorbance) and C the concentration in g/L).

The method was validated by analyzing artificial samples presenting accurately known concentrations of dextrose, and comparing the results with the known value and with value obtained by polarimetry. Recoveries were in the range 96.6–100.2%, and the difference with the polarimetric analysis was in the range 0.1–3.3%. Precision (R.S.D.,%) was better than 2.4%.

Sampling frequency of the system was 90 samples/h, with a reagent consumption of 0.14 mL per sample.

Keywords: Multicommutation flow analysis; Dextrose; Enzymatic determination; Parenteral solutions; Hemodialysis solutions

1. Introduction

Quality control of the production of contemporary pharmaceutical laboratories involves a large number of analytical determinations comprising many analytes in a number of different matrixes. The growth in the quantity of lots manufactured, and the simultaneous pressure for increased productivity and reduced costs, forces the adoption of automation in the laboratory [1,2].

Besides the obvious advantages of faster analysis and reduced personnel need, automation may convey some fringe benefits such as enhanced precision, reduced reagent consumption and waste generation, and less glassware usage.

Quality control laboratories in the pharmaceutical industry tend to adhere to well-established official methods such as those published in the pharmacopeias, for instance, the United States Pharmacopeia [3]. However, the possibility of automation is not excluded, as long as equivalence of results can be demonstrated through validation.

Among the pharmaceutical products whose control benefits from automation, large-volume parenteral solutions and hemodialysis concentrate solutions have an important place. These products are used in large amounts especially in hospital environments, and many lots are produced daily in the pharmaceutical industry.

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These products consist of aqueous solutions of one or more of several salts such as sodium, potassium, calcium and magnesium chlorides, sodium acetate, sodium lactate, and may contain dextrose and other substances.

The determination of dextrose is usually carried out either by polarimetry, or by a volumetric titration based on its reducing properties. These methods are slow, difficult to automate and their selectivity is questionable. Thus, there exists the need for selective automatic methods for the determination of this analyte.

Among the techniques available for attaining automation with a reduced investment, flow-injection analysis [4,5] has found widespread application in pharmaceutical analysis as reflected in the literature [6–13], reviews [14,15] and a book specifically dedicated to this theme [16].

A different flow technique introduced more recently called multicommutation flow analysis (MCFA) [17–20] shows interesting advantages for automation, and is also being used in the pharmaceutical and related fields [21–25]. Flow-injection analysis is based primarily on the injection of a well-defined volume of sample (and eventually of reagents) in a carrier stream by means of one or more injection valves fitted with sampling loops. On the other hand, MCFA is based on flow systems, where a number of independent commutators (usually solenoid valves) are used to configure a flow network, each commutator being independently actuated under computer control. MCFA allows the implementation of a new sampling technique called binary sampling [17], where a number of segments of sample, reagent and carrier are successively inserted in the analytical path. When compared with FIA, this approach may be more flexible, allowing modifications of the sample and reagent volumes to be carried out easily. Besides, changes in the system and even the implementation of different systems can be carried out under computer control without the need of modifying physically the connections.

Methods exploiting the use of flow-injection analysis for the determination of metals in parenteral and hemodialysis solutions have already been published either for the determination of those formulated as active substances, such as metallic salts [26] as well as of contaminants [27]. However, in a revision we have found no reference to publications pertaining to the application of flow-based methods to the automation of the determination of dextrose in these pharmaceuticals.

In this work, a system based on the concept of multicommutated flow analysis was developed for the selective automated determination of dextrose in parenteral and hemodialysis solutions. In order to attain a high selectivity, it was decided to explore the use of a method exploiting an enzymatic reaction.

The method developed was based on work by Trinder [28]. This enzymatic method for the determination of dextrose is widely used in clinical analysis. A number of commercial kits are available for this determination. The reactions involved in Trinder's method, as applied to the current work are:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{4-hydroxybenzoate} \xrightarrow{\text{POD}} \text{Quinoneimine}$$

where GOD stands for glucose oxidase and POD for peroxidase.

The quinoneimine formed is a colored substance with maximum absorption at 505 nm, allowing the use of colorimetric detection.

2. Experimental

2.1. Instruments

A Shimadzu (Kyoto, Japan) UV-240 recording spectrophotometer was used as detector. It was fitted with an 80 μL quartz flow cell (Hellma, Müllheim, Germany).

The instrument was set at 505 nm and was operated in the time scan mode. Recordings of the signals were obtained from the graphic recorder–printer of the instrument, which also provided peak-height measurements.

An Alitea (Stockholm, Sweden) C8/2-XV peristaltic pump, fitted with Tygon® tubing was used for pumping the carrier, sample and reagent.

The MCFA system employed NResearch (West Caldwell, NJ, USA) 161T031 3-way 12 V solenoid valves. These valves were controlled using the individual bits of the parallel LPT1 port of the computer via a lab-made DC transistor interface.

Reactors and connections were made from stock Teflon FEP tubing (0.8 mm internal diameter).

A thermostatic bath set at 37 °C was used to keep the reactor's temperature constant throughout the experiments.

Polarimetric measurements were made in a Zuzi 412 automatic polarimeter.

2.2. Computer control

The MCFA system was operated by means of purpose-written software compiled in QuickBASIC 4.0 language and running under MS-DOS 6.0 in a notebook computer.

2.3. MCFA system

The MCFA system (Fig. 1) consisted of a peristaltic pump (P), three 3-way solenoid valves (V1–V3), reactor R1 (in a thermostatic bath) and spectrophotometer used as detector (D).

Valve V1 was used for the introduction of the sample (S), V2 for the reagent (R) and V3 for water, which was used as carrier (C). When not being introduced to the system, sample

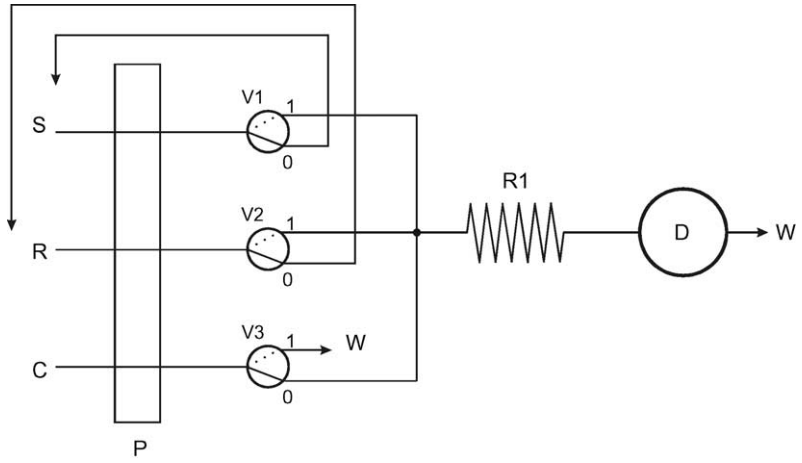

Fig. 1. Flow system used in the determination of dextrose by multicommutation flow analysis. P: peristaltic pump. S: sample, 0.7 mL/min. R: reagent, 0.7 mL/min. C: water, 1.5 mL/min. V1, V2, V3: 3-way solenoid valves. R1: reactor. D: detector (spectrophotometer at 505 nm). W: waste.

and reagent were recycled to the respective bottles, while water was sent to waste.

2.4. Reagents

A commercial kit for enzymatic determination of glycemia (Wiener Laboratorios, Rosario, Argentina, "Serie AA") was used. The reagent was purchased ready for use.

D-(+)-Glucose, biochemical grade (Merck, Darmstadt, Germany) was used as received, for the preparation of calibration standards and artificial samples.

Other reagents were of analytical reagent grade.

Distilled water obtained from an all-glass still (Aquatron A-4000, Bibby Sterilin, Staffordshire, UK) was used throughout.

2.5. Artificial samples

For validation purposes, several synthetic samples representative of widely used parenteral and hemodialysis formulations, both from USP and from the local market were prepared:

- Ringer's injection with dextrose, USP (NaCl, KCl, $CaCl_2{\cdot}2H_2O$, dextrose, water).
- Lactated Ringer's injection with dextrose, USP (NaCl, KCl, $CaCl_2{\cdot}2H_2O$, sodium lactate trihydrate, dextrose, water).
- Saline solution 1/3 (formulation from the local market containing NaCl dextrose, water).
- Hemodialysis concentrate solutions with dextrose (formulation from the local market containing NaCl, KCl, sodium acetate trihydrate, $CaCl_2{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, dextrose, water).
- Hemodialysis acidic concentrate solution with dextrose (formulation from the local market containing NaCl, KCl, acetic acid, $CaCl_2{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, dextrose, water).

2.6. Methods

Calibration was performed by injecting dextrose standard solutions in the range 0–1 g/L. Calibration curves were fitted by means of least-squares regression analysis.

Samples were diluted by weight (in the range 1:100–1:50) before introduction in the MCFA system.

2.7. Influence of operating conditions

The following variables of the flow system were studied: sample and reagent segment size, number of segments, reactor length and temperature of reactor R1. The rest of the variables, including flow rates, were fixed beforehand aiming at maximizing the sampling frequency.

Preliminary exploration of the influence of the variables was performed by means of a three-level central composite design.

For studying the influence of segment size, the activation time for the sample valve V1 (t_{V1}) was kept at 0.2 s (segment volume 2.3 μL) and the activation time of the reagent valve V2 (t_{V2}) was varied in the range 0.3–1.0 s (segment volume 3.5–11.7 μL). The number of segments was kept at 8.

For studying the influence of the number of segments, t_{V1} and t_{V2} were fixed at 0.2 s (2.3 μL) and 0.7 s (8.2 μL), respectively, and the number of segments was varied in the range 10–25.

The influence of the temperature of reactor R1 was studied taking into consideration that, according to the manufacturer of the kit, the optimum temperature for batch use is 37 °C. The influence of temperature in the flow system was confirmed in preliminary experiments by studying the response of the system in the range 22–45 °C.

2.8. Validation

Figures of merit evaluated were linearity, accuracy, precision and sampling frequency.

Verification of accuracy was carried out by means of synthetic samples representative of large-scale parenteral solutions and hemodialysis concentrate solutions, containing accurately known amounts of dextrose. These samples were prepared spanning a range of compendial and commercial preparations.

The content of dextrose was determined in these solutions by the proposed method as well as by polarimetry. The recovery of the proposed method was calculated based on the known composition of the solutions (recovery = 100 × amount found/amount put). Results obtained by the proposed method were also compared with those obtained by polarimetry.

Precision was estimated as the standard deviation of the results from the determinations performed on the artificial solutions ($n = 5$).

3. Results and discussion

3.1. Influence of operating conditions

From the results of the experiment involving a central composite design, and considering precision, peak shape and sampling frequency, it was decided to fix the reactor length at 200 cm as this value provided a compromise. The evaluation of the influence of operating temperature showed that below 37 °C, the response diminished significantly, while above 37 °C, there was little gain in sensitivity, at the same time observing an increase in the appearance of bubbles due to dissolved air. For this reason, 37 °C was chosen as operating temperature, confirming the recommendation of the manufacturer.

On the other hand, according to specifications of the manufacturer, response time of the solenoid valves can be as high as 30 ms. Thus, in order to minimize the uncertainty in activation time, a minimum value of 0.2 s (equivalent to a segment volume of 2.3 μL) was adopted as activation time. The influence of reagent valve activation time was studied with sample valve activation time kept at 0.2 s (2.3 μL). Results are shown in Fig. 2. It can be seen that with activation times above 0.7 s (8.2 μL) no significant gain in response could be obtained. As a compromise between sensitivity and sampling frequency, a value of 0.6 s (7.0 μL) was chosen, ensuring that excess reagent is available for the reaction.

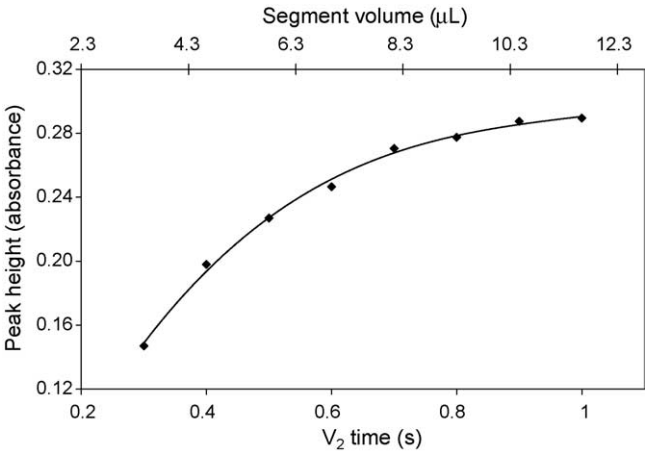

Fig. 2. Variation of response (peak-height, absorbance) with length of reagent segment (V2 activation time, seconds, also expressed as segment volume, μL). Sample segment (V1 activation time) was kept fixed at 0.2 s and the number of segments at 8.

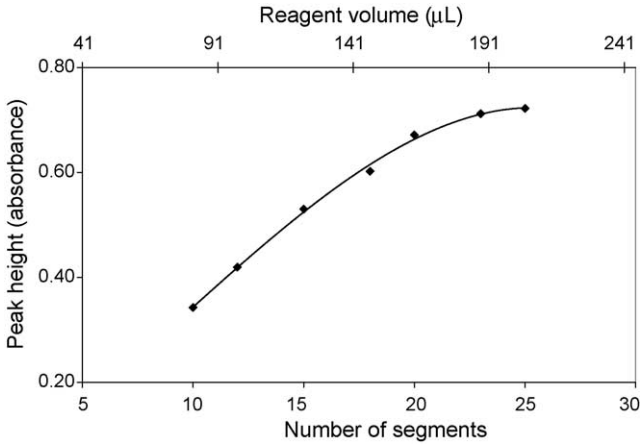

Fig. 3. Variation of response (peak-height, absorbance) with number of segments (also expressed as reagent volume, μL). Length of sample and reagent segments was kept at 0.2 and 0.7 s, respectively.

Variation of response with number of segments was studied using 0.2 and 0.7 s as activation times for sample and reagent valves, equivalent to sample and reagent volumes of 2.3 and 8.2 μL, respectively. Results are shown in Fig. 3. Twenty segments were chosen, as this value ensured a high response with a reasonable peak width and hence a high sampling frequency.

3.2. Linearity

Calibration curves in the range 0–1 g/L showed a slight curvature and a second-degree regression model was a slightly better fit than a linear one ($h = -0.0632C^2 + 0.6039C + 0.166$, $r^2 = 0.9973$, h being the peak-height (absorbance) and C the concentration in g/L). Thus, a second-degree model was chosen for calibration.

A typical plot of the signal corresponding to a calibration curve can be seen in Fig. 4.

3.3. Accuracy and precision

The specific formulations of the artificial samples used in this work were chosen because they are representative of widely used parenteral and hemodialysis solutions containing dextrose. The composition of these formulations present different saline concentrations and pH values.

The results obtained when analyzing the artificial samples with the proposed method were compared with “true”

Table 1
Results of determinations carried out with artificial samples representative of hemodialysis and parenteral solutions by MCFA and polarimetry

Formulation	Known concentration (g/L)	MCFA – found (g/L)/recovery ± R.S.D. (%)	Polarimetry – found (g/L)/recovery (%)
Hemodialysis concentrate solution with dextrose	74.13	73.4 (99.0 ± 1.9)	73.5 (99.1)
Hemodialysis acidic concentrate solution with dextrose	73.88	72.4 (98.0 ± 1.7)	72.4 (98.1)
Lactated Ringer's Injection with dextrose, USP	58.08	57.1 (98.3 ± 2.4)	58.3 (100.4)
Saline solution 1/3	36.02	36.1 (100.2 ± 1.3)	35.6 (98.9)
Ringer's Injection with dextrose, USP	56.98	55.1 (96.6 ± 1.4)	56.9 (99.9)

Recoveries (%) are calculated relative to the "true" concentration and expressed ± their relative standard deviation (R.S.D., %)

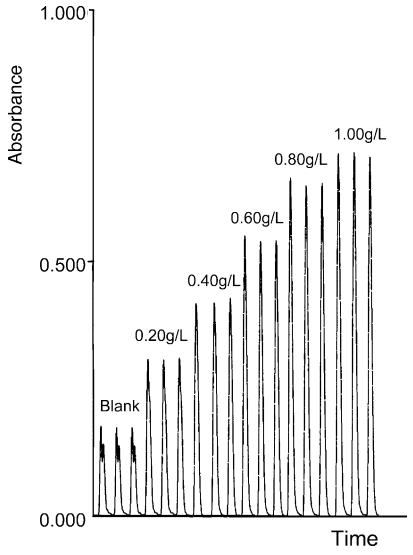

Fig. 4. Plot of a typical calibration curve.

values corresponding to the preparation of the formulations (Table 1). The results were also compared with those obtained by polarimetry.

Recoveries were close to 100% (96.6–100.2%) and the results agreed closely with those obtained by polarimetry (0.1–3.3%). Precision (relative standard deviation) was better than 2.4%.

3.4. Sampling frequency and reagent consumption

With the conditions selected, a sampling frequency of about 90 samples per hour could be attained. Reagent consumption was 0.14 mL per sample.

Table 2 resumes the figures of merit obtained with the system operating at the selected conditions.

Table 2
Analytical figures of merit of the proposed method

Sampling throughput (samples per hour)	90
Detection limit (g/L)	0.020
Quantification limit (g/L)	0.067
Dynamic response range (g/L)	0.067–1.6
Precision (R.S.D., %)	1.7
Reagent consumption (mL per determination)	0.140
Waste volume generated (mL per determination)	1.44

4. Conclusions

MCFA has been applied successfully to the automation of the determination of dextrose in parenteral and hemodialysis solutions.

When applied to the analysis of formulations representative of commercial parenteral and hemodialysis solutions, the proposed method turned out to be fast, accurate and precise, with a low consumption of reagent. Only one manual dilution was necessary, thus minimizing glassware usage and risk of contamination or losses.

It is concluded that the proposed method is appropriate for quality control of the contents of dextrose in parenteral and hemodialysis solutions.

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