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Review

Various techniques for the routine evaluation of the degradation of glucose in parenteral solutions — a critical study

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Abstract: A practical approach is described for studying the influence of various physicochemical factors on the degradation of glucose in parenteral solutions sterilized by heating in an autoclave.

Five routine analytical methods are discussed: pH determination; direct ultraviolet absorption measurement (BP) method; liquid chromatography of 5-HMF; thin-layer chromatography of sugars, carboxylic acids and carbonyl species; and enzymatic determination of glucose.

The effects of various factors on the degradation of glucose were studied: glucose concentration (10%, 30%, 50%); pH of solution before sterilization (2–10); sterilization cycle (103 min at 110°C, 20 min at 120°C, 3 min at 134°C; same *Fo*); time of heating at 120°C (30, 40, 60 min); and the presence of salts (sodium acetate, sodium lactate, sodium chloride).

The results demonstrate the importance of these factors in influencing the rate of glucose degradation during sterilization. In the presence of salts, 5-HMF is not the most important product of degradation and the BP assay is not suitable for evaluation of glucose breakdown.

The authors propose two control procedures. For simple solutions of glucose, the BP method is suitable. In the presence of salts the glucose oxidase method should be used.

Keywords: *Glucose parenterals; glucose degradation; 5-HMF.*

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Introduction

Autoclaving is the most common method of sterilization of parenteral solutions of glucose. In a few autoclaved solutions a pale yellow to brown colour develops, whose intensity increases with glucose concentration, pH of the solution after autoclaving, the presence of salts, and sterilization temperature.

Knowledge of the products that are formed during sterilization is important. In 1958 Webb *et al.* suggested that 5-hydroxymethylfurfural (5-HMF) was the principal degradation product of glucose during heating [1]. Spectrophotometric determination of 5-HMF and related substances at 284 nm in glucose parenterals is described in the *British Pharmacopoeia* (BP) [2]. Many new techniques have been developed, and numerous mechanisms of degradation have been suggested. New oxidization products have been found [3–6] and an alternative route of 5-HMF decomposition has been proposed [6]. It is considered that absorbance measurement at 284 nm is no longer suitable for the study of the degradation of glucose parenterals during heating. The aims of the present study were to evaluate routine techniques to determine glucose after autoclaving in different infusion fluids.

Liquid chromatography (LC) of 5-HMF

LC of 5-HMF is widely used in the study of honey adulteration [7, 8] and in the analysis of caramel, spirits and fruit juices.

A high concentration of 5-HMF in these products indicates either poor preservation or excessive heating during concentration or pasteurization. Jeuring *et al.* determined 5-HMF in honey and cognac by reversed-phase LC using isocratic methanol–water (10:90, v/v) as eluent with spectrophotometric determination at 285 nm [9]. Alfonso *et al.* separated 5-HMF from caramel solution by reversed-phase LC with a water–methanol gradient and spectrophotometric detection at 277 nm [10], LC was used by Cilliers *et al.* to determine 5-HMF in fruit juice concentrates after separation on two columns [11].

A LC technique was used for the analysis of glucose parenterals since it appeared to have considerable value as a rapid and sensitive method for routine procedures.

Absorbance at 284 nm

In 1968 the BP added in its monograph on dextrose injection an assay for the determination of 5-HMF and related substances. The absorbance of a diluted aqueous solution (1 g/250 ml) is measured at about 284 nm; the absorbance is no greater than 0.25.

pH determination

A decrease in pH has been observed in various autoclaved glucose solutions. A simple measurement of the pH of glucose solutions before and after autoclaving permits a good estimation of the degradation [12].

Thin-layer chromatography (TLC)

TLC can be used to detect sugars. The observed decrease in pH after sterilization of glucose solutions containing acetates suggests the formation of carboxylic acids; these products have been identified under similar conditions [13] by TLC. Investigation by TLC of the degradation of glucose has shown the formation of oxidization products, mainly carbonyl compounds (as 2.4-dinitrophenylhydrazones).

Experimental

Preliminary experiments on the determination of glucose

Polarimetric determination. Since glucose is an optically active substance its concentration can be determined by measurement of its specific rotation [2, 14]. A RSD of 3.12% (n = 10) was found.

However, it has been shown that in alkaline solution, glucose yields isomeric sugars such as mannose or fructose; under these conditions the measured specific rotation represents the total optical rotation of different sugars.

Reduction of Fehling's solution. This classical method [15] is not specific for the determination of glucose since all reducing sugars react with Fehling's solution. A RSD of 5.72% (n = 10) was found for this method.

Determination by orthotoluidine. Glucose gives with *o*-toluidine in a hot acid medium (acetic acid) a green compound (a furfural derivative) which can be determined spectrophotometrically. The main disadvantage of this method is the possible presence of glucose breakdown products containing an aldehyde function (e.g. 5-HMF), that might react easily with *o*-toluidine. A RSD coefficient of 4.22% (n = 10) was found for this method.

Enzymatic determination by the glucose oxidase method. Glucose is converted by glucose oxidase into gluconic acid and hydrogen peroxide; the latter compound, in the presence of a peroxidase, oxidizes a chromogen (4-aminophenazone in phenol) to a red compound which can be determined spectrophotometrically at 505 nm. The first step of this assay is very specific; however the second step is not. The presence of hydrogen peroxide could result in an error. Vuorinen [16] pointed out that during oxidation in alkaline solution, glucose enolates *in situ* with oxygen, to form D-arabino-hexo-2-ulose and hydrogen peroxide. A RSD of 3.21% (n = 10) was found for this method.

Selection of the method for determination of glucose. The glucose oxidase method was chosen because of its simplicity, its good reproducibility and better specificity. Moreover, under the conditions used, no hydrogen peroxide was detected in glucose solutions.

Determination of 5-HMF by LC

Apparatus. The chromatographic system comprised: a pump (Knauer 64); universal injector (Rheodyne 7125, 20 μl); stainless steel column (300 × 4.6 mm i.d.) packed with Microbondapak (10 μm) (Waters); and a variable wavelength detector (Kratos 773). A Chromatopac CR 1A integrator (Shimadzu, Kyoto, Japan) was used for recording the signal.

Standard solutions. The internal standard solution was an aqueous pyrazinamide solution (1 mg/ml) (Aldrich, France).

A standard preparation of 5-HMF was made by weighing 48.0 ± 0.1 mg of 5-HMF in a 100-ml standard flask, dissolving in demineralised water and diluting to volume.

Solutions for preparation of a calibration curve. For preparation of a calibration curve the range of concentrations of 5-HMF was 0.480–5.760 μg/ml.

Chromatographic conditions. The eluent was water–acetonitrile (98:2, v/v). The detector was set at 284 nm (0.08 a.u.f.s.); 20 μl of the calibration sample was injected on the column and eluted at a flow rate of 1.7 ml/min. The chromatographic peaks were recorded and integrated. Quantitative analysis was achieved by use of a standard preparation.

Chromatograms, reproducibility and linearity of assay. As shown in Fig. 1, 5-HMF was distinctly separated from pyrazinamide (internal standard); the retention times were 8.4 and 6.5 min, respectively.

Blank samples tested by the same procedure showed no significant interfering peaks. The precision and accuracy of the method were demonstrated by replicate analyses (n = 10) of the solutions containing known concentrations of 5-HMF. The overall RSD ranged from 1.7 to 2.5%.

The calibration curve, based on peak-area ratios of 5-HMF and internal standard (pyrazinamide), was linear over a range of 10–115 ng per injection (R = 0.9998).

Absorbance at 284 nm

All measurements were made in 1-cm quartz cells using a Hewlett–Packard 8451A diode array spectrophotometer and recorded on a Hewlett–Packard X–Y plotter.

pH determination

The pH meter (Methrom 632) was standardized at 25.0°C.

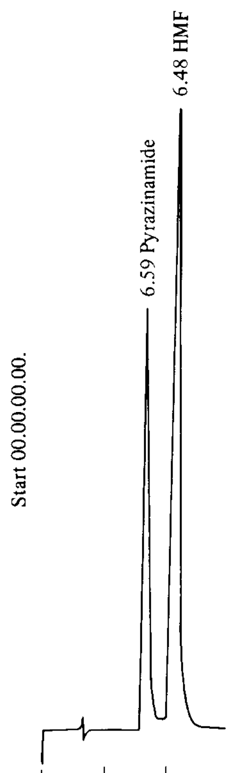

Figure 1
LC analysis of 5-HMF (1.52 μg/ml).

TLC

Screening of glucose degradation products was performed by three TLC systems.

TLC of sugars (*system* 1). A stationary phase of silica gel was used [17, 18]. The developing solvent was a mixture (35:65, v/v) of ethyl acetate with isopropanol–water (2:1, v/v).

Detection was achieved by spraying with a mixture of 1,3-dihydroxynaphthalene and phosphoric acid [18, 19].

TLC of carboxylic acids (*system* 2). The stationary phase was silica. The developing solvent was ethylmethylketone–ethanol–ammonia (17% m/m) (56:15:28, v/v/v).

Detection was achieved by spraying with 0.04% bromocresol green solution in 0.1 M sodium hydroxide [20].

TLC of 2,4-dinitrophenylhydrazones (*system* 3). The carbonyl compounds were subjected to chromatography as 2,4-dinitrophenylhydrazones on silica gel [21] with chloroform–methanol (100:10, v/v).

Results

Influence of various parameters on degradation of glucose

The degradation of autoclaved glucose solutions depends on various factors: glucose concentration, pH, the presence of salts, the sterilization cycle and temperature. The effects of each of these factors were evaluated.

Glucose concentration. 10, 30 and 50% solutions of glucose (w/v) in double-distilled water were prepared by vigorous stirring at room temperature, in neutral glass flasks of 125 ml. The solutions were then autoclaved (in a Lequeux autoclave) at 120 ± 1°C for 20 min (±1%).

The solution to be analysed was weighed and diluted with double-distilled water.

Results indicated that under these conditions of sterilization, the rate of degradation was enhanced as the concentration of the glucose solution was increased: 1.7% degradation for 10% and 30% solutions; 7.2% degradation for 50% solutions (n = 10).

Therefore 50% glucose solutions were used to study the degradation of glucose parenterals, although such a high concentration of glucose is not usually employed in clinical practice.

pH. Hydrochloric acid and sodium hydroxide solutions were used to adjust the pH of 50% glucose solutions within a range of 2–10.

The solutions were sterilized according to the procedure previously described (120°C for 20 min). Results are shown in Table 1. The rate of glucose decomposition was particularly accelerated at pH >8. An increase in acidity was observed after sterilization, especially for an initial pH >4.

Sterilization cycle: choice of an optimal cycle. This study was conducted with the three sterilization cycles commonly used in France: 103 min at 110°C; 20 min at 120°C and 3 min at 134°C.

These sterilizing cycles are not thermodynamically equivalent but have the same *Fo*

Table 1
pH changes in solutions* during sterilization at 120°C for 20 min

Sample ($n = 5$)	pH before sterilization	pH after sterilization	Glucose degradation after sterilization (%)	Colour† of solution after sterilization
1	2	1.92	9.2	–
2	3.1	3.01	10.5	–
3	4.01	4.01	12.3	–
4	4.8	4.05	15.8	+
5	6.05	4.18	22.5	+
6	7.03	4.26	28.2	+
7	7.97	4.08	32.5	+
8	9.14	4.40	37.5	++
9	10	4.62	45.5	+++

*Initial glucose concentration: 48.05% m/v.
† –, no colour; +, pale yellow; ++, yellow; +++, brown.

Table 2
Effects of sterilization at various temperatures

Temperature	Glucose concentration before sterilization (g/l)	Glucose concentration after sterilization (g/l)	Degradation (%)
110°C	522.6	504.6	3.4
120°C	522.6	487.5	6.7
134°C	522.6	477.5	8.62

Table 3
Effects of time of sterilization at 120°C

Time at 120°C	Glucose concentration	Absorbance at 284 nm	pH	Degradation (%)
Before sterilization	567.5	0	5.10	0
30 min	531.3	0.36	4.60	6.3
40 min	502.5	1.092	4.20	11.4
60 min	442.3	1.423	3.80	22.1

value. An increase in the glucose degradation rate was observed with an elevation of the autoclave temperature as shown in Table 2.

The effect of time of sterilization at 120°C on glucose breakdown was examined. The results which are reported in Table 3 show an increase in the rate of degradation of glucose with temperature.

Effects of salts. Many investigators [1, 5, 22] have studied the effect of the presence and concentration of various salts on the degradation of glucose solution during autoclaving since physicians often require the presence of electrolytes in such solutions.

Sodium lactate, sodium acetate and sodium chloride, which are widely used in hospital pharmaceutical formulations, were used to test the effect of various concentrations of salts on the rate of glucose breakdown.

The following glucose solutions were sterilized at 120°C for 20 min. The concentrations of these salts chosen were those commonly used in infusion fluids.

Solution 1 was glucose 522 g/l; Solution 2 was glucose 477.5 g/l and sodium acetate trihydrate 168 g/l (1.23 M); Solution 3 was glucose 459.5 g/l and sodium lactate 168 g/l (1.50 M); Solution 4 was glucose 477.5 g/l and sodium chloride 117 g/l (2 M); and Solution 5 was glucose 450.5 g/l, sodium chloride 117 g/l (2 M) and sodium lactate 168 g/l (1.50 M).

All the salts except sodium chloride increased the degradation rate of glucose (Table 4). A further investigation of the effects of sterilization on glucose solutions containing sodium chloride showed a decrease of pH and an increase of colour compared with a simple glucose solution (Table 5).

Evaluation of routine techniques for determination of glucose degradation

A study was carried out to evaluate pH, TLC, spectrophotometry and LC as routine techniques for the determination of the degradation of glucose after autoclaving.

pH. pH measurements of glucose before and after autoclaving did not permit a proper evaluation of glucose degradation (Table 6) since the degradation rate was not found to be consistent with pH changes.

TLC. The identification of the degradation products of glucose, such as carboxylic acids and furfural derivatives, was carried out by use of the three TLC systems described above.

System 1

The reference solutions used were 10% glucose solution (adjusted to pH 9) and 5-HMF solution. The test 10% glucose solution (pH 9) was autoclaved at 110°C for 30 min.

Samples of 5 and 100 μl of each solution were applied to the silica gel plate. One single spot (*Rf* 0.57) was observed for the reference solution and the glucose solution; the *Rf* value of 5-HMF was 0.93 and was observed only for the reference solution of 5-HMF.

System 2

No organic acids were detected in the autoclaved test solution.

System 3

The dinitrophenylhydrazone of the carbonyl compound of the autoclaved glucose solution was isolated by precipitation and then dissolved in methanol. These methanolic solutions were then applied to silica gel plates. Five yellow spots were detected for all the glucose solutions. None of the degradation products of glucose was detected by the three TLC systems.

Spectrophotometry and LC. An attempt was made to establish a correlation between results obtained by the specific determination by LC of 5-HMF and the spectroscopic technique of the BP (Tables 7 and 8).

From these results it is concluded that the main degradation product of glucose solution during heat sterilization is 5-HMF and that the BP technique appears to be a good routine method for the determination of this product since similar values for 5-HMF concentration were obtained by LC.

Table 4
Effects of electrolytes on the degradation of glucose solution (50%) sterilized at 120°C for 20 min

Solution	Salts present	Mean glucose concentration before sterilization (g/l)	Mean glucose concentration after sterilization (g/l)	Degradation (%)	Mean pH before sterilization	Mean pH after sterilization
1	None	522.6	487.5	6.7	4.64	4.26
2	Sodium acetate	477.5	315.3	33.9	7.69	5.73
3	Sodium acetate	459.5	405.4	11.8	5.77	5.37
4	Sodium chloride	477.5	459.5	3.8	5.30	3.90
5	Sodium chloride and sodium lactate	450.5	342.4	24.0	5.50	5.17

Table 5
Influence of sodium chloride on glucose degradation, pH and discolouration after sterilization at 120°C for 20 min

	Solution 1 (glucose)	Solution 2 (glucose + sodium chloride)
Glucose concentration (g/l) before sterilization (n = 5)	522.6	477.5
Glucose concentration (g/l) after sterilization (n = 5)	487.5	459.5
% degradation	6.7	3.8
pH before sterilization (n = 5)	4.64	5.3
pH after sterilization (n = 5)	0.426	3.9
pH change	0.38	1.4
Absorbance of 284 nm of a dilute solution (1:5, v/v) (n = 5)	0.357	1.094

Table 6
Influence of sterilization at 120°C for 20 min on pH for various concentrations of glucose

Concentration of glucose	pH before sterilization	pH after sterilization	pH change
10%	6.13	4.20	1.93
30%	5.40	4.08	1.32
50%	4.64	4.26	0.38

Table 7
LC determination of 5-HMF compared with UV determination (BP) for various concentrations of glucose solutions sterilized at 120°C for 20 min

Glucose concentration (n = 10)	UV determination of 5-HMF (mg/1) mean	LC determination of 5-HMF (mg/1) mean	Significance $P < 0.05$
10%	7.94	8.34	NS
30%	23.79	24.23	NS
50%	36.46	36.70	NS

Table 8
LC and UV determination of 5-HMF in 50% glucose solutions sterilised at 120°C for various times

Time (min)	UV determination of 5-HMF	LC determination of 5-HMF	Significance $P < 0.05$
30	2.46	1.86	NS
40	7.66	7.27	NS
60	40.01	40.14	NS

Table 9
LC and UV determination of 5-HMF in 50% glucose solution with various salts

Glucose solution (n = 10)	Mean of 5-HMF determination (mg/1)		Significance $P < 0.05$
	LC	spectrophotometry	
Without salts	36.70	39.98	NS
Sodium acetate	4.32	875.82	S
Sodium lactate	42.19	132.15	S
Sodium chloride	124.37	122.87	S
Sodium chloride and lactate	38.16	80.66	S

However, in glucose solutions containing salts the BP method detected at 284 nm not only 5-HMF but also related compounds (Table 9).

Conclusions

For autoclaved solutions of glucose alone the technique of the BP is suitable and reliable for the evaluation of the degradation of glucose. However, for solutions containing glucose and salts, the glucose oxidase method appears preferable since there is a good correlation between the degradation rate of glucose and the stability of the formulation during autoclaving. Since 5-HMF is a minor degradation product in complex mixtures that contain glucose and salts, it would be of great interest to determine the nature of the other products formed in the degradation of glucose.

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