AN EXAMINATION OF THE DECOMPOSITION OF DEXTROSE SOLUTION DURING STERILISATION

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A method is described for assessing the degree of decomposition of dextrose in solutions by measuring absorptiometrically the blue colour produced by a reaction with Folin-Ciocalteu reagent. The amount of decomposition depends on the duration and temperature of the sterilisation process, the concentration of dextrose and the presence of added substances. It is minimal at about pH 3. A product other than 5-hydroxymethylfurfural is formed. Two breakdown products of dextrose, gluconic acid and 5-hydroxymethyfurfural, also progressively alter in composition when their solutions are autoclaved for increasing periods either separately or as a mixture.

Solutions of dextrose are known to undergo change when heated: this is often associated with the development of a straw colour. Webb and others[1] found that 5-hydroxymethylfurfural arose from a general acid-base hydrolysis of dextrose in solution and that the rate of reaction was inversely proportional to the initial concentration of the dextrose. Singh and others[2] state that the degree of colouration paralleled the extent of formation of 5-hydroxymethylfurfural, and Scallat and Gardner[3] have suggested that the colour is at least partly due to the polymerisation of this substance. Hirayama and Kubuta[4] have reported negligible decomposition at 100° which rapidly increases when solutions are sterilised under pressure. Hudson and Tarlowski[5] noted the lowering of the pH of dextrose solutions as a result of sterilisation, and Singh and others[2] found decomposition was minimum at pH 3. Griffen and Marie[6] showed that in the presence of sodium lactate the decomposition was increased, but was at a minimum at pH 5. Hornauer[7] has shown that the pH falls with increasing rise of temperature and strength of solution.

Colour Reaction Developed by Sterilised Dextrose Solutions with Folin-Ciocalteu Reagent

Sterilised solutions of dextrose react with Folin-Ciocalteu reagent to give a stable blue colour, and this has been adapted for measuring the degree of decomposition of these solutions. To 4 ml. of a solution of dextrose in a test tube was added 0·6 ml. of Folin-Ciocalteu reagent and 1·0 ml. of 25 per cent solution of sodium carbonate and the whole mixed. The tube was placed in a water bath at 37° and heated for 15 minutes. A blank solution of 4 ml. of water was similarly treated. Readings were then made in a Spekker absorptiometer using filter No. 608.

Action of Added Substances upon Folin-Ciocalteu Reaction

To determine whether substances used with dextrose given by intravenous infusion affected the colour, measurements were made with a

solution of phenol 0·001 per cent w/v containing the added substances in the concentration used in solutions of dextrose. Sodium acid citrate, potassium phosphate, sodium lactate and hydrochloric acid did not alter the colour reaction. Sodium metabisulphite effected a much deeper colour and therefore solutions containing it cannot be determined by this method.

Measurements of solutions of 5-hydroxymethylfurfural of known strength and of unheated solutions of dextrose 5 per cent containing the same concentrations of 5-hydroxymethylfurfural were also made. The absorption readings for the combined solutions were found to be additive.

Nature of the Reaction

As the decomposition of dextrose solutions has been related to the formation of 5-hydroxymethylfurfural, solutions of the latter were subjected to the method described and were found to give a blue colour

TABLE I

COMPARISON OF TWO METHODS OF ASSESSING THE DECOMPOSITION OF DEXTROSE SOLUTIONS BY REFERENCE TO THE CONTENT OF 5-HYDROXYMETHYLFURFURAL

Sample No.	Strength of solution	5-HMF content per cent w/v.	
		(*a*) Direct absorption method	(*b*) Folin-Ciocalteu reaction method
322	5 per cent w/v	0·00014	0·465
329	10 per cent w/v	0·000365	0·72
301	20 per cent w/v	0·00084	1·095

which was directly proportional to the strength of the solution. The colour changed slightly on long standing, whereas that produced by dextrose solutions remained unchanged. By relating the colours produced in dextrose and 5-hydroxymethylfurfural solutions the degree of decomposition after sterilising appeared far in excess of that reported by others. The method was therefore compared with direct absorption in a Beckmann D.K.2 spectrophotometer. As seen in Table I, the results obtained by direct absorption indicated the formation of much smaller amounts of 5-hydroxymethylfurfural than those estimated by the method using Folin-Ciocalteu reagent. This suggested that at least one other substance which produces a much more intense blue colour with this reagent is present, and it appears to be a further product of decomposition of dextrose.

Solutions of gluconic acid and 5-hydroxymethylfurfural, both decomposition products of dextrose, produce a blue or more intense blue colouration with Folin-Ciocalteu reagent after autoclaving. Separately and in mixtures both substances were found to change progressively after autoclaving for 30 minutes successively 8 times, producing another substance which may be similar to that formed during the sterilisation of dextrose solutions. With gluconic acid, the blue colour develops after heating a solution under pressure. Further autoclaving, as with 5-hydroxymethylfurfural, produces a more intense blue colour.

Examination of Sterilised Dextrose Solutions

Using the method detailed, solutions of dextrose were examined and the readings used to compare the degree of decomposition. As many of the solutions were in containers fitted with rubber closures, water and normal saline solutions prepared under similar conditions were also examined. The results in Table II show that the blue colour arises almost entirely from the decomposed dextrose and that the occasional detection of extracted substance from rubber-capped containers arises probably

TABLE II

EXAMINATION OF THE DEGREE OF DECOMPOSITION OF STERILISED DEXTROSE SOLUTIONS

Sample No.	Type of solution	Container	Reading or calculated reading	
31	Normal saline	R.C.	0·000	
32	Normal saline	R.C.	0·060	Manufacturer A
68	Normal saline	R.C.	0·003	Manufacturer B
53	Isotonic Sod. Lactate.	R.C.	0·008	Manufacturer C
55	Isotonic Sod. Lactate.	R.C.	0·013	
13	4·3 per cent Dextrose with 0·18 per cent sod. chloride	R.C.	0·466	
33	4·3 per cent Dextrose with 0·18 per cent sod. chloride	R.C.	0·537	Manufacturer A
70	4·3 per cent Dextrose with 0·18 per cent sod. chloride	R.C.	0·271	Manufacturer B
17	5 per cent Dextrose	R.C.	0·377	
214	5 per cent Dextrose	R.C.	0·670	Manufacturer A
218	5 per cent Dextrose	R.C.	0·368	Manufacturer B
51	5 per cent Dextrose	R.C.	0·215	Manufacturer C
472	5 per cent Dextrose	R.C.	0·362	Manufacturer C
26	50 per cent Dextrose	Ampoules	1·335	Manufacturer D
45	50 per cent Dextrose	Ampoules	1·04	Manufacturer D
46	50 per cent Dextrose	Ampoules	2·35	Manufacturer E

R.C.—fitted with rubber closure.

TABLE III

EXAMINATION OF UNHEATED SOLUTIONS OF DEXTROSE OF COMMERCE

Sample No.	Type	Strength per cent	Reading	pH of solution
25	Dextrose B.P.	5 w/v	0·040	
127	Dextrose B.P.	5 w/v	0·035	
431	Dextrose B.P.	10 w/v	0·223	5·18
432	Dextrose B.P.	10 w/v	0·176	
433	Dextrose B.P.	10 w/v	0·230	5·26
434	Dextrose B.P.	10 w/v	0·194	
435	Dextrose B.P.	10 w/v	0·220	5·20
436	Dextrose B.P.	10 w/v	0·197	
451	Dextrose B.P.	10 w/v	0·090	
437	Dextrose analytical grade	10 w/v	0·142	5·13
438	Dextrose analytical grade	10 w/v	0·118	
439	Dextrose monohydrate	10 w/v	0·115	5·20
440	Dextrose monohydrate	10 w/v	0·107	

from the inadequate treatment of closures before use, and contributes only a small amount of blue colour to dextrose solutions.

To determine what factors influenced the formation of the decomposition product giving a blue colour with Folin-Ciocalteu reagent a systematic examination was undertaken.

Dextrose Powder

Solutions of Dextrose B.P. and the monohydrate prepared without heat were examined for the substance yielding a blue colour to Folin-Ciocalteu

reagent. Table III shows that variable amounts are present and could influence the results obtained with the sterilised solutions.

Effect of Concentration of Dextrose

An aqueous 20 per cent solution of dextrose was prepared without heat. This together with dilutions containing 5 per cent and 10 per cent dextrose were filled into 500 ml. containers which were either sterilised for 30 minutes at 10 lb. or heated for 1 hour at 98–100°. The results in Table IV show that the amount of decomposition is much increased by raising the

TABLE IV

EFFECT OF CONCENTRATION AND TEMPERATURE ON THE DECOMPOSITION OF DEXTROSE SOLUTIONS

Sample No.	Strength of solution per cent w/v	(*a*) Unheated solution	(*b*) Autoclaved for 30 min. at 10 lb. pressure		(*c*) Heated for 1 hour at 98–100°	
			Reading	(*b*)–(*a*)	Reading	(*c*)–(*a*)
323	5	0·110	0·428	0·318	—	—
330	10	0·220	0·651	0·431	—	—
304	20	0·448	0·962	0·514	—	—
324	5	0·110	—	—	0·157	0·047
347	10	0·220	—	—	0·380	0·160
305	20	0·448	—	—	0·679	0·231

TABLE V

RELATIONSHIP OF DECOMPOSITION TO LENGTH OF TIME OF THE HEATING PROCESS IN SOLUTIONS OF DEXTROSE 5 PER CENT

Sample No.	Heating	Reading
35	30 min. at 10 lb. pressure	0·302
37	45 min. at 10 lb. pressure	0·347
36	30 min. at 10 lb. pressure and overnight storage in autoclave	0·424
38	45 min. at 10 lb. pressure and overnight storage in autoclave	0·405
43	45 min. at 10 lb. pressure and overnight storage in full autoclave	0·583
131, 132, 147, 148, 219,	30 min. at 10 lb. in ampoule	0·392 (average)
133, 134, 149, 150, 220,	2 × 30 min. at 10 lb. in ampoule	0·554 (average)
135, 136, 151, 152, 221,	4 × 30 min. at 10 lb. in ampoule	0·833 (average)
137, 138, 153, 154.	8 × 30 min. at 10 lb. in ampoule	1·392 (average)

temperature of the solution, and that the effect of increasing the concentration of the dextrose is to raise the total amount of decomposition product but to reduce the rate of formation. This is in agreement with Webb and others[1] and Hirayama and Kubuta[4].

Time Factor

During the examination of 5 per cent solutions of dextrose, more decomposition appeared after autoclaving for 45 minutes, than for 30 minutes. Also more decomposition occurred when batches were left to stand overnight in the autoclave instead of removing as soon as practicable. This is seen in Table V.

Further investigation with ampoules of 5 per cent dextrose solution subjected to autoclaving at 10 lb. pressure for 30 minutes for one, two,

four and eight times, showed that the amount of decomposition was directly related to the length of time of autoclaving (Table V).

Presence of other Substances and Effect of pH

Singh and others[2] showed that the decomposition of dextrose solutions as measured by 5-hydroxymethylfurfural formation was least in solutions with a pH of 3 before autoclaving. Webb and others[1] also showed that the rate of decomposition was related to the concentration of buffer at a constant pH.

Hydrochloric acid and a number of substances commonly used in intravenous infusions were added to dextrose solutions. The solution containing 0·01 per cent w/v HCl showed less than one-quarter of the decomposition shown by an unmodified solution of dextrose, the pH remaining constant at 2·62. A solution with 0·0001 N HCl was less stable than the above but more stable than the unmodified solution, the pH moving from 4·16 before to 3·73 after autoclaving. Sodium chloride

TABLE VI

EFFECT OF ADDED SUBSTANCES ON THE STABILITY OF DEXTROSE SOLUTIONS

Sample No.	Strength of dextrose w/v	Strength of added substance w/v per cent	pH		Reading or calculated reading		(*a*)-(*b*)
			Before autoclaving	After autoclaving	(*a*) Autoclaved solution	(*b*) Unheated solution	
323	5	—	5·28	3·97	0·428	0·11	0·318
342	5	0·01 HCl	2·62	2·62	0·183	0·11	0·073
353	5	0·000365 HCl	4·16	3·73	0·330	0·11	0·220
333	5	0·9 NaCl	5·40	3·94	0·448	0·11	0·338
319	5	0·22 Sod. lactate	5·93	5·41	0·964	0·11	0·854
315	5	0·05 K_2HPO_4	7·89	5·09	4·420	0·11	4·310
311	5	4·18 Sod. acid citrate	4·92	4·91	0·586	0·11	0·476
343	2·5	2·09 Sod. acid citrate	5·04	5·08	0·195	0·055	0·140

0·9 per cent has no effect on decomposition or the pH shift. Potassium phosphate at 0·05 per cent w/v caused a marked decomposition associated with the development of a definite brown colour. As the initial pH was 7·89 this was to be expected. A solution containing 0·22 per cent w/v of sodium lactate also caused increased decomposition. Sodium acid citrate used with dextrose at the concentration used for collecting blood produces a fairly stable solution. However, by comparing this solution with one of twice its strength it was found that the increase in concentration of the buffer accentuates the decomposition of the dextrose. Also, although the pH remained constant, heating for half an hour at 20 lb. pressure produced a twenty-five-fold increase in the decomposition as measured by the method detailed.

Unbuffered dextrose solutions normally become more acid during sterilisation to a point about pH 4. The addition of buffers in low concentration capable of maintaining the pH at 4 provides some stability, but higher concentrations increase the rate of decomposition. Added substances giving a pH which alters during sterilisation also appear to increase the formation of breakdown products of dextrose (Table VI).

Discussion

It may be deduced that the decomposition of dextrose is directly related to the length and temperature of the sterilising process. Also, samples of dextrose powder vary in the amount of the product or products of degradation present, although in all experiments this was small.

The addition of hydrochloric acid to pH 3, as shown by other workers, also appears to be the most satisfactory means of reducing decomposition chemically. Because of the increase in decomposition, sterilisation of dextrose solutions in the presence of other substances is inadvisable. To attain minimum decomposition care must be taken to ensure that the heating process is not prolonged beyond the time necessary for sterilisation, and that no overheating takes place. At the same time it is an advantage to cool the solution as quickly as possible after sterilising.

With Folin-Ciocalteu reagent, results showed the presence of a substance other than 5-hydroxymethylfurfural, and it appears that another detectable decomposition product is present, probably in amounts comparable with the amount of 5-hydroxymethylfurfural determined by direct spectrophotometric absorption. Certainly the results obtained in this work parallel observations made by others who related decomposition to the formation of 5-hydroxymethylfurfural. The development of more intense blue colour reactions in solutions of both gluconic acid and 5-hydroxymethylfurfural, subjected to progressively longer periods of sterilisation, show also that these products of dextrose decomposition produce other substances during such treatment. However, as relatively strong solutions of these substances were used, this may only be taken as an indication, and not the explanation of what is happening in the alteration of the dextrose molecule under the conditions of sterilisation of solutions of that substance.

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After Mr. Wing presented the paper there was a DISCUSSION.