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Identification of some acids produced during autoclaving of D-glucose solutions using HPLC

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Summary

Using ion-pairing HPLC methods two acids have been detected as being present in autoclaved D-glucose solutions. On the basis of their ultraviolet spectra and chromatographic behaviour in ion pairing reverse-phase liquid chromatography these acids have been identified as 5-hydroxymethylfuroic acid and furan-2,5-dicarboxylic acid. The synthesis of the standard acids employed is described and the mechanism of the reaction producing the acids is discussed.

Introduction

While it is well established that 5-hydroxymethylfurfuraldehyde (5-HMF) is a major product in the decomposition of D-glucose solution during autoclaving prior to its use as an infusion fluid, the nature of the acidic products causing the observed decrease in pH has not been well authenticated. The acidity may arise either from the decomposition of D-glucose before 5-HMF production, as a parallel reaction, or may be produced by subsequent decomposition of the 5-HMF once formed.

The literature contains many references to acidic products being formed from D-glucose. The direct formation of organic acids usually involves strongly oxidizing conditions and results in the loss of one or more carbon atoms to produce aldonic and other acids (Coffey, 1967). It has also been shown that under alkaline conditions D-glucose may form various isomeric saccharinic acids (Ferrier and Collins, 1972).

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This latter type of reaction has been suggested as the source of the D-glucose acidity in one chromatographic investigation of the autoclaving degradation (Taylor and Sood, 1978). It has also been established that 5-HMF, on heating at high temperature in very acid (0.5 M) solution, is converted to approximately equimolar proportions of levulinic and formic acids (Teunissen, 1930). This very early work has been generally taken to account for the acidity produced under autoclaving conditions. An alternative route of 5-HMF decomposition has been suggested as opening of the furan ring with subsequent decarboxylation and oxidation to produce acetylacrylic acid (Tahir and Cates, 1974). This suggestion, based on spectrophotometric and mechanistic grounds, was also interpreted as accounting for the yellow colour occasionally observed in degraded D-glucose solutions.

A major difficulty in the investigation of species produced during autoclaving of D-glucose lies in the relatively small proportion of the sugar decomposed and the subsequent low concentrations of impurities produced. This renders isolation and subsequent characterization of products, other than the main 5-HMF product, extremely difficult especially in the presence of the very large excess of unreacted sugar. It was intended in this work to investigate the acidic species formed during the heating of initially neutral solutions of D-glucose using HPLC with the inclusion of ion pairing agents in the mobile phase. Such a technique should enable characterization, as acids, certain products obtained during the decomposition by demonstrating a marked increase in their retention on addition of cationic pairing ion to the eluent. It was also intended to identify the acid products by comparison with standard acids both with respect to their ultraviolet spectra and the variation of their chromatographic capacity factors with pairing ion concentration. The variation of the chromatographic capacity factor with pairing ion concentration in the mobile phase has been shown (Hung and Taylor, 1980) to be very characteristic of the lipophilicity of a given acid anion. Such a comparison with standards may yield a much more unequivocal identification procedure than that of directly comparing retention times under unique chromatographic conditions. Such HPLC methods also offer considerable advantages over the widely used spectrophotometric methods in that a much higher degree of specificity and thus spectral purity can be obtained by the method of stopped flow scanning.

Materials and methods

Decomposition

D-Glucose (May and Baker) 5% w/v solution in distilled water was heated either in sealed 1 cm^3 glass ampoules in an air oven (Taylor et al., 1972) for various periods of time to produce varying amounts of decomposition products or autoclaved in infusion fluid containers by a normal autoclaving cycle. Both methods of decomposition yielded the same products although the former gave a greater degree of control over the concentration of decomposition products produced. The solution containing D-glucose and degradation products was injected directly on to the chromatography column.

Chromatography

A Waters Associates M6000A solvent delivery system coupled with a Waters M440 (254 nm) ultraviolet detector was used. The chromatographic solvent was distilled and Millipore filtered water. When required tetraethylammonium (TEA) or tetrabutylammonium (TBA) phosphate (Aldrich Chemicals) was included in the eluent as pairing ion together with disodium hydrogen phosphate (Fisons Scientific) as buffer and counterion. Chromatographic columns were stainless steel, slurry packed at 600 bar with ODS-Hypersil, 5 μm, (Shandon Southern Products). Columns were 4.6 mm i.d. and of varying lengths from 100 to 200 mm depending upon the magnitude of the capacity factor. Injection of a standard 20 μl sample was by a Rheodyne 7125 valve and loop system. Stopped flow measurements were made using a Cecil CE 558 spectrophotometer adapted to take a 10 μl flow cell.

Synthesis and characterization of the standard acids

Since the acidic products anticipated, namely 5-hydroxymethylfuroic acid (5-HMFA) and furan-2,5-dicarboxylic acid (FDA), could not be obtained commercially, these compounds were synthesized from 5-HMF and characterized for use as standards as follows.

(a) 5-HMFA. 5HMF (Sigma London Chemicals) (500 mg, 0.004 M) in 10 cm^3 methanol was mixed with a suspension of silver oxide prepared from silver nitrate (1.4 g, 0.008 M) and sodium hydroxide (0.6 g, 0.016 M) in 10 cm^3 water. The mixture was heated at 65°C for 3 h (Reichstein, 1926). The supernatant was cooled, filtered and acidified with dilute HCl. Extraction into ether gave, after exaporation, 5-HMFA and traces of a second organic product.

Characterization of 5-HMFA. Recrystallized from ether m.p. 162°C; literature value 165°C (Blanksma, 1910). UV λ_{max} (ethanol) 247 nm (ϵ 1.2×10^4). IR (nujol) cm^{-1}: 3250 (OH broad), 2700–2500 (OH, acid), 1665 (C = 0, acid, broad). PMR (60 MHz, d_6-DMSO, TMS): δ4.46 (singlet, 2H, methylene), 6.46 (doublet, 1H, J = 4Hz, furyl), 7.14 (doublet, 1H, J = 4Hz furyl), 6.73 (broad, 2H, hydrogen bonded OH). ms M/e (% base): 142 (42, M^+), 125 (12), 123 (18), 113 (12), 97 (100). Accurate mass measurement $M^+ = 142.0264$; $C_6H_6O_4$ requires 142.0266.

(b) FDA. Crude 5-HMF (Haworth and Jones, 1944) (3.8 g, 0.03 M) and silver nitrate (25 g, 0.15 M) were dissolved in 50 cm^3 water and sodium hydroxide (12.5 g, 0.31 M) in 50 cm^3 water added. A strongly exothermic reaction ensued with rapid deposition of silver. The temperature was maintained on a stream bath for 1 h. The supernatant was filtered and acidified to pH 3.0 with concentrated HCl. On cooling, crystals and FDA were deposited.

Characterization of FDA. Recrystallized from water as needles m.p. > 300°C; literature value m.p. > 320°C (Dict. Org. Chem., 1965). UV λ_{max} (ethanol): 259 nm (ϵ 1.6×10^4). IR (nujol) cm^{-1}: 2700—2500, 1690, 1670. PMR (60 MHz, NaOD, trimethylsilylpropanesulphonic acid): δ6.9 (singlet, furyl protons), 4.6 (singlet, solvent exchanged protons). ms M/e (% base): 156 (M^+, 100), 139 (58), 119 (14), 117 (15), 112 (12), 97 (18), 95 (21), 91 (17). Accurate mass measurement M^+ 156.0056; $C_6H_4O_5$ requires 156.0057.

Results and discussion

Location of acid products

Typical chromatograms under non-ion-pairing and ion-pairing conditions are shown in Fig. 1a and b. It can be seen that two peaks, A and B, which are very rapidly eluted under purely aqueous conditions are extensively retained when TBA is added to the eluent as ion pairing agent. It was found that the compounds producing peaks A and B behaved in a very similar manner to that observed to be general for several carboxylic acids (Hung and Taylor, 1980). That is: (a) they showed a maximum in the curve showing the variation of their capacity factors with pairing ion concentration. This is demonstrated in a later figure where their behaviour is compared quantitatively with that of standard acids; and (b) the effect of added counterion in the form of phosphate was to decrease the capacity factors of both A and B which is again consistent with current ideas on ion pairing.

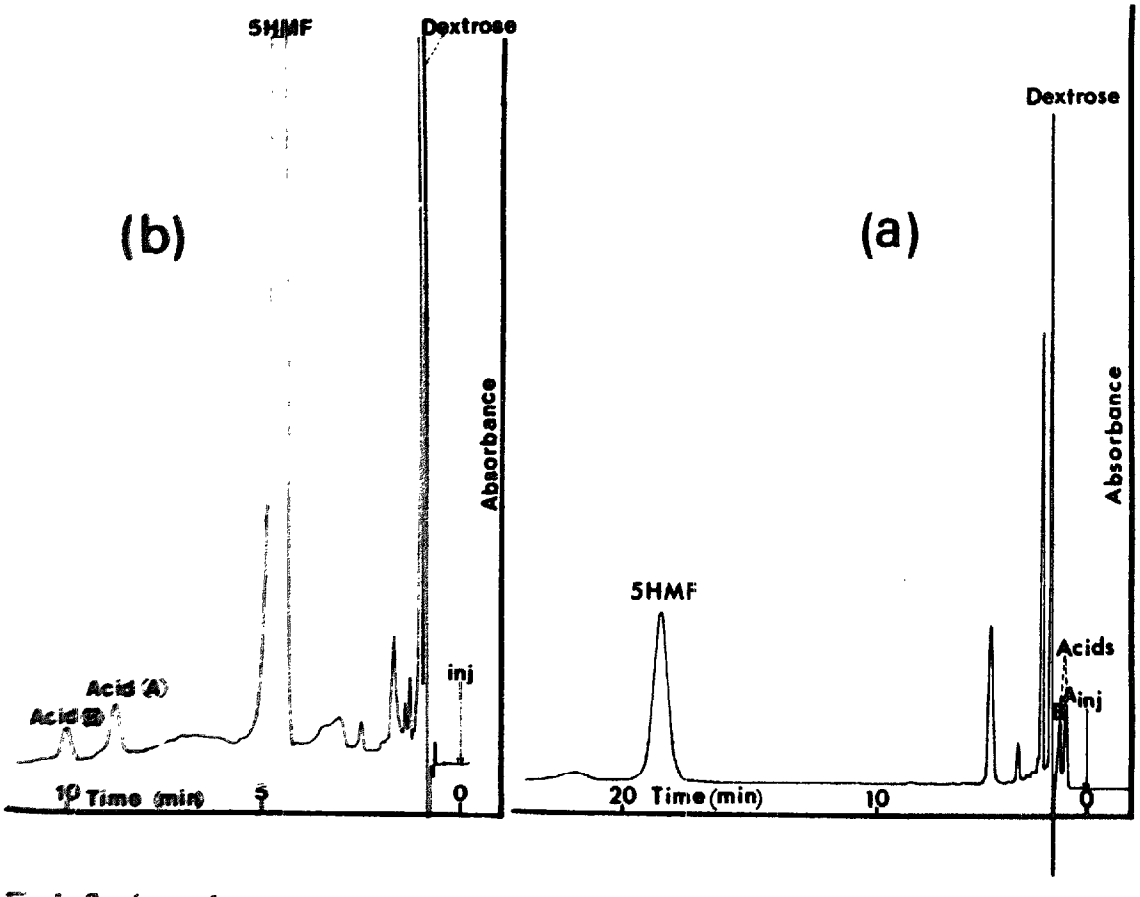

Fig. 1. Specimen chromatograms of degraded D-glucose solutions. Chromatographic conditions: (a) 200×4.6 mm Hypersil ODS column at 2.0 $cm^3 \cdot min^{-1}$ flow rate; solvent distilled water; full scale deflection 0.5. (b) 100×4.5 mm Hypersil ODS column at 2.0 $cm^3 \cdot min^{-1}$ flow rate; solvent 20 mM tetrabutylammonium phosphate in 0.01 M disodium hydrogen phosphate buffer (pH 7); full scale deflection 0.05.

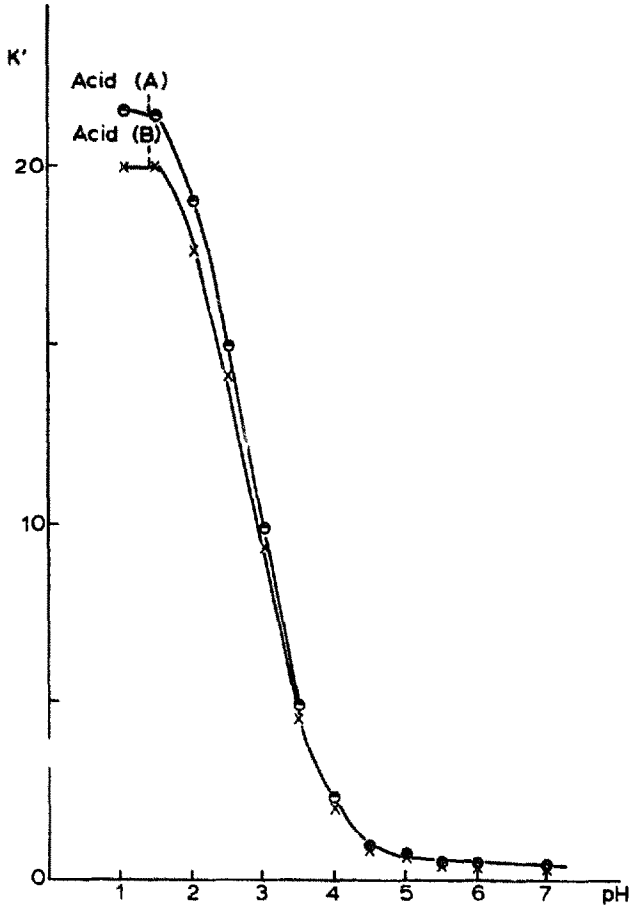

Fig. 2. Plots showing the variation of capacity factors with pH for the acids A and B derived from D-glucose.

The acidic natures of A and B can further be demonstrated by the technique of ion suppression. Fig. 2 shows the variation in capacity factor as a function of pH for both acids. It can be seen that at low pH when the acids are undissociated capacity factors are high, while at high pH the fully dissociated anions are rapidly eluted.

These results indicate that A and B are two separate acid species formed during the heating of essentially neutral sealed solutions of D-glucose. The behaviour of the major decomposition product, 5-HMF, exhibits the opposite behaviour under ion-pairing conditions in that its capacity factor decreases constantly with added pairing ion concentration. This is characteristic of uncharged solutes under ion-pairing chromatographic conditions (Graham and Rogers, 1980) and further verifies that peaks A and B are the only acid anions present in the degraded D-glucose solution which exhibit appreciable ultraviolet absorption.

Identification of acid products

Preliminary measurements on the variation of the capacity factors of A and B with pairing ion concentration (Figs. 3 and 4) indicated that neither of these species corresponded to formic or levulinic acids. Fig. 3 shows that in TEA solutions acid A

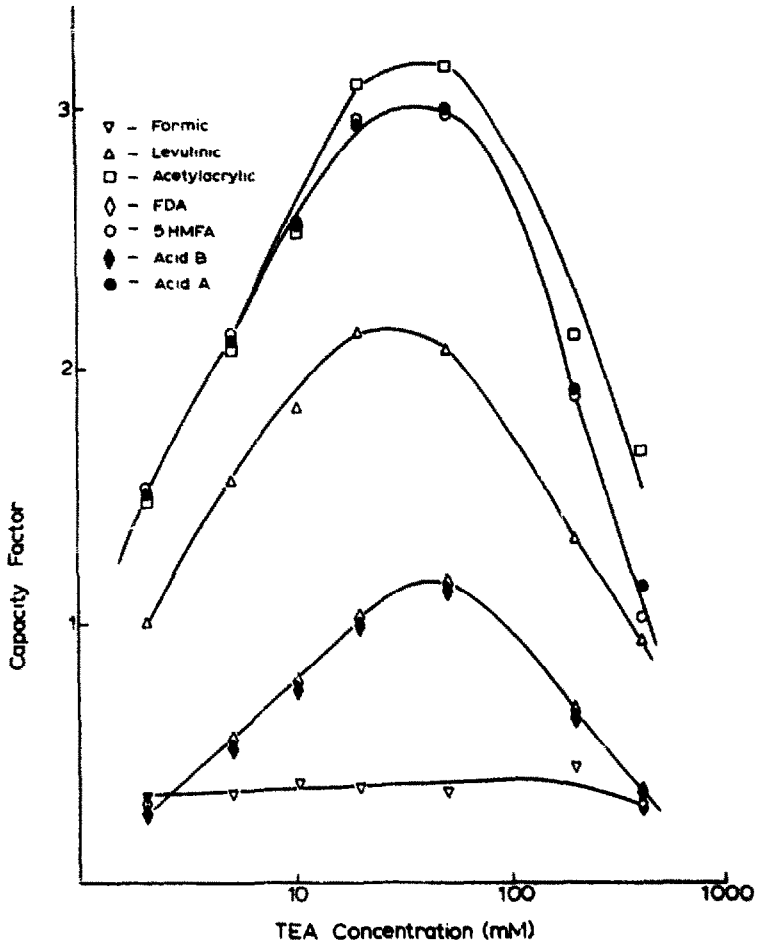

Fig. 3. Plots showing the variation of capacity factor for various acids as a function of tetraethylammonium phosphate concentration.

has a similar behaviour to acetylacrylic acid. The corresponding curves in Fig. 4, however, using the more strongly adsorbed TBA as pairing ion show that acetylacrylic acid does not correspond with A. The plots shown in Figs. 3 and 4 are very characteristic of a particular anion. Not only are the capacity factors altered in a relative sense between different pairing ions but the order of elution of the acid species A and B is different in each pairing ion. Also, the magnitude of the change in retention produced by a variation in pairing ion concentration is characteristic of a particular solute. While it is as yet not possible to predict retention in such systems from a knowledge of chemical structure alone, such curves would appear to provide a very sensitive and unique test for identity from chromatographic data provided standards of known chemical composition are available. The complexity of acid retention behaviour in presence of different pairing ions has recently been demonstrated by Deelder et al. (1981).

Following the observation that degraded samples of D-glucose solution left for some time at room temperature after brief exposure to air showed an increase in acid product at the expense of 5-HMF (Hung et al., 1982), it was considered that A and

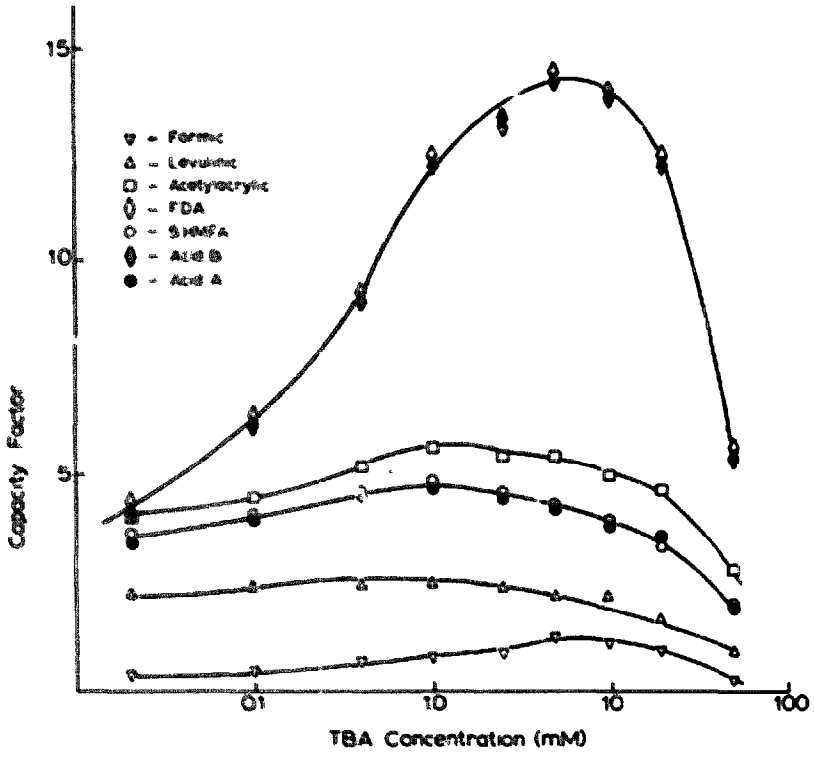

Fig. 4. Plot showing the variation of capacity factor for various acids as a function of tetrabutylammonium phosphate concentration.

B might be produced from 5-HMF by some mild oxidation reaction. To investigate this effect further, 5-HMF was oxidized with aqueous alkaline silver oxide at 65°C (Reichstein, 1926) to 5-hydroxymethylfuroic acid (5-HMFA) plus traces of a second acidic product. While the melting point of this major product was in good agreement with the literature value (Blanksma, 1910), its structure was unequivocally established on a spectral basis as 5-HMFA. The PMR spectrum was consistent with a 2,5-disubstituted furylmethylene system with non-equivalent furyl protons resonating as a pair of doublets (δ7.14 and 6.46) with the 5-methylene protons giving rise to a singlet (δ4.46). The protons of the acid and alcohol groups as a result of time-averaged exchange give rise to a single broad peak (δ6.73). The infrared spectrum showed absorption at 3250 cm^{-1} (hydrogen-bonded hydroxyl group) and $\approx$ 2600 cm^{-1}, 1665 cm^{-1} (hydrogen-bonded acid and carbonyl groups, respectively). An accurate mass measurement of the molecular ion peak M/e 142 in the mass spectrum was in excellent agreement with that expected for 5-HMFA with a molecular formula $C_6H_6O_4$.

Oxidation of 5-HMF under more vigorous conditions (larger excess of oxidizing reagent and higher temperature) resulted in formation and subsequent isolation of the second acid in highly purified form. Spectral characteristics were consistent with an assignment as furan-2,5-dicarboxylic acid (FDA). Accurate mass measurement of the molecular ion (M/e 156) in the mass spectrum established a molecular formula of $C_6H_4O_5$. The low solubility of the acid in more usual deuterated solvents

necessitated recording the PMR spectrum in NaOD. The simplicity of this spectrum was consistent with a highly symmetrical molecular structure. A singlet resonance (δ6.9) was due to the equivalent C-3/C-4 furyl ring protons, while traces of H_2O in sample/solvent as well as solvent-exchanged acid protons gave rise to a single peak at δ4.6. The absence of any signal due to methylene protons indicated the transformation of the 5-hydroxymethyl substituent to a second acid group. This is also supported by an absence of any hydroxyl group absorption at $\approx 3200\ cm^{-1}$ in the intrared spectrum of this acid. The shift of λ_{max} in the ultraviolet spectrum from 247 nm for the 2-monoacid to 259 nm for the 2,5-diacid is that expected due to an extension of conjugation in the π system and is supported by observation in analogous heterocyclic systems (Scott, 1964). While no melting point could be ascertained (> 300°C), this was also consistent with a previous recorded observation on FDA (Dict. Org. Chem., 1965).

Following the observation that degraded samples of D-glucose solution left for some time at room temperature after brief exposure to air showed an increase in acid product at the expense of 5-HMF (Hung et al., 1982) it was considered that A and B might be produced from 5-HMF by some mild reaction.

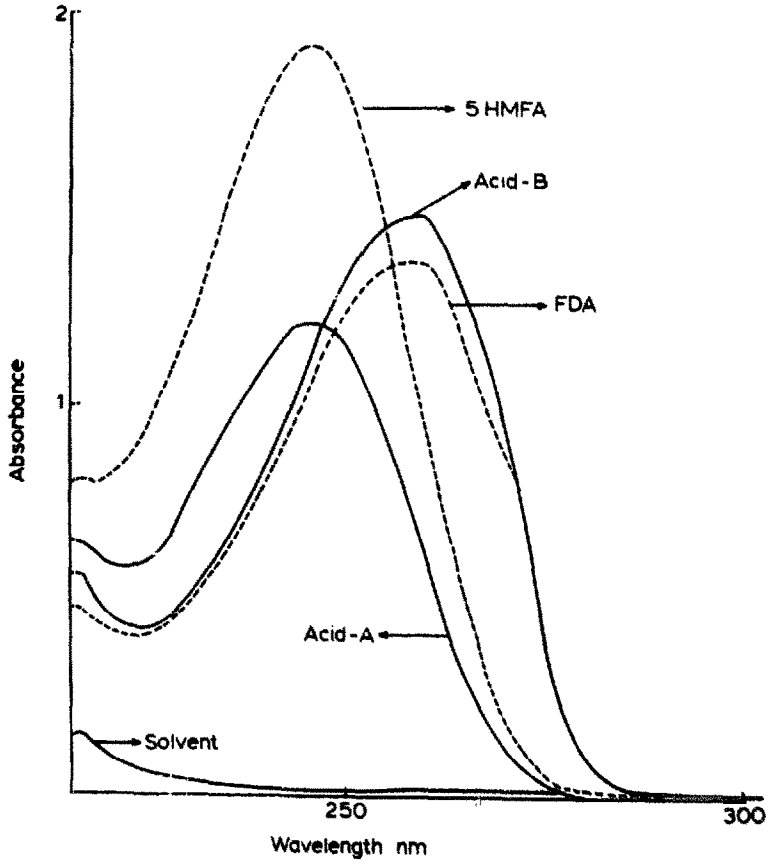

Fig. 5. Ultraviolet spectra of the acids A and B produced in degraded D-glucose solution compared with standard 5-HMFA and FDA obtained by stopped flow technique in 50 mM TEA solution 0.01 M in disodium hydrogen phosphate (pH 7).

TABLE 1

COMPARISON OF ABSORPTION MAXIMA AND ABSORBANCE RATIOS FOR STANDARD AND UNKNOWN ACIDS

Acid	λ_{max} (nm)	$\frac{\lambda_{247}}{\lambda_{225}}$	$\frac{\lambda_{247}}{\lambda_{265}}$	$\frac{\lambda_{259}}{\lambda_{225}}$	$\frac{\lambda_{259}}{\lambda_{270}}$
5-HMFA	247	2.14	3.97		
FDA	259	-		3.72	1.57
Acid-A	247	2.15	3.77		
Acid-B	259	-		3.91	1.58

Comparison of the variation of the capacity factors of 5-HMFA and FDA with those of A and B in Figs. 3 and 4 shows identical behaviour. The stopped flow scanning technique was used to obtain the ultraviolet spectra of A and B in the degraded D-glucose solution. Fig. 5 shows the correspondence of 5-HMFA and A and FDA to B in that the spectra are identical in shape and position of maximum also the values of absorbance ratios shown in Table 1 are very close. This is at variance with previous findings (Taylor and Sood, 1978) using HPLC under such solutions where the unretained acid peak was reported as showing no distinct absorption maximum above 200 nm. This discrepancy can only be attributed to the vastly improved resolution of the columns currently being used together with the separation into two distinct components using ion pairing methods.

The present findings are supported by previous workers (Turner et al., 1954) who indicated that the presence of 5-HMFA could distort the spectrum of 5-HMF in sugar solutions following oxidation. It is suggested that acidity in autoclaved D-glucose solution arises by oxidation of 5-HMF to give 5-HMFA, a process analogous to the known autoxidation of aromatic aldehydes such as benzaldehyde to benzoic acid (Cram and Hammond, 1959). The mechanism for the parallel oxidation step of alcohol group to acid under autoclaving conditions remains obscure. No chromatographic data could be obtained which supported the existence of a UV absorbing ring-opened acidic product, although this mechanism of furan degradation has been well established (Tahir and Cates, 1974).

Conclusion

From the above results the autoclaving of D-glucose, as well as producing 5-HMF as the major decomposition product in sealed conditions, also gives rise to acidic products. Two acids produced have been shown to be 5-hydroxymethylfuroic acid and furan-2,5-dicarboxylic acid on the basis of their chromatographic and ultraviolet spectral characteristics by comparison with standards. While it cannot be stated unequivocally that formic and levulinic acids are not formed (since unlike acetylacrylic their molar absorptivities are very low), it would appear unlikely that

these acids are formed under the very mild decomposition conditions of autoclaving. It is also shown that the variation of the capacity factor with ion pairing agent concentration can provide a useful check identification technique provided authentic samples of material are available.

Acknowledgements

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