Trade Science Inc.

ISSN : 0974-7419 Volume 10 Issue 10

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(10) 2011 [651-656]

A modified DNPH derivatization method in RPLC for quantification and determination of dihydroxyacetone and formic acid in formose samples

Zafar Iqbal[1,2]

[1]Department of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6, (CANADA)

[2]Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh - 2202, (BANGLADESH)

E-mail: Zafar_Iqbal@carleton.ca; zafar.ftri007@gmail.com

Received: 24th April, 2011 ; Accepted: 24th May, 2011

ABSTRACT

Dihydroxyacetone (DHA) and formic acid (HCOOH) in formose samples were analyzed using reversed-phase liquid chromatography (RPLC) utilizing a modified chromatographic and 2, 4-Dinitrophynylhydrazine (DNPH)-derivatization techniques. Especial evaporation technique was developed to remove excess of unconverted substance from the formose mixture to facilitate the reduction of excess of reagent used for derivatization. Usage of derivatization reagent (DNPH) was reduced by a factor of 60. The optimum conditions for separation of DHA-hydrazone were (5-90)% ACN at 1 ml/min. Sodium borohydride ($NaBH_4$) reduction of aldehydes, ketones and sugars helped to analyze HCOOH-hydrazone quantitatively in formose sample.

KEYWORDS

RPLC;
DNPH-derivatization;
Dihydroxyacetone;
Formic acid;
Reduction of formose.

INTRODUCTION

The formose reaction (FR) itself is a special type of one of the most fundamental reactions for carbohydrate chemistry and natural product chemistry. This reaction is of great importance to the question of origin of life because it is considered as a potential synthesis route for the generation of complex monosaccharides, a non-enzymatic source of sugars, starting with the simple C_1 compound HCHO. In addition, it may play a big role in long manned space missions, where regenerative life support systems providing carbohydrates are necessary (see Figure 1). Such closed system employing carbohydrate production must be developed.

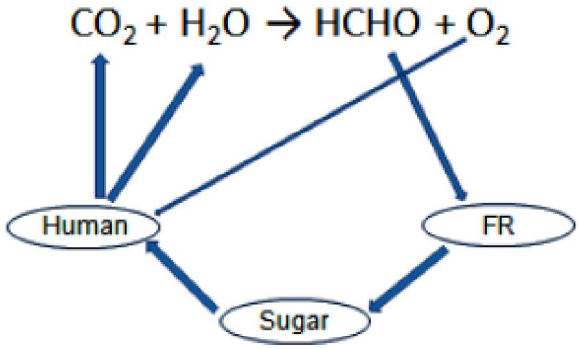

Figure 1 : Scheme for the production of formose sugar (synthetic sugar) from formaldehyde.

This reaction is an autocatalytic anionic polymerization of formaldehyde (HCHO) to carbohydrates, which requires a base, for example calcium hydroxide

($Ca(OH)_2$), as catalyst and a carbohydrate containing α-hydrogen such as gylcolaldehyde (GA) or glyceraldehyde (GCA) in trace amounts acting as an initiator. The reaction product contains at least 30 different compounds including straight chain- and BC-carbohydrates, organic acids and sugar degradation products.

Analysis of formose sugar by HPLC is a challenging issue to the chromatographer since long period. The reaction products are complex mixtures that need challenges to analyze by using available analytical techniques. Gas chromatography-mass spectrometry (GC-MS) and thin layer chromatography (TLC) were used for this task. Qualitative measurement and structure elucidation of formose products were done previously by TLC and GC-MS, respectively as reported[15]. Currently, there is no HPLC technique existing for quantification of individual substances (for instance, DHA) in formose sample. In ion chromatography, analysis of HCOOH in formose sample is quite problematic due to the bad peak resolution comes from interference with other organic acids. With regards to the present state-of-the-art of formose sugar analysis, only two recent publications[2,14] describe a HPLC method with a DNPH derivatization procedure. A detailed analytical investigation regarding analysis of individual compounds in formose does not exist either. Of course the derivatization method is well established for analysis of common monosaccharides and formaldehyde (HCHO)[1-19]. In this context, up to now, the methods presented in the earlier publication[12] are the well-developed one.

In this report, a modification of the previous methods and application for the analysis of DHA and HCOOH in formose samples are presented. Particular sample preparation and chromatographic techniques applied are discussed.

EXPERIMENTAL

Formose reaction

Experiments were conducted using a batch reactor-system. PF solution was prepared by dissolving 200 g powdered-PF in 400 mL water (50°C), refluxing for 4 h followed by filtering[18].

A calculated amount (mentioned as below) of HCHO was transferred into a vessel of high quality (HQ)-water to a final volume of 25 mL. For dissolving a calculated amount of GA initiator, an aliquot of the prepared HCHO-solution was removed. After completing the dissolution of the initiator, the aliquot was transferred back into the vessel containing HCHO. After dissolving the $Ca(OH)_2$ powder in 25 mL of water in another vessel, the reactants were pre-heated to the reaction temperature [40(±2)°C and 98(±0.5)°C]. Water-bath and oil bath were used for increasing and maintaining temperature in moderate and high temperatures, respectively. The reaction was started by transferring the HCHO-solution containing the pre-dissolved initiator into a 100 mL reaction vessel and adding the pre-dissolved $Ca(OH)_2$. By applying N_2, inert reaction conditions were maintained.

In the case of 40(±2)°C reaction temperature, 46 gL^{-1} of HCHO, 14 gL^{-1} of calcium hydroxide [$Ca(OH)_2$] and 2.5 gL^{-1} of glycolaldehyde (GA) were taken for the reaction. In the other case when reaction was conducted at high temperature [98(±0.5)°C], 54.6 gL^{-1} of HCHO and 8.47 gL^{-1} of $Ca(OH)_2$ were taken. The reactions were stopped by decreasing the pH to 5.0 by addition of a pre-determined amount of HNO_3 (13% HNO_3).

Evaporation of formose sample - procedure

Addition of DHA and HCHO standards with known quantities in the evaporated formose model samples followed by four steps evaporation and subsequent analysis were conducted. All samples were diluted to factor 5 prior to evaporation experiments those finally reach to the original volume after evaporation. Loss of several volatile compounds during evaporation depends on the sample's matrix. DHA and HCHO both are volatiles and evaporation of DHA is interfered by the HCHO in the matrix therefore, it is necessary to add a certain amount of both standards to optimize the evaporation rate of DHA from the samples and to establish recovery. Rotary vacuum evaporator was used for all evaporation experiments. Evaporation was done at 0.1 bars and 80°C with a rotating speed and evaporation time of 80 rpm and 20 min, respectively.

Reduction of formose samples - procedure

For conducting reduction reaction, particular alkaline pH (8-10) was necessary. Therefore, 1 ml of formose sample (produced by high temperature reac-

tion, sample pH was 7.0) was diluted to 5.62 ml with de-ionized water followed by addition of 100 µL of 1% NH_4OH aqueous solution to rise the pH up to 9.0 to facilitate reduction. As FR further starts if pH exceeds 10.0 therefore, care should be taken to maintain the specific pH. Reduction was started for 24 h at ambient temperature by addition of 54.6 mg sodium borohydride ($NaBH_4$). Reactant was stirred in a closed-glass tube during the reaction period. Production of certain amount of H_2 gas inside the tube was not problematic. Reaction was stopped by addition of 220 µL of 10% HCl. Final volume of the reduced sample was 6 ml.

Analysis

(a) Chemicals and reagents

$Ca(OH)_2$, GA, sugar standards, absolute ethanol, HCOOH and DNPH were purchased from Sigma Aldrich, Vienna, Austria. 36% HCHO aqueous solution (containing approximately 10% CH_3OH as stabilizer), paraformaldehyde (PF), CH_3OH, H_2SO_4, acetonitrile (CH_3CN) and HNO_3 were obtained from Carl Roth, Graz, Austria. NH_4OH (25% solution) was obtained from Alfa Aesar, Vienna, Austria. Sodium borohydride ($NaBH_4$) was purchased from Merck, Vienna, Austria. All standards, chemicals and reagents meet HPLC-grade purity.

(b) Chromatographic conditions

Hitachi Lachrom Elite HPLC system (VWR International, Darmstadt, Germany) equipped with L-2130 pump, L-2450 DAD, containing a Hg lamp with 13 µL flow cells and set at 360 nm was used for analysis of the derivatized samples. Three Chromolith RP-C18 columns each (100 X 4.6 mm) with Chromolith guard cartridge (5 X 4.6 mm) (all from VWR International) were used for determination of the aforementioned analytes. Different gradients ((5-90) and (5-100)%) and flow rates (0.8 and 1.0 ml/min) were used for the analysis of DHA. Gradient was applied for 65 min run time. For HCOOH analysis, the conditions were: (5-36.6)% ACN for 20 min at 1 ml/min.

(c) Preparation and analysis of standards and samples

HCHO, DHA and HCOOH each 10 gL^{-1} stocks were prepared by diluting the corresponding reagents in de-ionized water. Stocks were stored in 4°C. Formose model samples were prepared following the same technique. To maintain the DNPH/sample mole ratio approximately 30:1, DNPH solutions were prepared by dissolving the reagent in H_2SO_4 as 100 mg/0.5 ml of acid and then diluted to 50 ml with absolute ethanol. The prepared reagents were 17.0, 2.0, 1.0, 0.75, 0.5 and 0.35 gL^{-1}as required for the derivatization protocol. Particular derivatization technique was followed as described in the earlier work[12]. Formose samples were derivatized with 17.0 and 1.5 gL^{-1} of reagent for the analysis of HCOOH in non-reduced and reduced samples (see Figure 5), respectively. Other mentioned concentrations of reagent were used in the samples intended to use for DHA analysis. All samples were diluted to 1:500 before derivatization.

RESULTS AND DISCUSSION

Optimization of DHA analysis

Figure 2a, b, c and d represent results in which chromatograms produced by using 0.35 gL^{-1} of DNPH seemed to be the best one (see Figure 2d). The derivatization reactions appeared as incomplete when using the concentrations of DNPH below 0.35 gL^{-1}.

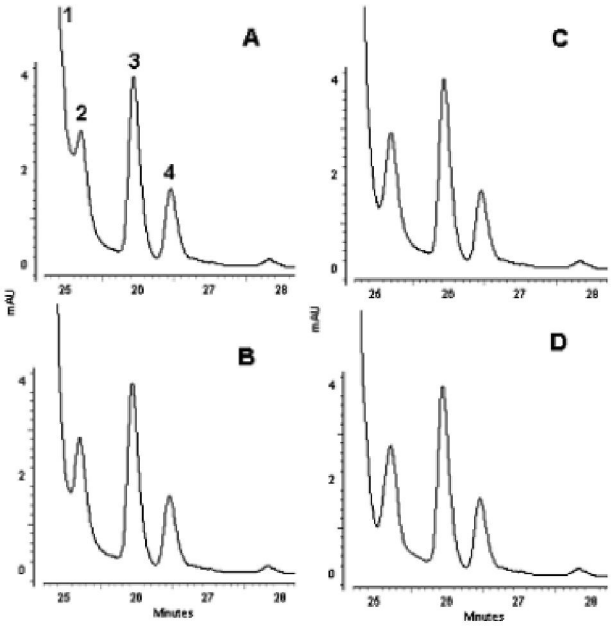

Figure 2 : A. Analysis of DHA in RPLC; c(DNPH): 1.0 gL^{-1}, CH_3CN/water gradient used: (5-100)% CH_3CN for 60 min at 1 ml/min; Peak assignments: 1: Unreacted DNPH, 2: DHA-hydrazone, 3: GA-hydrazone, 4: hydrosulphate; B. c(DNPH): 0.75 gL^{-1}; C. c(DNPH): 0.5 gL^{-1}; D. c(DNPH): 0.35 gL^{-1}; other conditions for B., C, and D. are as A

Full Paper

(5-100)% ACN at 1 ml/min as appeared in the previous work was used for the separation. Reduction of flow rate results worse resolution in DHA peak (see Figure 3a). Among all of the optimization trials, the best chromatographic conditions were found as in Figure 3b where (5-90)% ACN at 1 ml/min was used for the separation.

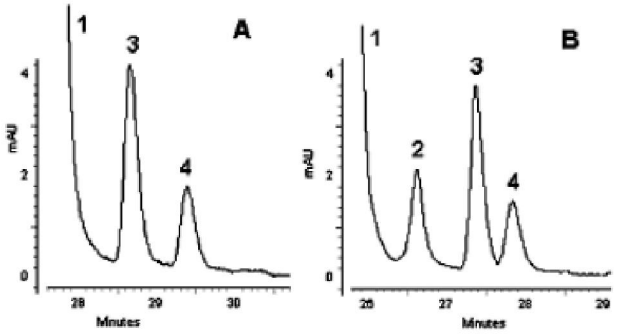

Figure 3 : A. Analysis of DHA in RPLC; c(DNPH): 0.35 gL^{-1}, CH_3CN/water gradient used: (5-100)% CH_3CN for 60 min at 0.8 ml/min; Peak assignments: 1: Unreacted DNPH, 2: DHA-hydrazone, 3: GA-hydrazone, 4: hydrosulphate; B. CH_3CN/water gradient used: (5-90)% CH_3CN for 60 min at 1.0 ml/min; c(DNPH): 0.35 gL^{-}1 and peak assignments: as A

Optimization of DHA evaporation

As it is mentioned in the earlier work[12] the DHA peak appears on the tailing of 1st Unreacted DNPH peak therefore, quantification was not possible within the same run together with other compounds. Removal of excess amount of Unreacted DNPH is essential to analyze the DHA. To eliminate this problem, the technique mentioned in section 2.2 was followed. Though a hint made regarding the usage of evaporation technique in the previous work, the analytical data for DHA presented was an approximation from the GCA quantification yet. DHA produces out of FR through isomerization of GCA in a particular proportion, while approximation is possible. However, this ratio is depending on the reaction parameters. Therefore, precise quantification is necessary for this substance.

Figure 4 is a plot of percent calculated DHA in vapour phase (VP) and corresponding concentration (gL^{-1}) in liquid phase (LP) of 4 different observations. The Figure constructed as resemble with the 'McCabe-Thiele' diagram (method for the analysis of binary distillation) for the analysis of volatile substances. It is necessary to be mentioned here the DHA is a volatile compound in the formose sample as HCHO therefore, evaporation of the matrix containing these compounds results certain losses of the amount in the vapour phase. It is then obvious to take into account the losses. According to 'Henry's law', the equilibrium of DHA will be interfered by other volatile substances in the matrix. Value of DHA in VP was calculated by using the developed curve. Relevant analytical and calculated data are provided in TABLE 1, 2 and 3. However, it is mentionable here that 'McCabe-Thiele' diagram is limited for specific reaction samples. Detailed investigations by varying the concentrations of different substances in the model solution are necessary to optimize a real 'vapour-liquid phase diagram' for DHA analysis in formose sample. This is reasonably a future outlook for this work.

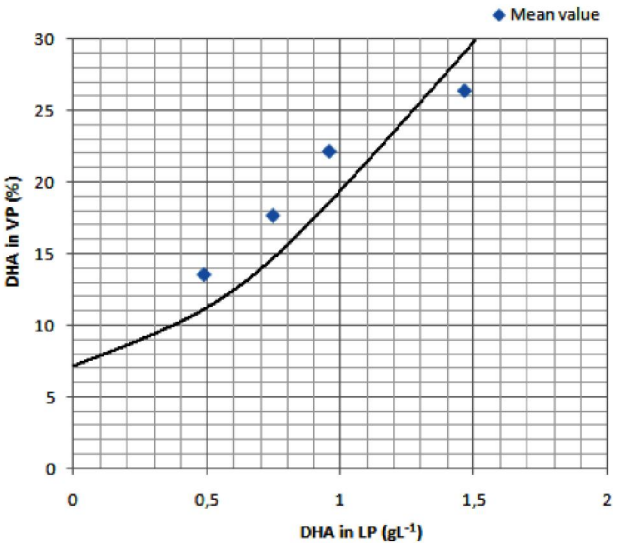

Figure 4 : Developed diagram for the analysis of DHA in RPLC system; thick line represents the 'line of constant response' for all the values measured; used data are provided in TABLE 4

TABLE 1 : Data on 1st event of evaporation experiments. HCHO and DHA in model and formose samples; quantities added before evaporation and corresponding observed values after evaporation; FD = formose model sample

Samples	DHA added [g/l]	HCHO added [g/l]	DHA observed [g/l]	HCHO observed [g/l]
FD_1	2.00	32	1.56	11.90
FD_2	1.00	32	0.86	9.60
FD_3	0.50	32	0.70	10.80
FD_4	1.50	20	1.05	7.12
Formose	0	0	0.25	6.55

TABLE 2 : Cumulative data on 1st, 2nd and 3rd events of evaporation experiments were obtained in RPLC. Three steps evaporation of the same samples of four different formose model samples were conducted. HCHO and DHA observed in liquid phase and calculated in vapor phase in corresponding samples; VP = vapor phase

DHA in 1st observation* [g/l]	DHA in VP (%)	HCHO in 1st observation* [g/l]	HCHO in VP (%)
1.56	30.45	11.90	69.13
0.86	30.81	9.60	75.10
0.70	32.71	10.80	71.98
1.05	39.76	7.12	73.18
DHA in 2nd observation [g/l]	**DHA in VP (%)**	**HCHO in 2nd observation [g/l]**	**HCHO in VP (%)**
1.56	30.00	5.62	52.77
0.70	18.61	2.90	69.80
0.50	0	5.34	50.56
1.05	0	5.07	28.80
DHA in 3rd observation [g/l]	**DHA in VP (%)**	**HCHO in 3rd observation [g/l]**	**HCHO in VP (%)**
1.27	18.60	2.67	52.50
0.68	3.60	1.50	48.28
0.46	8.00	2.90	45.70
0.77	26.67	2.22	56.21

***Corresponding data on 1st event of evaporation experiments from TABLE 1**

TABLE 3 : Data arranged from TABLE 2 to construct Figure 3

1st value of DHA observed [g/l]	Mean value of DHA observed [g/l]	1st value of DHA in VP (%)	Mean value of DHA in VP (%)
1.56		30.45	
1.56	1.463	30.00	26.350
1.27		18.60	
2nd value		**2nd value**	
0.86		30.81	
0.70	0.745	18.61	17.673
0.68		3.60	
3rd value		**3rd value**	
0.50		32.71	
0.50	0.487	0	13.570
0.46		8.00	
4th value		**4th value**	
1.05		39.76	
1.05	0.957	0	22.143
0.77		26.67	

Finally, DHA was precisely quantified in 4 min formose sample as 0.643 gL^{-1} using the optimized evaporation and chromatographic conditions, while the HCHO conversion was 23.7 gL^{-1}.

Optimization of HCOOH analysis

Formose samples produced at high temperature reaction were taken under reduction (as described in section 2.3) for the ease of HCOOH analysis. Reduced samples were analyzed by using the chromatographic conditions as in section 2.4.2. Figure 5 shows the chromatogram overlays of non-reduced and reduced formose samples. Non-reduced samples possessed larger peak areas due to emerging either sugars or other analogues of sugars in it whereas, reduction of those sugars privileged the precise quantification of HCOOH. The optimized method is a unique for the quantification of HCOOH in formose sample since quantification of this acid is interfered by other organic acid's peaks in ion chromatography. It is mentionable, including HCOOH; some other organic acids are produced in formose reaction indeed.

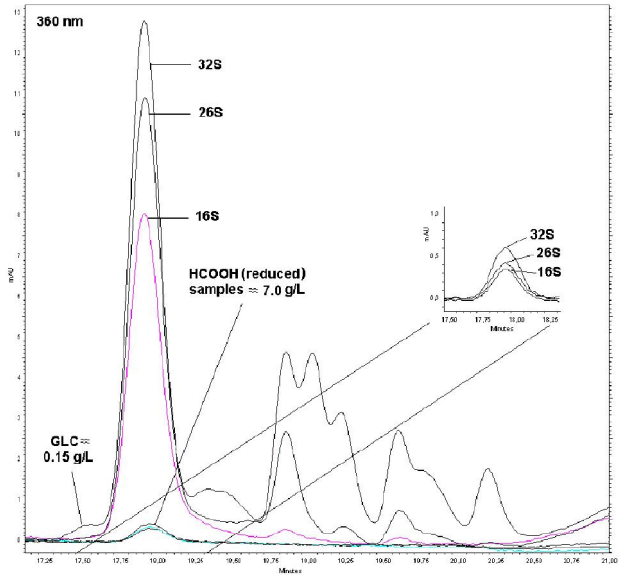

Figure 5 : Chromatogram overlays of non-reduced and reduced formose samples; 16S, 26S and 32S are the 16, 26 and 32 sec reaction samples respectively; quantified data as in legend; chromatographic conditions as in section 2.4.2; sample dilutions and DNPH concentrations as in section 2.4.3

CONCLUSIONS

A modified DNPH derivatization method in RPLC for quantification and determination of DHA and HCOOH in formose samples as presented in this manu-

Full Paper

script is an additional crucial contribution in the field of formose chemistry. Particular evaporation technique and diagram was developed for the DHA analysis, which could be helpful to analyze this specific substance in the formose samples. Analysis of HCOOH in formose sample by the developed technique can be considered as a better one over ion chromatography due to no interference comes from other substances in the sample during analysis.

ACKNOWLEDGEMENTS

Financial support from OEAD Austria is gratefully acknowledged. Author thanks the department of Food Science and Technology, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria for providing him research scope. Author also thanks the Bangladesh Agricultural University, Mymensingh, Bangladesh, for his sabbatical leave.

REFERENCES

[1] A.Levart, M.Veber; Chemosphere, **44**, 701 **(2001)**.
[2] A.N.Simonov, O.P.Pestunova, L.G.Matvienko, V.N.Parmon; Kinet.Catal., **48**, 245 **(2007)**.
[3] D.R.Cardoso, S.M.Bettin, R.V.Reche, B.S.Lima-Neto, D.W.Franco; J.Food Compos.Anal., **16**, 563 **(2003)**.
[4] F.Edelkraut, U.Brockmann; Chromatographia, **30**, 221 **(1990)**.
[5] F.Lipari, S.J.Swarin; J.Chromatogr., **247**, 297 **(1982)**.
[6] F.M.Lamari, R.Kuhn, N.K.Karamanos; J.Chromatogr.B, **793**, 15 **(2003)**.
[7] G.Burini, R.Coli; Anal.Chim.Acta, **511**, 155 **(2004)**.
[8] H.J.Zhang, J.F.Huang, H.Wang, Y.Q.Feng; Anal. Chim.Acta, **565**, 129 **(2006)**.
[9] H.Zegota; J.Chromatogr.A, **863**, 227 **(1999)**.
[10] I.D.Miguel, V.Rieumajou, D.Betbeder; Carbohydr. Res., **319**, 17 **(1999)**.
[11] J.Li, J.Z.Md, L.Y.Md; Asia Pac.J.Clin.Nutr., **16**, 127 **(2007)**.
[12] M.Z.Iqbal, S.Novalin; J.Chromatogr.A, **1216**, 5116 **(2009)**.
[13] N.K.Karamanos, T.Tsegenidis, C.A.Antonopoulos; J.Chromatogr., **405**, 221 **(1987)**.
[14] O.Pestunova, A.Simonov, V.Snytnikov, V.Stoyanovsky, V.Parmon; Adv.Space Res., **36**, 214 **(2005)**.
[15] T.Mizuno, A.H.Weiss; Adv.Carbohydr.Chem. Biochem., Academic Press, 173 **(1974)**.
[16] T.Okuda, S.Saito; Tetrahedron, **30**, 1187 **(1974)**.
[17] X.H.H.Huang, H.S.S.Ip, J.Z.Yu; Anal.Chim.Acta, **604**, 134 **(2007)**.
[18] Y.Shigemasa, T.Fujitani, C.Sakazawa, T.Matsuura; Bull.Chem.Soc.Jpn., **50**, 1527 **(1977)**.
[19] Y.V.Heyden, A.N.M.Nguyet, M.R.Detaevernier, D.L.Massart, J.Plaizier-Vercammen; J.Chromatogr. A, **958**, 191 **(2002)**.