Quantitative Determination of Sugars with Ammonium Metavanadate Reagent: Micro Analysis of Medicinal Compounds

Kumari Anita Sinha[1], AfshanSuraiya[1]and R. P. S. Chauhan[2,*]

[1](Department of Chemistry, G. B. M. College, Gaya, Magadh University, Bodh Gaya, Bihar 824234)
[2](PG Department of Chemistry, Magadh University, Bodh Gaya, Bihar 824234)
Corresponding author: R. P. S. Chauhan

Abstract:*The current study shows that taking advantage of the oxidizing capacity of ammonium metavanadate (v) with certain organic compounds, the oxidation of certain sugars was studied an accurate and quantitative procedure has been described for the determination of certain mono and disaccharides on micro scale. 1-5 mg of sample was allowed to react with excess of ammonium metavanadate (v) for about 30 (monosaccharides) to 40 (disaccharides) minutes at 40°C temp. The unreacted ammonium metavandate (v) was titrated against ferrous ammonium sulphate using N-Phenylanthranilic acid as indicator. A blank was also run under identical conditions and the recovery of the sugars was calculated. For establishing exact reaction condition following variables were carried out.*

Background:*Drugs are substances or products that are used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient. It is only logical that these drugs might arise from several sources. The sources of drugs have travelled through a complete arc, being derived mainly from natural sources in the early centuries to being synthetically manufactured today. Lindberg and Missionryemployed sodium borohydride as the reductant for determining carbohydrates. The sample was dissolved in water and treated with solution of sodium borohydride. After the reaction sis completed the residual borohydride is determined by measuring the hydrogen evolved upon the addition of acid. Peat, Whelan and Roberts' determined the degree of polymerization of reducing oligosaccharides, by means of sodium borohydride.*

Materials and Methods: *Aliquots, containing 1.5 mg of the sample were taken in 100 ml Erlenmeyer flask followed by the addition of 1 ml of 0.3 N, V(v) reagent and 5 ml of 10 N sulphuric acid. The reaction contents were shaken gently and kept on a boiling water bath for 30 minutes. After the reaction was over the reaction mixture was cooled at room temperature. The unconsumed V(v) reagent was titrated against 0.025N ferrous ammonium sulphate using N-phenyl anthranilic acid as an indicator.*

Results: *In the view of the above reactions and considering the amount of ammonium metavanadate (v) consumed per mole of the sugars the following reaction mechanism are proposed for complete oxidation of mono and disaccharides. It has been found that sugars containing aldehydic group are oxidized to formic acid only. In case of Ketonic sugars also formic acid is the end product but ketonic group appear to be oxidized to carbon dioxide. Xylose which is a pentose is oxidized as follows with V(v) reagent. Disaccharide consume 26 equivalents of V(v). On the basis of the following oxidation reaction is proposed for disaccharides. The proposed method has been applied for the determination of monosaccharide and disaccharide using 0.3N, V(v) reagent. The sample size of determination ranges from 1 to 5 mg and error does not exceed + 1%.*

Conclusion:*The current study shows that taking advantage of the oxidizing capacity of ammonium metavanadate (v) with certain organic compounds, the oxidation of certain sugars was studied an accurate and quantitative procedure has been described for the determination of certain mono and disaccharides on micro scale. 1-5 mg of sample was allowed to react with excess of ammonium metavanadate (v) for about 30 (monosaccharides) to 40 (disaccharides) minutes at 40°C temp. The unreacted ammonium metavandate (v) was titrated against ferrous ammonium sulphate using N-Phenyl anthranilic acid as indicator. A blank was also run under identical conditions and the recovery of the sugars was calculated. For establishing exact reaction condition following variables were carried out.*

I. Introduction

Drugs are substances or products that are used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient. These drugs might arise from several sources. The sources of drugs have travelled through a complete arc, being derived mainly from natural sources in the early centuries to being synthetically manufactured today.
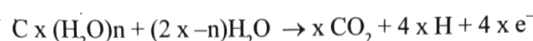
Lindberg and Missionry[1,2] employed sodium borohydride as the reductant for determining carbohydrates. The sample was dissolved in water and treated with solution of sodium borohydride. After the reaction sis completed the residual borohydride is determined by measuring the hydrogen evolved upon the addition of acid. Peat, Whelan and Roberts' determined the degree of polymerization of reducing oligosaccharides, by means of sodium borohydride.

Colorimetric methods are also available for the determination of sugars. The first procedures were developed by Floin and Wu[4] in 1919 which is based in the conversion of cupric ions to the cuprous sulphate by the presence of reducing sugars. The cuprous ions subsequently reduce phosphotungstic acid to a blue complex which is measured calorimetrically. Dearing' described a micro method for the estimation of cellulose Wahba and Coworkers[6] converted glucose to glucosazone and measured the yellow colour of its solution. Shallenberger and Mooros[7] developed the colour with a reagent containing copper sulphate and arsenomolybdates and measured the solution at 500mg Various phenolic compounds have been recommended for the colorimetric determination of carbohydrates. Dubois[8] used phenol for the determination of reducing sugars. Tillmans[9] determined the reducing sugars with the use of thymol. Sorenson, Fisher and Lindh 10-12 used resorcinol and phloroglucinol for the determination of reducing sugars.

Livingstin, Maurmeyer and Worthman[13] described a specific method for fructose which is based on the formation of green colour when the sample is treated with concentrated sulphuric acid and phenol thendiluted with glacial acetic acid. Nitro compounds have also been used for the determination of carbohydrates'as the colorimetric reagents. Kestermann used picric acid for the determination of carbohydrates. While others used 3,4- dinitrobenzoic acid, dinitrosalicylicacid,2,4-dinitropheny-lesulphoner, p-aminosalicylic acid' and o-aminodipheny119 for the determination ofaldose. Anthrone has also been used for the quantitative analysis of carbohydrates.Helbert and Brownreported that colour is unstable and varies with temperature and time.Cheronis and Co-workers2' determined reducing sugar using tetrazolium salts. Hass and Lynch[22] developed a method for the

determination of carbohydrates. Mayer and Isbe1[23] employed radioactivity and determined end groups in carbohydrates. Browne[24] determined carbohydrates by biological methods. Figueirede[25] have developed an improved complexometric method for the analysis of reducing sugars. Amongst the various oxidimetric methods for the determination of sugars, cuprimetry has been of great use from early times. Divalent copper complexed with tartarate or citrate in alkaline medium known as Fehling's solution[26-29] and Benedict's solution respectively.
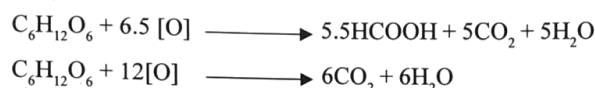
A number of attempts have been made to modify the cuprimetrywith greater advantages. Esohmann[32] and Eschmann and Co-workers replaced tartaric and citrate with EDTA and claimed to get better results. In a series of papers published by Ablov and Batyr[34-38] and Kats and Shoikhet[37] trihydroxy glutarate complex of Cun has been used as oxidising agent for sugars where in many irregularities attending Fehling's and Benedict's method have been claimed to be removed. Likewise,Rebega[38] and Rlbega and coworkers[39] used cuprithiosalicylate as oxidising agent and have determined sucrose, fructose and glucose empirically. Celsi and Sarrailoxidised glucose, surcose and lactose with Cu(14) in the mixture of K_2CO_3 and KCNS. The precipitated CuSCN was estimated using Fe(III) and Ag(I). Defrates and castles estimated reducing sugars with Fehling's solution using electrometric end point detector. Potassium ferricyanide has been widely used in determining sugar[42, 48]. According to Blom and Rosted[49] this method of estimation so sugars with ferricynide is much inferior to cuprimetry method in as much as ferricyanide procedures, side reactions and it attacks other organic substances present in the sugar. Lately there have been good deal of attempts to regularise the oxidation of sugars with ferricyanide better results have been claimed when sugar is treated against a boiling alkaline Solution of hexacyanoferrate (111)[50]. Yamagishi and Yoko& using phosphate buffer instead of carbonate and bicarbonate showed enhanced regularity in the determination of sugars. They also found that under these conditions, sucrose, tretose and cellulose were oxidized with ferricynitZelawski and Co-workers , Wollslegier modification in which the quantity ofphosphate buffer and $ZnSO_4$-K[1] mixture solution were reduced to 25% and 40% respectively of those in procedure of Fuzita and Iwatake unsuitable because of incomplete oxidation of sugars, and hence Fuzita and Iwatake used phosphate buffer for oxidation of sugars, is not recommended with ferricyanide. Friedemann and co-workers demonstrated that the irregularities in the oxidation of sugars observed earlier due to ferricyanide and sodium carbonate ratio presence of $NaHCO_3$ size of test tube, volume of the reaction mixture, time of standing before heating and cooling become insignificant when sodium carbonate and ferricyanide ratio was greater than 10:1 They further gave an equation relating concentration of ferricyanide and sugars.

II. Material and Methods

Aliquots, containing 1.5 mg of the sample were taken in 100 ml Erlenmeyer flask followed by the addition of 1 ml of 0.3 N, V(v) reagent and 5 ml of 10 N sulphuric acid. The reaction contents were shaken gently and kept on a boiling water bath for 30 minutes. After the reaction was over the reaction mixture was cooled at room temperature. The unconsumed V(v) reagent was titrated against 0.025N ferrous ammonium sulphate using N-phenyl anthranilic acid as an indicator.

For testing the quantitative validity of reaction,alkaline Solution of hexacyanoferrate (111)[50]. Yamagishi and Yoko&using phosphate buffer instead of carbonate and bicarbonate showedenhanced regularity in the determination of sugars. They also found thatunder these conditions, sucrose, tretose and cellulose were oxidized with ferricynitZelawski and Co-workers , Wollslegier modificationin which the quantity ofphosphate buffer and $ZnSO_4$-K1 mixture solutionwere reduced to 25% and 40% respectively of those in procedure ofFuzita and Iwatake unsuitable because of incomplete oxidation ofsugars, and hence Fuzita and Iwatake used phosphate buffer for oxidationof sugars, is not recommended with ferricyanide. Friedemann andco-workers demonstrated that the irregularities in the oxidation ofsugars observed earlier due to ferricyanide and sodium carbonate ratiopresence of $NaHCO_3$ size of test tube, volume of the reaction mixture,time of standing before heating and cooling become insignificant whensodium carbonate and ferricyanide ratio was greater than 10:1 Theyfurther gave an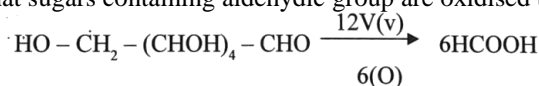 equation relating concentration of ferricyanide and sugars.Keeping the amount of glucose, concentration of V(v) reagentand sulphuric acid as constant, the reaction time was varied from 1 to45 minutes. Aliquots containing 1 to 5 mg of glucose were taken inflask and 1 ml of 0.3 N, V(v) reagent and 5 ml of 10 N sulphuricacidwas added to it. The reaction was carried out on a boiling water bathfor 5, 10, 15, 20, 25, 30, 35, 40, 45 minutes. After the prescribed reactiontime contents were cooled at room temperature and the unconsumedV(v) reagent was determined. It was observed that the recovery of thesample become Constant within the reaction time of30 minutes.Keeping the amount of sample reaction time and theconcentration of V(v) reagent as constant. The effect of varyingconcentration of sulphuric acid was studied. Concentration rangingfrom 1 N to 15 N was added to the reaction mixture and allowed toreact on a boiling water bath. After prescribed reaction time the reactionmixture was cooled at room temperature and unconsumed V(v) reagentwas determined titrimetrically. It was observed that the most accurateresults were obtained with 10Nsulphuric acid.

III. Resultand Discussion

With the recommended procedure the determination of mono and dihydric alcohols has successfully been achieved the present study has revealed that ammonium metavanadate(v) is a good oxidising reagent for mono and disaccharides. Thedetermination was done by using different variables such as reactiontime, temperature concentration of the reagent and amount of the reagentof the different samples like glucose, maltose, galactose, fructose,sorbose, xylose and sucrose.The result show thatthe percentage recovery of the sample is fairly constant with the varyingamount of 1-5 mg.

Various reaction mechanism has been proposed for sugarusing different reagent. Fleury and Co-workers[56] shows that formic acidand formaldehyde are the product when aldoses are oxidized whileglycolic acid is an additional product when ketoses were oxidized with periodic acid.
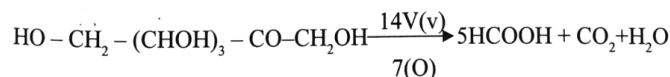
$$HO{-}CH_2{-}(CHOH)_4 - CHO \xrightarrow{5HIO_4} HCHO + 5HCOOH + 5HIO_3$$

Aldose

$$HO{-}CH_2{-}(CHOH)_3 {-}CO{-}CH_2OH \xrightarrow{4HIO_4} HCHO + 5HCOOH + \underset{CH_2OH}{\overset{HOOC}{|}} + 4HIO_3$$

Ketose

It is evident from the above reaction that varying amounts of periodate are consumed for the two categories of sugars.

Aldoses are smoothly attacked by hypoiodite and hypobromite consuming two equivalents of the oxidants. As early as 1897 Romijh[62] determined aldoses by oxidising with hypoiodite on the basis of consumption of two equivalents. He found that ketoses are not attacked by hypoiodite but methanol, formic acid, glycerine, mannitol and lactic acid interfered in the determination.

Yoshimura and Co-workers illustrated the consumption of 6 equivalents of hypobromite when oxidation was carried out in boiling condition instead of room temperature.

Nasulaev[76] determined glucose with the consumption of two equivalents of IBr. Becke[1-82]standardisedCu(III) against glucose at room temperature assuming the consumption of 8 equivalents of Cu(111) per mole of the glucose.

$$C x (H_2O)n + (2 x - n)H_2O \rightarrow x CO_2 + 4 x H + 4 x e^-$$

x = 5	n = 5	pentoses
x = 6	n = 6	hexoses
x = 12	n = 11	disaccharides

The present study has revealed that ammonium metavanadate(v) is a good oxidising reagent for mono and disaccharides. Thedetermination was done by using different variables such as reactiontime, temperature concentration of the reagent and amount of the reagentof the different samples like glucose, maltose, galactose, fructose,sorbose, xylose and sucrose.The result show thatthe percentage recovery of the sample is fairly constant with the varyingamount of 1-5 mg.Various reaction mechanism have been proposed for sugarusing different reagent. Fleury and Co-workers[56] shows that formic acidand formaldehyde are the product when aldoses are oxidized whileglycollic acid is an additional product when ketoses were oxidized with periodic acid.

Bhansali and Mathur[99] photochemically oxidised sugars with Ce(IV) sulphate with and without the use of catalyst they suggested the following reaction path.

$$C_6H_{12}O_6 + 6.5\,[O] \longrightarrow 5.5HCOOH + 5CO_2 + 5H_2O$$
$$C_6H_{12}O_6 + 12[O] \longrightarrow 6CO_2 + 6H_2O$$

In the view of the above reactions and considering the amountof ammonium metavanadate (v) consumed per mole of the sugars the following reaction mechanism are proposed for complete oxidation of mono and disaccharides. It has been found that sugars containing aldehydic group are oxidised to formic acid only.
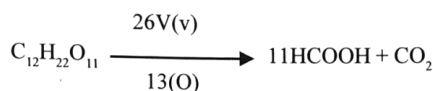
$$HO - CH_2 - (CHOH)_4 - CHO \xrightarrow[6(O)]{12V(v)} 6HCOOH$$

In case of Ketonic sugars also formic acid is the end product but ketonic group appear to be oxidized to carbon dioxide.

$$HO - CH_2 - (CHOH)_3 - CO-CH_2OH \xrightarrow[7(O)]{14V(v)} 5HCOOH + CO_2 + H_2O$$

Xylose which is a pentose is oxidized as follows with V(v) reagent.

$$C_6H_{10}O_5 \xrightarrow[5(O)]{10V(v)} 5HCOOH$$

Disaccharide consume 26 equivalents of V(v). On the basis of the following oxidation reaction is proposed for disaccharides.

$$C_{12}H_{22}O_{11} \xrightarrow[13(O)]{26V(v)} 11HCOOH + CO_2$$
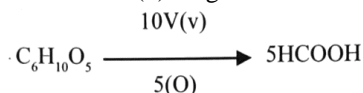
The proposed method has been applied for the determination of monosaccharide and disaccharide using 0.3N, V(v) reagent. The sample size of determination ranges from 1 to 5 mg and error does not exceed $\pm$ 1%.

IV. Conclusion

The current study shows that taking advantage of the oxidizing capacity of ammonium metavanadate (v) with certain organic compounds, the oxidation of certain sugars was studied an accurate and quantitative procedure has been described for the determination of certain mono and disaccharides on micro scale. 1-5 mg of sample was allowed to react with excess of ammonium metavanadate (v) for about 30 (monosaccharides) to 40 (disaccharides) minutes at 40°C temp. The unreacted ammonium metavandate (v) was titrated against ferrous ammonium sulphate using N-Phenylanthranilic acid as indicator. A blank was also run under identical conditions and the recovery of the sugars was calculated. For establishing exact reaction condition following variables were carried out.

References

[1]. lindberg. lt. &Missionry, A. Svcnsk, Papperstidn; 55, 13 (1952)
[2]. lindberg, 13. &Theander, Ibdi; 57, 83 (1954)
[3]. Peat, S.; Whelan, W. J. & J. Chem. Soc., 2258 (1956)

[4]. Floin, O. & Wu, M. J. Biochem., 38, 110 (1919)
[5]. Dearing, G. G. Nautre; 179, 579 (1957)
[6]. Wahba, N;.Hanna, S. Analyst, 81, 27 (1957).
[7]. Singh, R., D. Phil. Thesis A. U. (1979).
[8]. Paparia, Migue, Quint Anal; 30 (2), 93 (1976)9
[9]. Bondak, M.I., Lyutvort, S.G., Malikov, V.T., Pyatin, S., Skidan, Yu.A. (VennitsPolybelinic Institute) USSR 544, 917
[10]. Churasek, J., J. Chromatogr. Libr; 3 (1975)
[11]. El-Aggar, A.M. et al,   J. Chem. 19 (41, 499-503) (1970) (Eng.)
[12]. Srivanasan, M. et al, Z. Anal. Chem; 282(2), 143 (1976) (Eng.)
[13]. Kawati, Shohai et al,   J. Technol,Rep. Kansai Univ.18, 47-52 (1972) (Eng.)
[14]. Leenson, J:A.Sergeev G.B. et al, Z. Anal. Khim; 32 (5) (Russ) (1977).
[15]. Lucas, H. J., J. Amer. Chem. Soc; 52, 802 (1930).
[16]. Ritter, F. et al, J. Chem. Educ; 30, 395 (1953)
[17]. Litter, J. S. and Watters, W.A., J. Chem. Soc; 1299, 301, 4046 (1959).
[18]. Jones, J. R. and Wafters, W. A., J. Chem. Soc; 1629, 2068 (1962).
[19]. Srivastava, S and Gupta, V., J. Indian Chem. Soc; 83, 1103 (2006).
[20]. Rajni Mohanty K; Das, M; and Das, A., Indian J. Chem. Sec A. 37, 34 (1998).
[21]. Iloukhani, H; Zarie F., Asian J. Chem. 17(4) 2437 (2005).
[22]. Niknam, Khodabakhsh, Asian J. Chem. 17(4) 2513 (2005).
[23]. SerVi, S. and Acar, A., Molecules; 7 104 (2002) Reaction of Organic Compounds 212 (1962)
[24]. Reynold, C. Fuson, Reaction of Organic Compounds; 227 (1962)
[25]. Barakat, M. S. and M. F. A., El-Wahab, J. Amer. Chem. Soc., 75, 573 (1953).
[26]. Reynold, C. Fuson, J. Org. Chem; 21,478(1956)
[27]. Pappo, R., Allen, D. S. Lenienx, R.U.
[28]. Rigby, J., J. Chem. Soc. 1907 (1950)
[29]. Singh, S. M., D. Phil. Thesis A. U. (1978)
[30]. Barkat, M. Z., and Mousa et al, J. Pharm Phannacol; 4, 115(1952).
[31]. Kruse, P. F., Jr., Grist, K. L. et al Anal. Chem; 26, 1319 (1954)
[32]. Severin, M. et al, Bull, Rech Argon. Cemblaix, 1(2), 289; C.A. 66, 176188 (1966)
[33]. Sirot, JacquensandSirto et al 2, 87, 426 (Cl. G. 4 Feb. 1972) Appl. 18, 198,19 May 4pp (1970).
[34]. Naidu H. M. K. et al, Indian J.Chem. 13(a) 976-7 D. S.   (Eup) (1975).
[35]. Lotan, Noah, Ehrlich et al Microchem. J. 20(4) 534-8, (1975) Eng.
[36]. Dusic, Z., Inst. Anal. Chem. Fac. Pharm. Arh Farm. 25 (4), 233-40 (1975)
[37]. Singh, B. Singh A. et al, Indian Chem. Soc; 33, 778 (1956)
[38]. Beck, G, Mikrochemic, 38, 152 (1951)
[39]. Beck, G., Mikrochemic, 40, 258 (1953)
[40]. Stognushko, D. P. et al, Z. Anal. Khim; 33(1) 142-5 (1978) (Russ)
[41]. Naidu, H. M. K. and Mahadevappa, D. S., Curr. Sci; 45(6) 216-18 (1976) (Japan)
[42]. Land, Haring and William, A. W., J. Chem. Soci; 35, 111 (1960)
[43]. Swann, S. and Xanthakos T. S, J. Amer. Chem. Soc. 53 (1928)
[44]. Drummond, A. Y. and Waters, W. A., J. Chem. Soc; 3119, 3456 (1954), 217 (1955), 4312 (1957) 2129(1958)
[45]. Rama Krishnan, P. S.andChockalingam, J. Indian Chem. Soc; 70, 583 (1993).
[46]. Tuwar,S. M., bewoor, S. T. Raju, J. R., Nandi 69, 651 (1992)
[47]. Dutta, S Karki, R. Strivanda, P.G. and Udupa, N. I, J. Pharma Sci; 62, 381 (2000)
[48]. Kande, R. B., Kasture, A.V., and Wakodher, A., Ind. J. Pharm. Sci;64, 24 (2002)
[49]. Kumar, H.M.S, Anjaneyulu et al, J. Indian Chem. Soc; 84, 189 (2007).
[50]. Kartha, K.P. R., Aloui, M. and Robert, A. F., Tetrahedron; 37, 8807 (1996).