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ANALYSIS OF BENZALDEHYDE IN THE PRESENCE OF BENZOIC ACID, BENZOQUINONE AND MALEIC ACID

R. N. KUMAR, G. N. BHAT AND N. R. KULOOR

Department of Chemical Engineering, Indian Institute of Science, Bangalore

IN the course of investigations on the catalytic vapour phase oxidation of toluene, using vanadium pentoxide as the catalyst in a fluidized bed, the qualitative analysis of the mixture of reaction products obtained, indicated that it consists of benzoic acid, benzaldehyde, benzoquinone and maleic acid. Downie *et al.*[1] are reported to have analysed such a mixture using potentiometric and spectrophotometric techniques. However, when an attempt was made to analyse such a mixture using the well-known techniques of chemical analyses, certain difficulties were encountered. For instance, (1) the alkalimetric titration of the mixture, for the estimation of total acids, could not be employed, since benzoquinone produced a dark colouration with alkali; (2) the estimation of benzaldehyde by the oxime method[4] could not as such be used since this method involved ultimately the titration of hydrochloric acid liberated, against an alkali, and the presence of benzoquinone interferes in such a titration.

Solution (A) of a mixture of benzaldehyde, benzoic acid, benzoquinone and maleic acid was obtained in about 40 c.c. of ether. To solution (A), about 5 c.c. of 30% aqueous potassium iodide solution and a few drops of 30% sulphuric acid were added, and the mixture shaken well. Under these conditions, the benzoquinone present in the original mixture was quantitatively converted into hydroquinone, liberating an equivalent quantity of iodine.[2] The liberated iodine was primarily left in solution in the ether layer, and only a small quantity of it was left in the aqueous layer containing excess of unused potassium iodide. Known quantities of standard sodium thiosulphate solution were continually added to the reaction mixture until both the layers of the latter became colourless, indicating that sodium thiosulphate solution has been added in slight excess over that required to complete reaction with the liberated iodine. The resulting mixture was separated into a water layer (B) and an ether layer (C). Water layer (B) was titrated against standard iodine solution and the excess of thiosulphate present therein was determined. From the knowledge of the total amount of sodium thiosulphate added and its amount subsequently determined as excess, the amount of iodine liberated and hence the amount of benzoquinone present in the original mixture was estimated on the basis that one mole of iodine liberated corresponds to one mole of benzoquinone.

The ether layer (C) contained benzaldehyde, hydroquinone (equivalent in quantity to that obtained from benzoquinone), benzoic acid and maleic acid. The benzoic and the maleic acid constituents of this solution were extracted into a saturated solution of sodium bicarbonate, obtaining thereby a layer of aqueous bicarbonate solution (D) containing the two acids, and an ether layer (E) containing benzaldehyde and hydroquinone.

The bicarbonate layer (D) was acidified with 30% sulphuric acid and treated with ether, when a water layer (F) and an ether layer (G) were obtained. At room temperature, since benzoic acid is highly soluble in ether and only very slightly so in water, the benzoic acid was completely extracted by ether, from a mixture of benzoic and maleic acids, together with a small amount of maleic acid, during one single extraction step with a relatively small quantity of ether. The major portion of the maleic acid however remained in solution in the aqueous layer (F), since it is highly soluble in water.

The ether layer (G) contained total benzoic acid present and a small quantity of maleic acid. By determining the total acid content of this layer by titrating it against standard alkali and then estmating its maleic acid content by titrating against standard potassium permanganate solution,[3] the quantities of benzoic acid and maleic acid present in layer (G) were determined

Similarly, the quantity of maleic acid present in the aqueous layer (F) was determined permanganimetrically, and the total quantity of maleic acid present in layers (F) and (G) constituted the entire amount of maleic acid present in the original mixture.

The ether layer (G) contained benzaldehyde and hydroquinone, and the benzaldehyde was estimated from this mixture by the oxime method.[4]

Table I gives details of (1) the composition of the original mixture of benzoquinone, maleic acid, benzoic acid and benzal-

TABLE I

Component	Quantity in original mixture, g.	Quantity as estimated g.	% error
Benzoquinone ..	0·1096	0·1058	3·5
Maleic acid ..	0·2147	0·2062	4·0
Benzoic acid ..	0·4412	0·4263	3·4
Benzaldehyde ..	3·5053	3·4014	2·9

dehyde taken for analysis, (2) the amounts of the components as estimated by the analytical procedure reported above, and (3) the percentage errors involved in the various estimations. It was also observed that analytical errors of less than 1% were obtained for benzoquinone, when alcoholic solutions of the original mixture were iodometrically analysed.

1. Downie, J., *et al.*, *Can. J. Chem. Engg.*, Oct. 1961, **39** (5), 201.
2. (*a*) Valear, *Compt. Rend.*, 1899, **129**, 552.
   (*b*) Pargel, H. K., *Ph.D. Thesis*, University of Colorado.
3. (*a*) Marisic, M., *J. Am. Chem. Soc.*, 1940, **62**, 2313.
   (*b*) *Chem. Abs.*, 1928, **22**, 43.
4. Bryant, W. M. D. and Smith, D M., *J. Am. Chem. Soc.*, 1935, **57**, 57.

NITRITE ESTIMATION IN THE ASSAY OF CERTAIN ENZYME SYSTEMS

M. M. R. K. AFRIDI
Department of Botany, Aligarh Muslim University, Aligarh

ASSAYS of nitrate and nitrite reductase systems are usually done by colorimetric estimation of the nitrite formed or lost during the reaction period by the Griess-Ilosvay method.[1-4] Hydroxylamine reductase may also be assayed by this method in terms of residual hydroxylamine recovered as nitrite, after oxidation. Two major sources of error may occur in these assays. The first of these is caused by a non-enzymic reaction between reduced pyridine nucleotides and nitrite[1,5,6] and the second by the underestimation of nitrite due to the precipitation of protein on adding the acid sulphanilamide reagent. The non-enzymic loss of nitrite was first reported by Evans and Nason,[1] who, however, considered it to be small. Medina and Nicholas[6] noted that nitrite was lost slowly and emphasised that the error was serious when nitrite was being removed from the system (as in nitrite reductase assay) but not when it was being formed (as in nitrate reductase assay). It would be expected, however that the estimation of nitrite would be subject to the same errors when it is being removed as an end-product as when it is present as a substrate. In fact, Table I shows that in nitrate reductase systems the loss of nitrite due to the presence of reduced diphosphopyridine nucleotide (DPNH) may be as high as 70%. In studies of the adaptive formation of the enzyme[2,7,8] this error may become very serious. In standard nitrite solutions the relationship between nitrite present and the extinction curve of the coloured end-product after diazotisation approximated to Beer's Law up to 200 μ M of nitrite in 10 ml. in the presence of any given concentration of DPNH[5] but the values observed for nitrite decrease in a non-linear manner. Lastly it may be pointed out that the loss of nitrite in standard solutions may be up to 80% depending upon the quantity of DPNH present (Table I), and that, contrary to the observation of Medina and Nicholas[6] it takes place very rapidly.

TABLE I

DPNH Interference with NO_2 Estimation and its Prevention

Standard Nitrate Solution
(Values as % of NO_2 added)

Experiment No.	Untreated: Amount of DPNH present (mg.) 0	0·1	0·2	0·4	All three concentrations of DPNH treated with: Zn or Ba acetate and ethyl alcohol	ADH and acetaldehyde
1	100	60·2	40·1	20·05	100	100
2	100	60·0	41·0	20·00	100	100

Nitrate Reductase Assay System
Total enzyme activity in cauliflower leaves using 0·4 mg. DPNH

Experiment No.	Untreated	Residual DPNH removed by Zn or Ba acetate and ethyl alcohol	Residual DPNH removed by ADH and acetaldehyde
1	40·0	74·3	74·0
2	82·5	100·0	100·5
3	83·2	103·8	104·0

Note.—Sodium dodecyl sulphate was used in nitrate reductase assays.

Although reduced triphosphopyridine nucleotide (TPNH) may cause somewhat less interference than DPNH, its far greater cost and the existence of some DPNH-specific enzyme