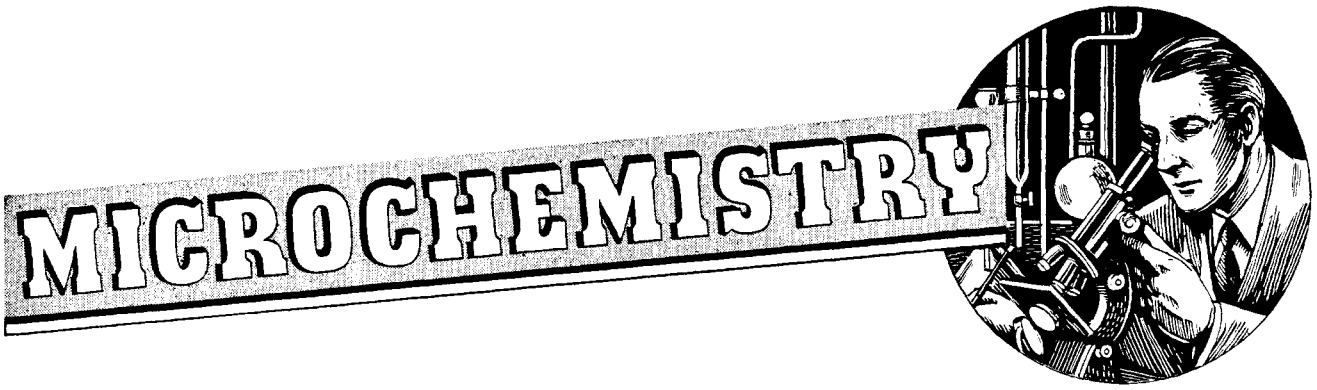

Quantitative Determination of Pentoses

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A convenient procedure is proposed for determining the furfural produced by the action of acids on pentoses and other substances. When a furfural-forming substance is refluxed with aqueous acid of the proper concentration in the presence of a suitable high-boiling, immiscible solvent, the furfural, as it is formed, is rapidly extracted by the solvent and to a considerable extent protected from further decomposition by the acid. This fact has made it possible to eliminate the continuous-distillation or steam-distillation procedure which is the time-consuming feature of most pentose determinations. When the refluxing is done it is necessary only to determine the concentration of furfural in the organic solvent in order to be in a position to calculate the total amount of furfural which has been formed. One hundred per cent conversion of *d*-xylose into furfural has been achieved under a variety of conditions.

FOLLOWING the observation that furfural in the presence of xylene is remarkably resistant to the action of boiling aqueous hydrochloric acid, a simplified method for pentose determination has been developed and is being used in this laboratory. [Foster (*3*) has employed immiscible solvents together with aqueous hydrochloric acid–containing salts to enhance the production of furfural from oat hulls.] The present report is concerned chiefly with the application of the procedure to the determination of the pentose xylose. The pentose is dissolved in dilute hydrochloric acid solution which is refluxed in the presence of a relatively large amount of xylene, and the furfural which is formed is determined colorimetrically in xylene solution. To convert the pentose to furfural it would be desirable to select a concentration of acid comparable to that of the usual distillation procedures. However, this is impossible for, as Pervier and Gortner (*9*) have pointed out, the distillation brings about a continuous change in concentration, so that the acid may increase from 12 to 20 per cent during a determination, while in the refluxing method the concentration of hydrochloric acid remains constant throughout the refluxing period. Satisfactory results have been obtained with acids containing 12 to 15 per cent of hydrochloric acid; however, over this range the rate of formation of furfural varies with the concentration of the acid.

A quantitative yield of furfural may be obtained from *d*-xylose. Determinations made on 0.4 to 1.0 mg. of xylose are reproducible to ±3 per cent, which is about the limit of accuracy of colorimetric observations. All other pentoses which have been investigated have given somewhat lower than the theoretical yields of furfural. This unfortunate shortcoming is shared by every method yet proposed for determining pentoses, with the exception of a recent macroprocedure of Hughes and Acree which, it is claimed, gives a quantitative yield of furfural from both arabinose and xylose (*6*, *7*).

The aniline acetate method for determining furfural is much less affected by methyl furfural and hydroxymethyl furfural than is the phloroglucide precipitation procedure (*12*) or the bromine titration method (*5*). For this reason in the analysis of pentose-methyl pentose mixtures by both the xylene and the Association of Official Agricultural Chemists methods (*1*, pp. 344–5) different results are to be expected. In this connection experiments have shown that the xylene procedure is a much better measure of the true pentose content of such a mixture than the phloroglucide procedure, even when the latter is supplemented by the tedious alcoholic extraction proposed by Ellett and Tollens (*2*).

It has been noted repeatedly that all pentose procedures should be applied to natural products with caution. The present method is no exception. Uronic acids are known to yield furfural under the conditions of the determination. Even pure glucose gives a substance which develops a slight pink color with the reagent; thus 100 mg. of glucose give a color comparable to that obtained from 0.1 mg. of xylose. Although all procedures yet proposed for the quantitative determination of pentoses have many obvious shortcomings, they are helpful tools for the investigation of natural products and are widely used for that purpose. The present method, which is less affected by methyl pentoses and hexoses and also allows a considerable saving of time and materials, is being used in the investigation of natural products containing pentose and uronic acid.

TABLE I. STABILITY OF FURFURAL IN XYLENE–13 PER CENT HYDROCHLORIC ACID

Time of refluxing, hours	0	1	2	3	5	10	15
Concn. of furfural in xylene layer, mg./cc.	0.0244	0.0250	0.0248	0.0250	0.0238	0.0242	0.0218
	0.0242	0.0247	...	0.0249	0.0244	0.0249	0.0242

TABLE II. FURFURAL FROM XYLOSE

(13% HCl, refluxing time 150 minutes)

Xylose	Furfural	
		% of theory
Mg.	*Mg.*	
0.404	0.253	97.5
0.807	0.515	99.5
1.0	0.644	100.5
1.0	0.648	101.0
0.972	0.618	99.2
10.0	6.60	103.0
100.0	60.6	95.8

Apparatus and Materials

The concentrations of the hydrochloric acid solutions used in the following experiments were determined at 20° to 24° C. by titration, together with specific gravity determinations.

Several lots of xylene (Merck reagent) were satisfactory without further purification; however, two lots gave a slight precipitate on long boiling with acid. This undesirable effect was removed by redistillation of the xylene. Because there are references in the literature (*10*) to harmful effects from continued exposure to xylene vapors, a rubber-ball aspirator is used in filling pipets with xylene solutions. It is strongly urged that any person using the xylene method as a routine procedure be provided with a similar device for protection from xylene vapors.

The sugars had the following specific optical rotations in water: *d*-xylose, +19.0°; *l*-arabinose, +103°; *d*-lyxose, −13.8°; *d*-ribose, −21.0°. (On four occasions low yields of furfural were obtained with xylose which had been stored for some time in a desiccator at room temperature. Theoretical yields were obtained after recrystallization of the xylose from alcohol.) Crystalline *d*-allomethylose, Eastman rhamnose-hydrate, and Bureau of Standards glucose were used.

Freshly distilled furfural was dissolved in xylene to make a stock solution containing about 1 mg. per cc. The stock solution was stored in the refrigerator, no change being noted during the course of 10 months. Standards containing 0.01 to 0.025 mg. per cc. were prepared by dilution of the stock solution with xylene.

The aniline acetate reagent was prepared by dissolving 1 cc. of colorless aniline in 50 cc. of glacial acetic acid and 50 cc. of 95 per cent alcohol. This reagent develops a slight yellow coloration after 2 or 3 days and should then be discarded. [Suminokura (*11*) reports that xylidine is preferable to aniline for the colorimetric determination of furfural in aqueous solution. McCance (*8*) recommends benzidine; however, in xylene solution the aniline reagent is superior to any of the other reagents the authors have tested.]

The refluxing is carried out in a 50-cc. round-bottomed flask attached to a reflux condenser by means of a small (T 14/35) ground-glass joint. Flasks of larger capacity should not be used because of excessive holdup of water droplets.

Colorimetric comparisons were made in a Bausch & Lomb, Duboscq-type colorimeter.

Distribution Coefficient for Furfural

The coefficient for the distribution of furfural between xylene and 13 per cent hydrochloric acid was determined at 24° C. The average of seven determinations gave the following result:

$$\frac{\text{Concn.}_{\text{acid}}}{\text{Concn.}_{\text{xylene}}} = 0.39$$

This value is used in all the calculations which follow. (A lower, and hence more favorable, distribution coefficient was obtained when *n*-butyl ether was substituted for xylene. However, the authors have been unable to find a color reaction for furfural which proceeds satisfactorily in this solvent.)

Procedure

The sample containing 0.4 to 1.0 mg. of pentose or its equivalent of furfural-yielding substance, dissolved in a known volume (3 to 10 cc.) of hydrochloric acid of the proper concentration, is introduced into the reaction flask together with 25 cc. of xylene and a small glass ebullition tube. (In studying the rate of formation of furfural the larger volumes of acid are more satisfactory because there is less change in concentration due to the holdup of water droplets in the condenser.) The flask is attached to the reflux condenser and the contents are kept at a moderate boil for the desired length of time. After the mixture has cooled to room temperature, the xylene layer is decanted and stirred with a little anhydrous sodium acetate to remove moisture and mineral acid. If, after filtration, the solution is colorless it is ready for the determination of furfural.

When large amounts of hexose (10 parts or more) accompany the pentose, the xylene solution at this stage often has a brown coloration which must be removed before the determination of furfural is made. To do this exactly 20 cc. of the solution are measured into a 125-cc. Claisen flask and distilled *in vacuo* into a well-cooled receiving flask with the aid of a water pump. The Claisen flask, bearing a small capillary, is evacuated and immersed in a water bath which is rapidly heated to boiling. When the distillation has gone to dryness the vacuum is broken and 3 cc. of xylene are introduced into the Claisen flask. Distillation is resumed and again carried to dryness to wash out remaining traces of furfural. The colorless distillate is transferred to a 25-cc. volumetric flask, using 2 cc. of xylene for rinsing, and made up to the mark with more solvent. The solution is now ready for the determination of furfural. The distillation procedure involves no measurable loss of furfural; however, the dilution from 20 to 25 cc. must be considered in subsequent calculations.

Determination of Furfural

To find the concentration of an unknown solution of furfural in xylene a standard is selected which does not differ from the unknown by more than 25 per cent. Five cubic centimeters of the unknown and the standard are set up in test tubes and to each in quick succession are added 5 cc. of the aniline acetate reagent. The red color develops rapidly for the first 15 minutes and increases slowly for the next 25 minutes. The comparison should be made about 20 minutes after addition of the reagent. Because the color is photosensitive, the tubes should be placed in the dark until the comparison is made and the readings should be taken as rapidly as possible, using a separate standard for each determination. When tubes containing the colored solution are placed in the relatively intense light of a photoelectric colorimeter, a rapid fading may be observed. Although the color developed by the aniline acetate reagent is satisfactory for use with the Duboscq colorimeter, it is possible that some other reagent would give a more stable color better suited for use with the photoelectric type of instrument.

The colorimetric determination gives the concentration of furfural in milligrams per cubic centimeter of the xylene solution, Concn._x. From this value it is possible to calculate the total furfural present.

1. When the distillation procedure is not used:

$$\begin{aligned}\text{Furfural in xylene} &= 25\ \text{Concn.}_x\\ \text{Furfural in water} &= \text{cc. of acid} \times 0.39 \times \text{Concn.}_x\\ \text{Total furfural} &= \text{furfural in xylene} + \text{furfural in acid}\end{aligned}$$

2. When the distillation procedure is used, it is necessary to multiply the observed Concn._x by 25/20 to allow for the dilution of the xylene solution.

Results

Table I shows that no appreciable decomposition of furfural occurs in 10 hours' refluxing with xylene–13 per cent hydrochloric acid. In the experiments shown 75 cc. of xylene containing 0.025 mg. of furfural per cc. were refluxed with 6 cc. of 13 per cent hydrochloric acid.

With 13 per cent hydrochloric acid and a refluxing time of 150 minutes xylose consistently yields the theoretical amount of furfural (Table II). (When more than 1.0 mg. of xylose was determined it was necessary to dilute the xylene solution at the end of the period of refluxing before making the furfural determination.)

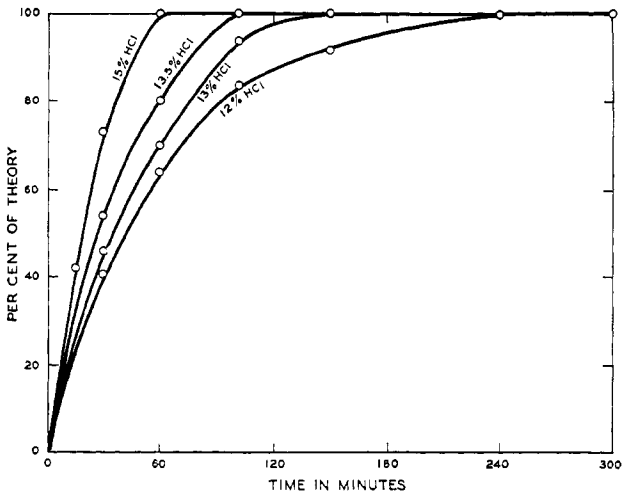

FIGURE 1. EFFECT OF CONCENTRATION OF HYDROCHLORIC ACID UPON RATE OF FORMATION OF FURFURAL FROM XLOSYE

TABLE III. FURFURAL FROM PENTOSES

(Refluxing time 150 minutes)

Concd. HCl, %	Furfural, Per Cent of Theory			
	d-Xylose	*l*-Arabinose	*d*-Lyxose	*d*-Ribose
12	92.2	65.5	80.3	..
13	100	81.7	91.0	81.0
14	..	83.2	..	..
15	100	82.4	..	..

TABLE IV. FURFURAL FROM XYLOSE–METHYL PENTOSE MIXTURES

(Xylene procedure, refluxing time 150 minutes, 13% HCl)

Sugars Used		Furfural Found	
Mg.		*Mg.*	% of theory
1.01 0.5	Xylose Rhamnose	0.639	98.6
1.01 0.5	Xylose *d*-Allomethylose	0.662	102.0

The rate of formation of furfural from xylose with various concentrations of hydrochloric acid is illustrated in Figure 1. It is apparent that 100 per cent conversion of xylose to furfural may be obtained under a variety of conditions. It does not follow that other pentoses or pentosans will give identical results under all the conditions which result in complete conversion of xylose to furfural.

In Table III are given the yields of furfural obtained from various pentoses after refluxing for 150 minutes with different concentrations of hydrochloric acid.

Experiments on synthetic mixtures of xylose–methyl pentose (Table IV) show that the xylene procedure can be used for determining 2 parts of xylose in the presence of 1 part of rhamnose or *d*-allomethylose. When similar mixtures were analyzed by the A. O. A. C. procedure, the amount of precipitate obtained was, of course, considerably more than would be anticipated from the xylose alone. In the authors' hands the extraction procedure of Ellett and Tollens (*2*) removed some of the methylfurfural phloroglucide, but the results as calculated from Kröber's tables (*4*, pp. 77–81) remained 15 per cent too high.

TABLE V. FURFURAL FROM XYLOSE-GLUCOSE MIXTURES

(Refluxing time 150 minutes, 13% HCl)

Xylose	Glucose	Furfural Found	
Mg.	*Mg.*	*Mg.*	% of theory
1.00	1.0	0.620	96.7
1.00	2.0	0.625	97.5
1.00	5.0	0.651	101.5
1.00	25.0[a]	0.636	98.3
0.97	50.0[a]	0.620	99.6

[a] Xylene solution of furfural distilled *in vacuo* to remove colored material.

Using the xylene procedure it was found that xylose can be determined with considerable accuracy even in the presence of large amounts of glucose (Table V).

Acknowledgment

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Flat-Bottomed Microcups as Weighing Bottles and Reaction Vessels for Kjeldahl Analyses of Viscous Liquids

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IN THE course of an extensive research study it became necessary to make a large number of quantitative nitrogen determinations of very viscous and sticky liquids. Attempts to weigh the samples in the ordinary manner and to transfer them afterwards to Kjeldahl digestion flasks were unsuccessful, as the samples could not be transferred completely to the flasks without using an excessively large amount of water. After various alternative procedures had been considered, it was found best to employ very small glass cups to hold the samples.

The microcups were made from ordinary 10-ml. specimen vials, of clear glass, with straight sides and flat bottoms. The lower ends of the vials were cut off by means of a hot-wire glass cutter so as to give cups of a capacity of approximately 1 ml. After careful cleaning and drying, the cups were weighed, filled with the sample, weighed again, and then placed in the Kjeldahl flasks for subsequent routine treatments.

These microcups gave very satisfactory results. They were used over again many times, as there was no breakage whatsoever during the digestion and distillation.