683

Water Sorption Studies on Rutin and the Determination of Moisture*

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Vapor phase water sorption studies were made on rutin of pharmaceutical grade and on highly purified rutin. The absorption and desorption curves for moisture at various relative humidities demonstrate the extremely hygroscopic nature of rutin. Several procedures for the determination of moisture in such a hygroscopic material were investigated. High temperature, vacuum, and an extremely dry atmosphere in the oven were found to be essential to insure complete drying. Special precautions were necessary to prevent rehydration of the samples after drying. A recommended procedure is described for determining moisture in rutin.

THE DRYING of rutin, either for obtaining anhydrous samples for analytical studies or for obtaining a value for moisture, has always been a difficult problem because of the extremely hygroscopic nature of the compound. Perkin (4, 5) found that rutin dried over concentrated sulfuric acid three weeks lost 5.76 per cent water ($2H_2O$ = 5.44%); after additional drying at 130°, it lost 1.49 per cent ($^1/_2$ H_2O = 1.43%); and finally after drying at 160° it lost an additional 1.67 per cent ($^1/_2$ H_2O = 1.47%), making a total of 3 molecules of water. He also observed that upon exposure to air the dried rutin quickly absorbed water. The extreme hygroscopic nature of rutin was demonstrated again in this laboratory during carbon and hydrogen analyses. Small dry samples of rutin picked up more than 2 per cent of their weight of water in the few seconds in which they were exposed to room air of 50 per cent relative humidity while being transferred from a micro weighing pig to the combustion tube. To obtain anhydrous samples for analysis, Porter, *et al.* (6), found it necessary to dry rutin under vacuum at 130° in the presence of magnesium perchlorate and in a specially designed drying vessel.

EXPERIMENTAL

Water Sorption Studies.—To determine the normal degree of hydration of rutin, vapor phase absorption studies were made using the technique reported for water absorption studies on proteins (2).

Rutin of N. F. IX (3) quality was further purified by crystallizing five times, alternately from absolute ethanol and water. The moles of water absorbed per mole of flavonol at various degrees of relative humidity are presented in Fig. 1.

The highly purified rutin absorbed up to 3.1 moles of water when exposed to high humidities while the N. F. rutin absorbed up to 4 moles of water. The absorption curves for the two types of rutin are different. The curve for highly purified rutin shows a large uptake of water at a low humidity while that for the N. F. grade rutin shows a gradual uptake with increasing humidity. The desorption curves for the two rutins are similar, both showing little loss of moisture until extremely low humidities are reached. These results show clearly why it is difficult to obtain an anhydrous sample.

To determine the effect of possible impurities on the absorption of water by rutin, the water absorption of quercetin[1] was investigated. The results are also presented in Fig. 1. Quercetin absorbed two moles of water, which is in agreement with

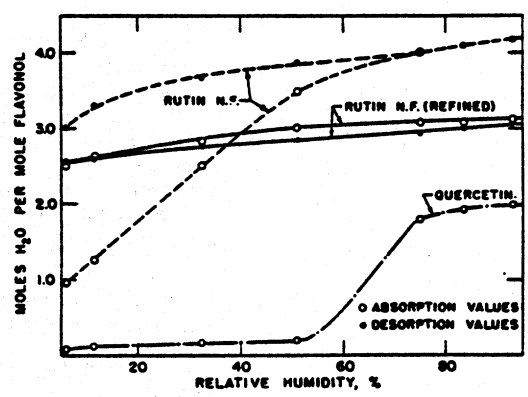

Fig. 1.—Water sorption isotherms for rutin and quercetin showing degree of hydration in relation to relative humidity.

reports in the literature (1, 8). The shape of the curve suggests that the presence of quercetin may be the cause for the lag in water absorption by the N. F. rutin. However, since less than 5% of quercetin is present in the N. F. rutin (3) it cannot account for the results observed. If the data for water absorption by the rutin and quercetin are plotted on a weight basis (Fig. 2) it will be observed that the N. F. rutin absorbs more water

* Received August 22, 1952, from the Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Philadelphia, Pa.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† The authors are indebted to Mary Jane Welsh and Frances C. Strolle for the moisture analyses.

[1] Quercetin was recrystallized three times from 70% ethanol.

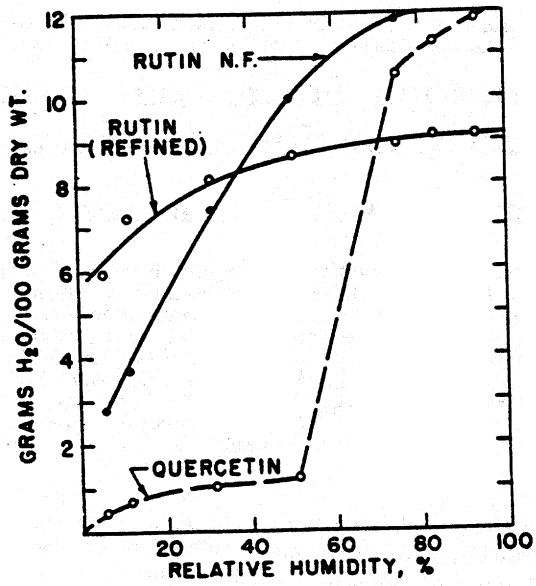

Fig. 2.—Water absorption of rutin and quercetin.

than even the quercetin. Either it contains small amounts of extremely hygroscopic impurities, or the presence of the quercetin disrupts the crystal structure sufficiently to permit the accumulation of free water. The latter is the more likely explanation.

Determination of Moisture in Rutin.—The results of sorption studies presented above were used to develop a simpler and more efficient procedure for the determination of moisture in rutin. To determine the best conditions for accurately measuring the moisture content of such a hygroscopic material, a series of experiments was performed, using a rutin sample which was shown by spectrophotometric analysis (6) to be of high purity. The rutin was equilibrated in a room maintained at 75 ± 1° F. and 50 ± 2% relative humidity, and all experiments were carried out in rooms having these same conditions. The drying conditions used and the results obtained are summarized in Table I.

The vacuum oven used in these experiments was the small chamber type with outlet and inlet valves on opposite sides of the oven and a vacuum gauge mounted in the self-sealing door. The inlet was connected to a drying train consisting of a gas wash bottle with fritted glass gas disperser, half filled with concentrated sulfuric acid, followed by a 2-ft. length of 60 mm. O. D. glass tubing containing a 14-in. section of anhydrous calcium sulfate followed by a 6-in. section of phosphorus pentoxide on pumice stone chips. With the drying train and separate outlet and inlet valves, the vacuum chamber can be swept continuously with dry air and the vacuum can be released with dry air at the end of the drying period. To sweep the oven during drying, an air inlet valve was opened slightly so that about 25 ml. of air per minute was drawn through the drying train. The principles involved in this procedure for drying were discussed by Willits (9).

The special weighing bottles (Fig. 3) used in experiments 3, 8, 9, and 11 were made by drilling a 2-mm. hole through the 24/12 ground glass joints of the Parr type weighing bottles. Drilling was done with the cap in place so that the holes in the cap and bottom coincided. When drying a sample in the special bottle the hole was left open to allow the moisture to escape from the sample chamber by diffusion through the opening. Upon removing the bottles from the oven the caps were immediately turned to close the hole. Thus the amount of moist laboratory air coming in contact with the sample after drying was limited to that which would diffuse through the hole during the short time required to remove the bottle from the oven and turn the cap.

The desiccant used in experiments 4 to 9 was anhydrous magnesium perchlorate placed in a shallow tray in the bottom of the vacuum oven. We do not recommend magnesium perchlorate for general use as an oven desiccant where there is a possibility of its picking up organic vapors. In these experiments, however, where it was known that the only volatile material present was water, magnesium perchlorate was considered to be a safe substitute for the anhydrous barium oxide used in the National Formulary procedure (3).

The data (Table I) show that drying in the air oven at 110° (experiment 1) left approximately 2% moisture in the rutin. The usual vacuum oven drying technique (experiment 2) at 110° gave a

TABLE I.—EVALUATION OF DRYING CONDITIONS FOR THE DETERMINATION OF MOISTURE IN RUTIN

	110° C.—16 Hrs.			125° C.—16 Hrs.							
Experiment No.	1	2	3	4[a]	5	6	7	8	9[b]	10	11[c]
Air oven	x	...	...	...	...	...	...	...	...	...	...
Vacuum oven	...	x	x	x	x	x	x	x	x	x	x
Desiccant in oven	...	...	...	x	x	x	x	x	x	...	...
Oven swept with dry air	...	...	x	...	...	...	...	...	...	x	x
Vacuum released with dry air	...	...	x	...	...	x	x	x	x	x	x
Vacuum released with room air	...	x	...	x	x	...	...	...	...	...	...
Parr type weighing bottle	x	x	...	x	x	x	x	...	...	x	...
Special weighing bottle	...	...	x	...	...	...	...	x	x	...	x
Sample weight (Gm.)	0.1	0.1	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.1	0.1
Av. % loss on drying	6.44	7.59	8.17	8.21	8.08	8.29	8.32	8.37	8.39	8.31	8.42
Standard deviation	0.11	0.06	0.15	0.01	0.21	0.02	0.04	0.02	0.04	0.03	0.01

[a] National Formulary procedure.
[b] Drying conditions same as recommended in reference 6.
[c] Recommended procedure (0.5 Gm. samples should be taken when a macro balance is used).

moisture figure about 0.8% low. Only when some more effective measure was taken to remove the water vapor from the vacuum oven chamber was the true moisture value approached.

In experiment 3 the moisture was removed from the vacuum chamber by a dry air sweep and the dry sample was protected by releasing the vacuum with dry air and by using the special weighing bottles. The moisture value of 8.17 is much better than that of experiment 2, but comparison with the value obtained by the same technique at 125° (experiment 11) shows that even under the best conditions a temperature of 110° is not quite sufficient. Experiment 4 is the same as the National Formulary procedure except that magnesium perchlorate was substituted for barium oxide. This substitution should have negligible effect on the results since the two desiccants are comparable, the barium oxide being recommended because of the possible hazard associated with the perchlorate. This procedure gave a value of 8.21% which was higher than the 8.08% obtained in experiment 5, where only 0.1-Gm. samples were used. This difference is due probably to the samples picking up moisture between the end of the drying phase and the final weighing. The amount of moisture absorbed would depend more on the degree of exposure of the sample to room air than on the sample weight. To reduce this possible source of error, the air used to release the vacuum in experiments 6 and 7 was passed through a drying train. The results of 0.1- and 0.5-Gm. samples were now in good agreement and were higher than those for the two preceding experiments. The procedure of using the dry air release was adopted. To protect the samples from moist air when the oven was opened, the further refinement of using the special weighing bottles was tested in experiments 8 and 9. Comparing the results obtained with those of experiments 6 and 7 shows that this expedient increased the moisture value obtained by approximately 0.1%.

It has been shown that it is necessary to release the vacuum with dry air; consequently, a drying train will need to be connected to the oven. Experiments 10 and 11 were conducted to compare the effectiveness of using dry air from this train to sweep the water vapor from the oven with that of placing a desiccant in the vacuum chamber. The values for experiments 10 and 7, which differ only in the method used for removing water vapor from the vacuum chamber, were 8.31 and 8.32, respectively. Similarly, the values for experiments 11 and 9 were 8.42 and 8.39, showing that the desiccant and dry air sweep methods were equally effective. The method of sweeping with dry air is preferred because it avoids the danger and trouble of placing a desiccant in the oven.

Increases in loss on drying resulting from using the dry air vacuum release and the special weighing bottle were both of the order of 0.1%. To determine if these differences were statistically significant, Student's t values (7) calculated from the data were compared with the critical t values. The significance of the effect of the special weighing bottle on the results was determined by pooling the data from experiments 8, 9, and 11 and comparing the mean with that of the pooled data from experiments 6, 7, and 10. This pooling of the data was done to obtain larger and more reliable samples for the statistical comparison and was justified because the sample size and the method for desiccating the oven chamber had no significant effect on the values under the conditions of these six experiments. The calculated t value, 2.54, was greater than the critical $t_{0.05}$, 2.10, showing that the difference was significant at the 95% level and the increase in the moisture values for rutin resulting from the use of the special weighing bottles was real. When the t test was used to compare the results of experiment 4 with the pooled data from experiments 6 and 7, releasing the vacuum with dry air was found to give significantly higher results even at the 99% level than those obtained when room air was used to release the vacuum [t (calcd.) = 5.44; $t_{0.01}$ = 3.06].

The standard deviation values shown in Table I were generally lower for 0.5-Gm. samples and this would have been much more pronounced had not all weighings been made to the nearest 0.01 mg., using a semimicro balance. The relatively standard deviations for experiments 1, 2, 3, and 5 are also a good indication that drying was not complete.

It has been assumed throughout this discussion that there was sufficient rutin to permit using at least 0.1-Gm. samples. If less material is available or if a small dry sample is desired for analysis, the special charging tube, Fig. 3, described by Porter, *et al.* (6), should be used. The dry air sweep (experiments 10 and 11) was equally as satisfactory as the desiccant method (experiments 6 to 9) used by Porter for drying rutin in the charging tubes.

To determine if the prolonged drying at 125° had any deleterious effect on the rutin, two samples dried according to the conditions of experiment 11 were analyzed by the spectrophotometric method of Porter, *et al.* (6), and found to be 99.6% and 99.9% pure. Two other samples of the same rutin were analyzed for purity without being dried and

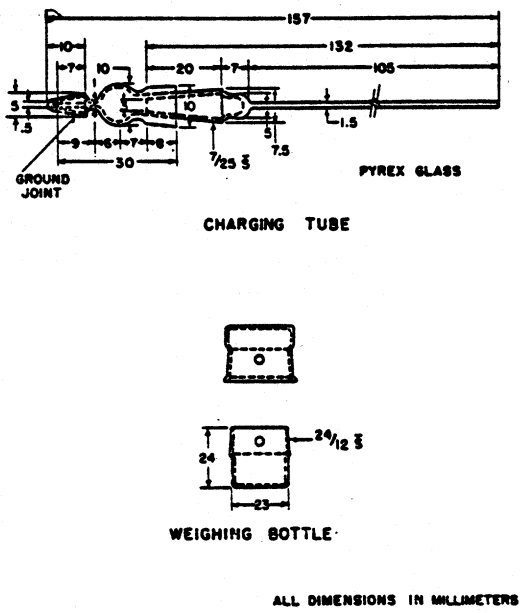

Fig. 3.—Special weighing bottle and charging tube used for determining moisture in hygroscopic materials.

the results were calculated to the moisture-free basis using the moisture value from Experiment 11. The values obtained, 99.8% and 100.1%, were within the normal experimental error of the results obtained on the dried samples and indicate that rutin is not measurably degraded by heating at 125° for sixteen hours. Rutin samples dried at 125° for sixteen, thirty-two, and forty-eight hours all showed the same per cent loss in weight, which is further evidence of the heat stability of rutin.

The following procedure is recommended for the determination of moisture in rutin. Dry the special weighing bottle (Fig. 3) in a vacuum oven for one-half hour and cool to room temperature in a desiccator containing an efficient desiccant (anhydrous calcium sulfate or better). Place approximately 0.5 Gm. of rutin in the tared bottle and weigh to the nearest 0.1 mg. Turn the bottle cap so that the hole is open and place in a vacuum oven at 125°. With a continuously operating vacuum pump, draw moisture-free air from the drying train described above through the oven at approximately 25 ml./min., controlling the air flow with the inlet valve. Continue sweeping with dry air for sixteen hours (overnight), close the outlet valve, and bring the oven to atmospheric pressure by bleeding dry air into the chamber (about ten min.). Remove the sample from the oven, immediately turn the cap of the weighing bottle to close the hole and place in the desiccator until it cools to room temperature. Weigh to the nearest 0.1 mg. and calculate the percentage loss in weight of the sample.

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