

WATER CONTENT OF PAPER AS A VARIABLE IN PAPER CHROMATOGRAPHY

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SUMMARY

The moisture content of a batch of papers may be measured indirectly either by weighing a reference paper or by reading a suitable recording humidigraph. Alternatively, papers can be equilibrated to an appropriate salt solution. The latter procedure eliminates the need for moisture content measurements and appears to be capable of eliminating water content of paper as a practical factor in R_F values; however, the time required for effective equilibration may be considered excessive for some types of studies.

A few years ago, this journal¹ carried a report from our laboratory on a technique for controlling the water content of chromatographic papers.

What we wanted most to communicate was our procedure for *obtaining* a standardized water content of paper. We equilibrated the papers to the constant vapor pressures from certain saturated aqueous salt solutions in contact with an excess of the solid salt. For instance, we usually spread thin layers of dry ammonium sulfate over the bottom of two or three large petri dishes, moisten the salt with a small amount of saturated solution of ammonium sulfate, and place the dishes in the bottom of the chromatography box during the equilibration period.

The published report of ours also carried a unique suggestion, upon which we will here expand. After we had purchased a recording humidigraph, we noted how slowly the instrument responded to changes in humidity, and we began to wonder how such a slow instrument could be of possible use to us. At this point we became impressed by the fact that the sensing element of the instrument consisted of wood fibers—or in other words cellulose, which was where the whole problem started. In Fig. 1 of the previous report, and in Fig. 1 of this report, are given some of the results we obtained by plotting both relative humidity readings and paper weight against time, the vertical scales being chosen to cause coincidence at both ends of the curves. The curves cannot be said to match exactly, but they are a reasonable fit, and enough so for our purposes. The curves of the figure of the previous reference were obtained in the absence of fanning, and the approach to equilibrium is the slowest we have observed. Curves can be made somewhat steeper by the use of more petri dishes of humidity control agent, and steeper yet by the use of a fan for 20% of the time, as shown in Fig. 1. Still steeper curves are obtained when the fan is

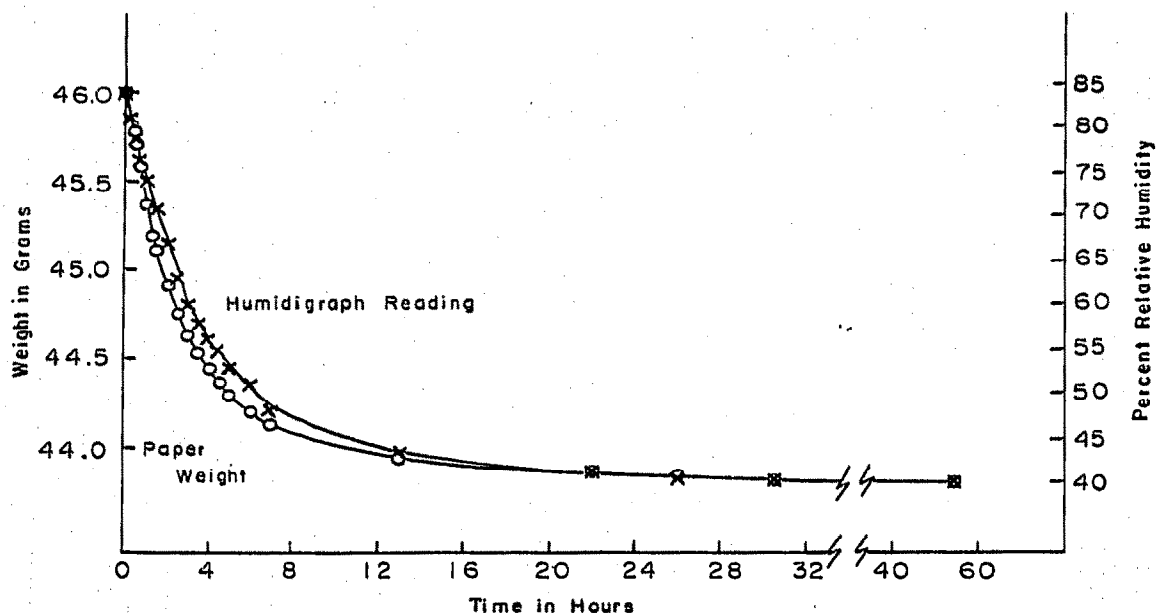


Fig. 1. Rate of equilibration to a humidity change. Fan used 20% of the time.

used continuously. When the fan is used, the exact number of petri dishes used becomes of negligible importance. Also, the curves have been run in the upward and the downward directions. The important point is that, with all these variations, the humidigraph remains an indicator of paper weight, within accuracy limits no worse than the two examples given. Thus, the measurement of the water content of paper does not require the weighing of individual papers. It can be done by taking a reading from an appropriate recording humidigraph, or (in absence of a humidigraph) by the method of HANES and co-workers², using the weight of a pre-calibrated reference paper as standard for whole batches of experimental papers. Of the two solvent systems studied, the phenol-water system¹ (Fig. 2 of ref. 1) showed more variation with humidity than did the *n*-butanol-propionic acid-water system¹. Of

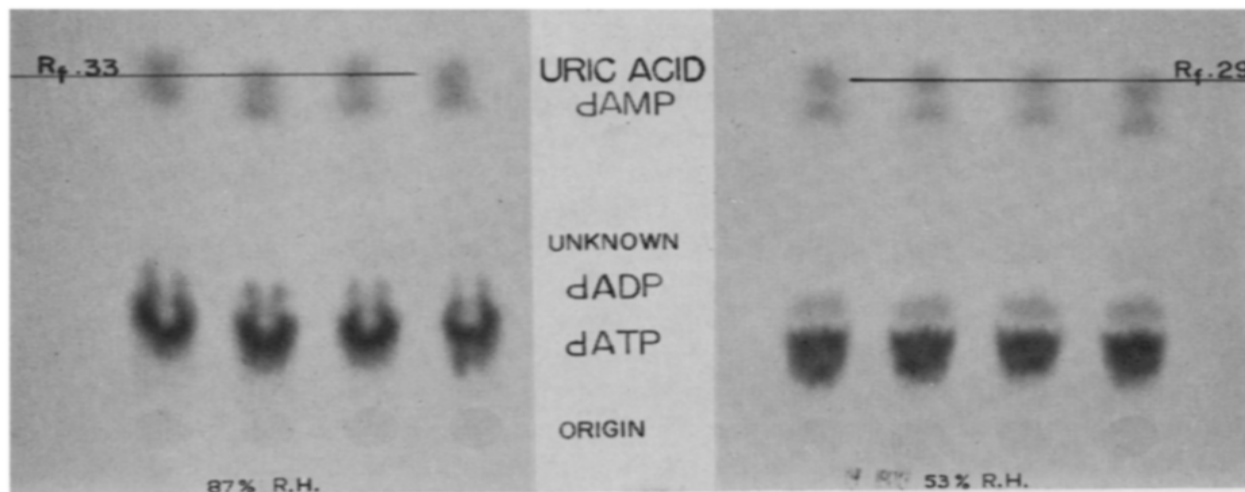


Fig. 2. Comparison of papers pre-equilibrated to different relative humidities. Schleicher and Schuell No. 589 orange ribbon paper; *n*-butanol-propionic acid-water solvent.

many compounds tested, the most variable was adenosine monophosphate; and for this extreme case the arbitrary limitation of chromatography experiments to the range of 50 to 75% relative humidity should limit the R_F variation to a maximum of 0.08 units on the R_F scale. Our experience has been that this degree of humidity control is well worth the effort.

Of course, as explained above, the papers could be equilibrated with a salt solution; and in that case neither weighings nor a humidigraph would be required. In theory, the variations in R_F of the AMP spot due to water content variation should then be within 0.01 unit on the R_F scale. We say in theory, because even in our limited experience with the equilibration procedure, it is clear enough that the total variation is several times 0.01 unit. Obviously, there are still more variables to be controlled. We do have some evidence that variation in Whatman No. 1 paper from one batch to another is an important remaining variable. In terms of work required, this procedure is about as simple as could be hoped for; but the equilibration time does delay completion of the experiment by at least 6 h, and in many cases the time is more important than the improved reproducibility. Perhaps more to the point, the time expended in obtaining this extra degree of water content control would be useful only in connection with careful attention to the other variables involved.

Less directly related to matters of reproducibility, we might add a remark or two on the use of water content control to improve separation of specific compound pairs. First, we would emphasize that no one relative humidity is best for all purposes. In the example of Fig. 2, our purpose was to separate uric acid from deoxyadenylic acid, and medium humidity was better than high. On the other hand, we also wanted to separate the di- and tri-phosphates of deoxyadenylic acid, and (for either solvent) the general opening up of the chromatographic pattern for slow moving spots on very humid papers is usually helpful. What this example shows is that the nature of the sample is a variable which itself varies according to the water content of the paper. These samples were taken from an enzymatic reaction mixture³, and the sucrose and buffer salts in the mixture are responsible for the smearing on the high humidity paper. A simple mixture of the di- and tri-phosphates of deoxyadenylic acid without the extra ingredients is not smeared in this manner.

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