CONTRIBUTED AND SELECTED

SENSITIVITY OF SOME COMMERCIAL LITMUS PAPERS.*

BY ARNO VIEHOEVER AND CARL O. EWING.

During the course of some experiments with drug plant extracts it was found that the different litmus papers on hand did not respond satisfactorily when solutions known to contain an excess of acid or alkali were tested. Since litmus is so often used in testing the reaction of solutions, and, furthermore, since it is prescribed frequently in pharmacopœial tests, it seemed advisable to examine the litmus papers on the market in order to determine their sensitivity. Samples of blue, neutral, and red litmus papers were therefore requested from seventeen American commercial firms which were considered most likely to make their own litmus papers. Nine of these firms submitted original samples. One sample obtained from a foreign firm was also examined. Another paper of foreign make, dyed only on one side and considered to be very sensitive, could not be obtained for comparison.

Some samples received were loose in envelopes, some were in unprotected booklets, some were in colorless bottles, and some in amber-colored bottles. The paper strips were removed from the original containers with forceps (to avoid contact with the hand) and tested by momentarily immersing one end in acid (H_2SO_4) and alkaline (NaOH) solutions of the following concentrations: $\frac{N}{10}$, $\frac{N}{50}$, $\frac{N}{100}$, $\frac{N}{250}$, $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{4000}$, and $\frac{N}{6000}$. In doubtful cases one end was dipped into distilled water.

The reaction of the distilled water used in all of the experiments was very close to absolute neutrality, as near as could be determined by the use of the indicators azolitmin, methyl orange, methyl red, and phenolphthalein. (A chart showing the relative position of these indicators on the hydrogen ion scale is given by Walpole.[1])

The results, which are very surprising, are given in the table, the papers of each type being arranged in the decreasing order of their sensitivity.

As can be seen by the table, there was considerable difference in the colors of the papers submitted. The differences may be partially accounted for by the fact that litmus is prepared from several different lichens, chiefly species of *Roccella, Lecanora,* and *Variolaria.* The conditions under which the fermentation of the lichens is carried on in the manufacture of the litmus also must have considerable influence. Different amounts of litmus dye used by different manufacturers will naturally cause differences in the shade and color intensity of the papers. Of other dyes added to litmus as an adulterant, only indigo has been mentioned in the literature.[2] According to Kunz-Krause,[3] indigo is a never-failing constituent of litmus. We believe that if it is present, it can be so only in very small amounts, and that its addition as an adulterant seems improbable at the present time in the light of a few experiments made with indigo solutions alone, in concentrations varying from 1 : 100 to 1 : 50000, and mixed with litmus solutions. The indigo

* Contribution from the Pharmacognosy Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C. Read before the Biological Section of the American Chemical Society, Urbana, Ill., April 22, 1916.

[1] Walpole: "Notes on Regulator Mixtures, Recent Indicators, etc." Biochem. J., 8, 628–640, 1914.

[2] Zörnig: Die Arzneidrogen II, 370–371, 1911.

[3] Hager's Handbuch der pharmaceutischen Praxis, Ergänzungsband, 442, 1908.

RELATIVE SENSITIVITY OF SOME COMMERCIAL LITMUS PAPERS.

	Lab. No.	Original color.*	Acid.									Alkali.								Remarks.
			N/10	N/50	N/100	N/250	N/500	N/1000	N/2000	N/4000	N/6000	Distilled water †	N/2000	N/1000	N/500	N/250	N/100	N/50	N/10	
Blue	F	Light plumbago gray	5‡	4	4	3	3	2	2	1−	0+	0	...	...	...	...	...	..	..	Excellent. Sharp color change.
	K	Light vinaceous gray	5	4	4	3	3	2	2−	1−	0+	0	...	...	...	...	...	..	..	Excellent. Sharp color change.
	D	Grayish blue violet	4	3	3	3	2	2	1−	0	...	0	...	...	...	...	...	..	..	Very good. Quick color change.
	I	Deep plumbago gray	4	3	3	3	2	1	1−	0	...	0	...	...	...	...	...	..	..	Very good.
	H	Deep plumbago blue	4	3	3	2	2	1	1−	0	...	0	...	...	...	...	...	..	..	Fair.
	E	Forget-me-not blue	3	3	3	2	1	1−	0	...	...	0	...	...	...	...	...	..	..	Fair.
	C	....	3	3	3	2	1	1−	0	...	...	0	...	...	...	...	...	..	..	Fair.
	B	Vinaceous gray	3	2	2	1	1	1−	0	...	...	0	...	...	...	...	...	..	..	Poor.
	G	Aniline lilac	3	2	2	1	1−	0	...	...	...	0	...	...	...	...	...	..	..	Very poor.
	A	Deep dull lavender	3	2	2	1	1−	0	...	...	...	0	...	...	...	...	...	..	..	Very poor.
Neutral	D	Heliotrope gray	4	3	3	2	2	1−	0+	0	...	0	...	0	1−	1	2	3	4	Very good, especially for acids.
	H	Light pinkish lilac	3	3	2	2	0+	0	...	...	...	0	0	0+	1−	2	3	3	3	Very good,
	E	Deep dull lavender	3	2	2	1−	0+	0	...	...	...	0	0	0+	1−	1	2	2	3	Fairly good.
	G	Pale lobelia violet	1	1	1−	1−	0+	0	...	...	...	0	0	1−	1−	2	2	2	3	Fair for alkali. Color change not intense for acids.
	C	Pale purplish vinaceous	1	1	1−	1−	0+	0	...	...	...	0	0	0+	1−	1−	1−	2	2	Poor, color change not distinct. Better for alkali.
	B	Light purplish vinaceous	1	1	1−	0	...	...	...	...	...	0	...	0	0+	0+	1−	2	2	Poor, color change too weak.
	A	Light brownish vinaceous	2	1	0	...	...	...	...	...	...	0	...	...	...	0	1	1	2	Very poor.
Red	F	Hydrangea pink	..	..	...	...	...	...	...	...	...	0	0	0+	1	2	3	4	5	Excellent. Sharp color change.
	K	Venetian pink	..	..	...	...	...	...	...	...	...	0	0	0+	1	2	3	4	5	Excellent. Sharp color change.
	H	Corinthian pink	..	..	...	...	...	...	...	...	...	0	...	0	0+	1	2	3	4	Good.
	D	Pinkish vinaceous	..	..	...	...	...	...	...	...	...	0	...	0	0+	1	2	3	4	Fair.
	C	Hydrangea pink	..	..	...	...	...	...	...	...	...	0	...	...	0	1	2	2	3	Rather poor.
	I	Brownish vinaceous	..	..	...	...	...	...	...	...	...	0	...	...	0	1	1	2	3	Rather poor.
	B	Pale vinaceous	..	..	...	...	...	...	...	...	...	0	...	...	0	1−	1	2	3	Poor. Color change not intense.
	E	Chatenay pink	..	..	...	...	...	...	...	...	...	0	...	...	0	0+	1	2	3	Poor. Original color good, but change not intense.
	A	Hydrangea pink	..	..	...	...	...	...	...	...	...	0	...	...	0	1−	1	2	3	Poor.
	G	Pale vinaceous pink	..	..	...	...	...	...	...	...	...	0	...	...	...	0	0+	1	1	Very poor.

* Color Standards and Nomenclature (1912). Ridgway.
† Reaction close to absolute neutrality.
‡ The figures do not represent exact ratios, but roughly indicate the relative intensity and speed of the color changes.

solutions alone did not change appreciably in color upon the addition of 5 N acid (H_2SO_4) or alkali (NaOH). When indigo and litmus solutions were mixed the color change of the latter was obscured.

We have been informed by a member of the trade that the medical profession demands deeply colored papers, apparently in the belief that they are more sensitive. It was found, however, that the lighter colored papers gave more speedy and more readily observable color changes than the more deeply colored ones. Walpole[4] suggests that the lesser sensitivity of thick, deeply-colored papers is due to "reaction inertia," the larger amount of dye present requiring a larger concentration of acid or alkali to produce a color change.

It will be noted that there was considerable variation even in testing the comparatively strong solutions $\frac{N}{10}$ to $\frac{N}{250}$. The most striking differences were observed in the more dilute solutions. On the whole, the blue papers were the best submitted, the limits of sensitivity ranging from $\frac{N}{500}$ (1 : 10000) to between $\frac{N}{4000}$ and $\frac{N}{6000}$ (approximately 1 : 100000) H_2SO_4. The limits for the red papers ranged from $\frac{N}{100}$ (1 : 2500) to $\frac{N}{1000}$ (1 : 25000) NaOH; and the limits for the neutral papers, which were the poorest submitted, ranged from $\frac{N}{50}$ (1 : 1000) to $\frac{N}{1000}$ (1 : 20000) on the acid side and from $\frac{N}{100}$ (1 : 2500) to $\frac{N}{1000}$ (1 : 25000) on the alkaline side.

It may be of interest to note that in a few experiments the sensitivity of taste fell far below that of the litmus papers, the limits observed for the taste being about $\frac{N}{250}$ for acid and $\frac{N}{50}$ for alkali when a drop was placed on the tongue.

In this connection attention is called to a simple and quite useful means of testing very dilute solutions by placing a drop on the middle of the broader papers. By this means the reaction of solutions more dilute than $\frac{N}{25000}$ (1 : 500000) H_2SO_4 and $\frac{N}{2000}$ (1 : 50000) NaOH could be determined. When the color change was not clearly distinct with one drop, a reaction could still be obtained by placing several drops, one by one, upon the same spot, allowing each one to evaporate before adding the next drop, thus in effect concentrating the solution to be tested. The color change appeared as a spot if the solution was rapidly absorbed or as a small ring if the absorption was slow, the color change in the latter case occurring chiefly at the circumference of the drop. When using this "spot test" it is well to make blank tests with distilled water.

The findings regarding the relative sensitivity of the blue and red papers conform in general with the statements of Dietrich,[5] whose figures for the limits of sensitivity of blue and red litmus papers show that the former are more sensitive.

It is interesting to note that one of the manufacturers stated on the label that the blue and red papers should respond quickly to $\frac{N}{250}$ acid or alkali, and that the neutral paper should respond to $\frac{N}{1000}$ acid or alkali. "Technical" dark blue and dark red papers from the same firm are claimed to respond to only $\frac{N}{100}$ acid or alkali.

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[4] Walpole: "The Use of Litmus Paper as a Quantitative Indicator of Reaction." Biochem. J., 7, 260–267, 1913.

[5] Neues Pharm. Manual, 1913, 73.

Most of the samples were prepared from slightly roughened filter paper, the texture of which varied from a hard, slowly absorbing paper to one that was soft, rough and rapidly absorbing. A paper about midway between these extremes gave the best results, especially in testing very dilute solutions, since the hard papers reacted too slowly and the soft papers absorbed the liquid too rapidly, thus distributing it over too large an area and decreasing the sensitivity of the "spot test."

The thickness of the papers varied from 0.09 to 0.17 mm., usually being about 0.10 to 0.12 mm. In general the thicker and smoother papers did not absorb the liquid satisfactorily. The very thin papers were not sufficiently opaque.

The width of the papers varied from about 5 to 17 mm., usually being about 6 or 7 mm. The broader papers were the most satisfactory, especially in testing the more dilute solutions, since the progressive zone of the color change could be more readily observed or the "spot test" could be better applied.

No experiments were made regarding the effect of the atmosphere, light, and the presence of salts in the paper upon the sensitivity. It is the common experience that laboratory fumes decidedly affect the papers. Light, according to Dietrich,[6] has a harmful influence upon the sensitivity of litmus papers. The presence of foreign salts obviously would have a deleterious effect, due to their effect upon the concentration of the reacting ions, especially if the tests are carried out in such a manner that the solution to be tested is allowed to stand and concentrate on the litmus paper. In fact, it has been observed that in some cases, for example, No. "A," a preliminary washing with distilled water increased the sensitivity of the paper.

Even on the basis of our limited experiments it would seem that litmus papers will prove to be satisfactory if they comply with the following specifications[7]:

Paper: Close formation, firm, medium hard, similar to Schleicher & Schull No. 589 (blue ribbon) filter paper.

Color: Blue—dull or grayish blue. Neutral—dull lavender or pinkish violet. Red—light pinkish red.

Width: About 12 to 15 mm.

Thickness: About 0.10 to 0.12 mm.

Sensitivity: Quick response to $\frac{N}{500}$ acid or alkaline solutions.

Preservation: Litmus papers should be kept in well-stoppered bottles, preferably amber-colored.

THE COLORING MATTER OF EGG-YOLK.

The principal pigment of the yolk of egg belongs to the xanthophyll group of plant pigments. The hen utilizes comparatively little carotin in the coloring of the egg. The same pigments are present in the body fat and blood serum. When hens were fed on food rich in carotin, but relatively poor in xanthophyll, no appreciable influence on the color of the yolks of the eggs was observed. When both carotin and xanthophyll-containing foods were withheld, the tint of the yolks became markedly paler. The color of the flesh and egg-yolks of fowls may, therefore, be controlled simply by giving or withholding xanthophyll-containing foods.—*J. Biolog. Chem.: Chem. Abstr.,* 1916, 10, 627.

[6] Hager's Handbuch der pharmaceutischen Praxis, II, 268–270, 1907.

[7] Directions for preparing very sensitive litmus papers are given by E. W. Rice, "Laboratory Preparation of Litmus Paper," J. Ind. Eng. Chem., *4*, 229, 1912.