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A STUDY OF THE HYDROGEN-ION CONCENTRATION OF TINCTURE OF DIGITALIS, TINCTURE OF ACONITE AND FLUIDEXTRACT OF ERGOT.*

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Introduction.—This investigation was undertaken for the Revision Committee of the U. S. P. A monograph on hydrogen-ion concentration has been tentatively accepted for admission to the U. S. P. XI. Thus, the hydrogen-ion concentration of official preparations will be stated where it is deemed desirable. The determination of this constant must, however, yield information pertinent to the therapeutic efficacy or stability of the product. The test outlined must be simple and yet reasonably accurate. The colorimetric method meets these fundamental requirements and is accepted.

The preparations that have been studied are: Tincture of Digitalis, Tincture

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of Aconite and Fluidextract of Ergot. The literature concerning the hydrogen-ion concentration of these preparations has been reviewed. Representative samples of the preparations as purchased and as experimentally prepared have been studied. As a result of these reviews and studies certain conclusions have been drawn and recommendations made.

EXPERIMENTAL.

Tincture of Digitalis.—The physicochemical work on the hydrogen-ion concentration of extractive preparations of digitalis began about a decade ago. Takahashi (1) showed that the addition of 0.05 per cent to 0.1 per cent of hydrochloric acid to the infusion of digitalis increased the stability as shown by the frog method. Tainter (2) showed the physiological activity of freshly prepared infusions of digitalis is independent of the hydrogen-ion concentration. He showed that the infusion may develop acid or alkaline reaction upon standing, depending upon the nature of the decomposition. The hydrogen-ion concentration of the tincture was found by Tainter to be the equivalent of $N/10{,}000$ hydrochloric acid, approximately p_H 4.6. Smith (3) in his studies on the determination of the hydrogen-ion concentration in alcoholic solutions investigated tincture of digitalis and found the range of p_H to be between 5.12 and 5.77. Of special interest is the work of Joachimaglu and Bose (4) who showed the stability of tincture of digitalis to be increased by the addition of 0.1 or 0.2 per cent of tartaric acid. These investigators found the p_H of tincture of digitalis to be 5.88, with 0.1 per cent tartaric acid the p_H was 5.44 and with 0.2 per cent 5.13. The stability of the heart-tonic value of the tincture was not markedly different when tartaric acid was added. In 1921, Joachimaglu (5) had observed that the addition of sodium bicarbonate to ticture of digitalis materially increased the speed of deterioration.

In 1930 Krantz (6) studied the buffer capacity of tincture of digitalis. In this study the p_H of seven samples of digitalis are reported.

No.	p_H.
1	5.88
2	5.67
3	5.70
4	5.67
5	5.68
6	5.73
7	5.61

The change in p_H upon aging of the tincture, when stored in indirect light is reported.

p_H When Prepared.	p_H After One Year.	p_H After Two Years.
5.88	5.66	5.38

In the same year, Krantz and Carr (7) studied the change of p_H of tincture of digitalis when stored in various colored glass in direct light.

	p_H.			
When Prepared.	Flint Glass.	Blue Glass.	Amber Glass.	Irradiated.
	5.59	5.59	5.59	5.59
42 days	5.09	5.18	5.20	5.19
70 days	4.89	4.87	4.98	....

In 1931 Krantz (8) studied the hydrogen-ion concentration of infusion of digitalis and reported the following p_H measurements.

No.	p_H Infusion.	p_H Tincture.
1	5.81	5.90
2	5.96	6.20
3	4.97	6.02
4	5.86	5.90

In 1931 Haag and Jarrett (9) found no constant relationship between the hydrogen-ion concentration and the heart tonic value of the tincture. The tinctures studied had a hydrogen-ion concentration generally below p_H 4.50.

Krantz and Munch (10, 11) in 1932 studied the influence of the alcoholic content of the menstruum upon the p_H of the tincture. They report an average p_H of four tinctures U. S. P. 5.75. The tinctures from the same drugs made with dehydrated alcohol showed an average p_H of 4.00. This vast increase in acidity is accompanied by a drop in heart-tonic value. However, the authors indicate that the diminution in potency is in all probability caused by the inefficiency of dehydrated alcohol in the extraction of the glucosides.

Scoville (12) in his recent studies has buffered the tincture with sodium acetate and reports increased stability.

Studies conducted by Munch (13), as chairman of the committee on bioassays of the AMERICAN PHARMACEUTICAL ASSOCIATION, seem to indicate that the rate of deterioration of the tincture is slow. Therapeutically, the stability scarcely changes in two years.

CONCLUSIONS.

From these data the following conclusions are drawn:

1. Tincture of digitalis made by the official process will have a p_H between 5.50 and 6.00.
2. This tincture (considering the manner of its dosage) is a stable product.
3. There is no unequivocal evidence to show that by buffering or adding acid to the product its stability can be increased.
4. It has been suggested, therefore, that no p_H range for tincture of digitalis be stated in the forthcoming Pharmacopœia.

Tincture of Aconite.—In 1924 Swanson (14) published some studies on the stability of the tincture and fluidextract of aconite. In this work the literature is quoted, indicating the stability of the alkaloid in acid solution. Swanson concluded that, "The rapid deterioration of tincture of aconite can be prevented by the addition of an acid to the finished percolate or menstruum." Further, "That the deterioration of the tinctures and fluidextracts is probably due to the decomposition or hydrolysis of the alkaloids, and may be a hydrogen-ion concentration factor."

In a previous communication Swanson (15) had recommended adding 2 per cent acetic acid or 0.1 per cent hydrochloric acid to the menstruum as a stabilizer for tincture of aconite.

In a subsequent communication Swanson and Hargreaves (16) showed a definite relationship between the stability and p_H of tincture and fluidextract of aconite. Without acid the p_H of tincture of aconite is 5.13. The stable p_H range lies between 2.5 and 3.0. The authors state, "The amount of acid required to produce the desired p_H depends upon the amount of alkaloids and inert material present in each lot of drug." About 0.03 cc. HCl per 100 cc. produces approximately this p_H. This is approximately 2.9 cc. of diluted hydrochloric acid per liter.

EXPERIMENTAL.

A sample of tincture of aconite was prepared by the official process. The drug was a composite sample of three commercial specimens of aconite. The p_H of the tincture was determined by the Wilson (17) type hydrogen electrode. The tincture was diluted fivefold with a mixture of 3 parts of alcohol and one part of water. At this concentration the tincture retains only a pale yellow color.

p_H Tincture.	p_H Dilution.
5.56	5.90

Twenty-five cc. portions of the tincture were treated with varying quantities of diluted hydrochloric acid. The p_H of these was determined and also the p_H of the fivefold dilution by means of the hydrogen electrode. These results are shown in the following table.

No.	Cc. Dil. HCl.	p_H Tincture.	p_H Dilution.
0	0	5.56	5.90
1	0.15	3.65	3.85
2	0.30	2.32	2.61
3	0.45	1.95	2.42
4	0.60	1.72	2.22
5	0.75	1.58	2.06
6	0.90	1.46	1.96
7	1.05	1.38	1.85
8	1.20	1.32	1.71

These results are plotted in Fig. 1, expressing the acid in moles.

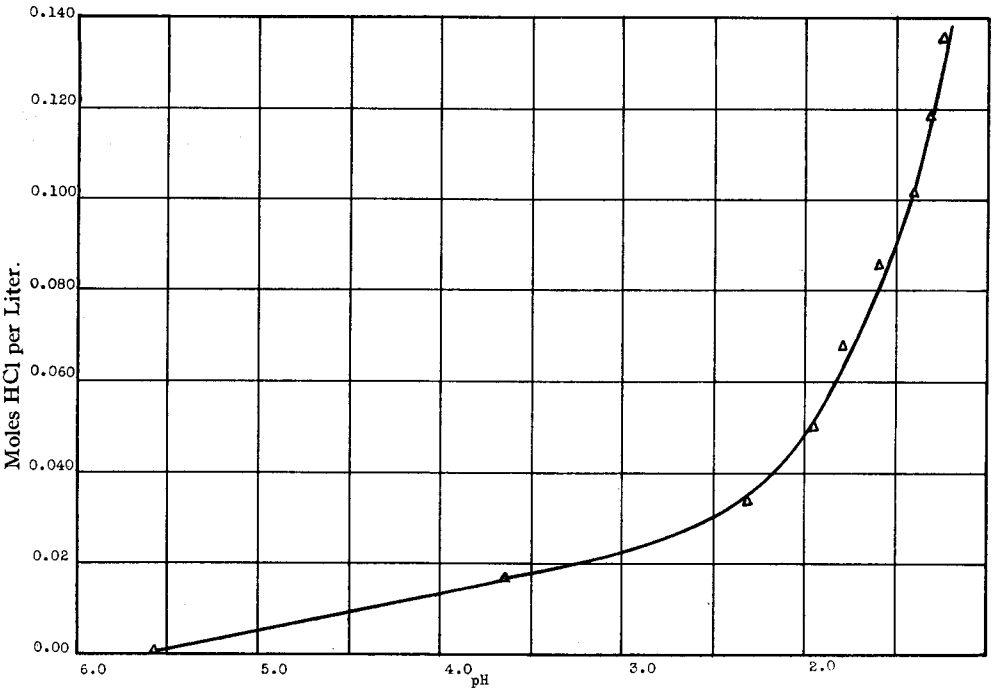

Fig. 1.—Influence of strongly dissociated acid on tincture of aconite.

The curve is a straight line between p_H 5.56 and 3.00. To change the p_H of this composite tincture 2.56 p_H units, 0.022 mole of HCl per liter was added to the buffer capacity of the tincture using the Van Slyke (18) ratio $\frac{-dB}{-dp_H} = \frac{-0.022}{-2.56} = 0.0086$. It is indeed of interest to note that the value obtained by Krantz (19) for tincture of digitalis was 0.0090.

For practical purposes, the indicator solutions thymol blue and methyl orange were selected. The p_H range of the former is 1.2 (red) to 2.6 (yellow); at p_H 2.2 an orange color is exhibited. With methyl orange the range is p_H 3.1 to 4.4. Thus, a solution acid to methyl orange (p_H 3.1) and showing an orange color with thymol blue will be between p_H 2.2 and 3.1, *i. e.*, the stable range suggested by Swanson.

The preparations in the foregoing table reacted to these indicators as follows:

No.	Thymol Blue.	Methyl Orange.
0	Yellow	Yellow
1	Yellow	Yellow
2	Orange	Red

No.	Thymol Blue.	Methyl Orange.
3	Red	Red
4	Red	Red
5	Red	Red
6	Red	Red
7	Red	Red
8	Red	Red

Thus, sample No. 2 meets the requirements and the p_H of this tincture is 2.32 electrometrically.

Accordingly the following statement is suggested for the Pharmacopœia under the tincture of aconite monograph.

"Add to the finished percolate diluted hydrochloric acid in 0.5 cc. volumes until 1 cc. of the tincture diluted to 5 cc. with a mixture of 3 volumes of alcohol and one volume of distilled water shows a red color, when 6 drops of methyl orange T.S. are added, but no deeper than an orange color, when 5 drops of thymol blue T.S. are added to another similar dilution of the tincture (p_H 2.0 to 3.0)."

Swanson's stability curve for the tincture shows a long, flattened portion of maximum stability which is well included within this p_H range.

As a matter of general interest, five commercial samples were purchased and the p_H determined as described previously. These values are shown in the following table.

A. p_H 3.48
B. p_H 3.05
C. p_H 4.03
D. p_H 2.75

Only sample "D" met the requirements of the test suggested, although each label stated that the hydrochloric acid had been added.

CONCLUSIONS.

1. A method has been described for determining the p_H of tincture of aconite which provides for variations in commercial samples of crude drug.
2. A colorimetric method of controlling the p_H of the tincture has been described.
3. The buffer capacity of the tincture has been determined.
4. The Van Slyke ratio was observed to closely approach the ratio for tincture of digitalis.
5. Samples of commercial tinctures showed a wide deviation from the suggested optimum p_H, which suggests the need for standardization.

Fluidextract of Ergot.—There seems to be no controversy regarding the favorable influence of acids upon the stability of fluidextract of ergot. The acetic acid employed in the menstruum of the U. S. P. VIII was changed to the more strongly ionized hydrochloric acid in the U. S. P. IX and this formula was in turn adopted by the revision committee of the 1920–1930 decade. During the last half decade, the interest in fluidextract of ergot, which had reached a condition of somnolent passivity, was revived and deepened by a variety of activities. The ergot investigation of 1929 instituted by a senatorial committee, the appearance of ergotamine tartrate on the market, the indictment and restoration to grace of the cock's comb assay and the influence of hydrogen-ion concentration upon the stability of extractive preparations of ergot have served to bring this drug an unusual degree of legal and pharmaceutical prominence. The results of the studies of

hydrogen-ion concentration on the stability of the fluidextract are, however, the chief concern of this report.

In 1929 Swanson (20) studied the standardization and stabilization of ergot preparations in relationship to the hydrogen-ion concentration factor. In this work he showed that the fluidextract of ergot requires a certain amount of acid to prevent deterioration. The graph set forth in Swanson's report shows that in the study of the fluidextract over a period of two years, a p_H of 3.00 or less is necessary to prevent deterioration. The importance of this, gleaned from the work of this investigator, cannot be overestimated, for at p_H 5.35, the fluidextract lost 90 per cent of its activity over a period of two years, whereas, those fluidextracts having a p_H less than 3.00 retained more than 90 per cent of their original activity over this same period.

In 1930 Thompson (21), in a comprehensive study of ergot and its extractive preparations, makes the following comment regarding the addition of acid:

"The hydrochloric acid of the prescribed menstruum plays a double rôle. *First*, it increases the efficiency of the menstruum in extracting the specific alkaloids, and *second*, it increases the stability of the product. Proper control of this acidity is therefore of vital importance. The use of organic acids, such as citric or tartaric, in place of hydrochloric, serves no good purpose, in that such departure from the U. S. P. method decreases the efficiency of the menstruum in extracting the alkaloids and also detracts from the stability of the finished product."

In a subsequent communication Thompson (22) studied the stability of various samples of fluidextract of ergot in relation to the hydrogen-ion concentration of the preparation. Thompson lists the following data.

No.	Present Age Approx.	Per Cent Deterioration in 18 Months.	p_H.
1	5 years	10.6	4.192
2	3 years	5.7	1.778
3	18 months	8.4	1.809

In this work Thompson calls attention to the fact that the fluidextract with the highest p_H showed the greatest degree of deterioration.

Rowe and Scoville (23), in 1931, made a study of the stability of fluidextract of ergot, using, respectively, hydrochloric acid and hypophosphorous acid as stabilizing agents. Most of the preparations studied by these workers deteriorated rather rapidly and because in most instances the p_H was practically the same, no conclusions can be drawn from this work regarding the influence of p_H on the stability of the fluidextract.

In 1932 Swanson (24) studied the stability of ergotamine tartrate crystals in hydroalcoholic solution in the presence of phosphate buffers. These workers conclude that a solution of pure ergotamine tartrate crystals in 40 per cent alcohol with a hydrogen-ion concentration of around p_H 3.00 appears to be the critical point, where there is the least deterioration.

Wokes and Elphick (25) observed that the efficiency of extraction of ergot with a 50 per cent neutral alcohol depended upon the acidity of the crude drug. With more acid specimens of ergot (p_H below 5.5) the neutral menstruum was quite as effective as one to which either hydrochloric acid or tartaric acid had been added. These workers suggest, that owing to the presence of certain phosphate buffers in the drug, the amount of acid required for each sample of ergot cannot be definitely established. Therefore, a control of the finished product by p_H adjustment seems to be advisable.

Swanson (26) reported in 1932 that over a period of three years, there was a distinct deterioration in eight samples of fluidextract of ergot studied. However, the samples with a p_H around 3.00 showed the least deterioration. In the conclusion of this paper, Swanson, *et al.*, set forth the following comment: "The data in this report show no definite conclusions that the deterioration of fluidextracts of ergot or a solution of pure ergotamine tartrate is prevented by definite hydrogen-ion concentration."

In a subsequent investigation from Swanson's laboratory, Powell (27) reported on a series of eighteen fluidextracts. These products were stored six months, at room temperature, and subsequently assayed by the Broom and Clark method and also by Smith's chemical method. The results of the two methods of assay show a remarkable agreement. Again, this worker observed that those fluidextracts, the p_H of which was close to 3, retained the greatest degree of original

potency. At the close of the account of this investigation Powell made the following statements. "Some fluidextracts of ergot deteriorate rapidly regardless of hydrogen-ion concentration," and also "The hydrogen-ion concentration still appears to have some influence on the stability of fluidextracts of ergot."

Smith and Stohlman (28) conclude from a series of extensive investigations that a variation of p_H from 5.2 to 2.2 does not favor the stability of fluidextract of ergot.

Swoap, *et al.* (29), reviewed the work of previous investigators in the field and in conjunction with studies which they conducted concluded that the manipulation of the p_H of the fluidextract did not favorably influence the stability of the product. Contrary, in a measure at least, to the findings of Swanson and his associates, Swoap states that, "p_H variations between the limits of 3.0 and 6.1 do not enhance the stability of fluidextract of ergot," furthermore, "manipulation of p_H in the finished fluidextract appears to be more harmful than beneficial."

CONCLUSIONS.

In summarizing the foregoing opinions of the authorities in the field of the pharmacy and pharmacology of ergot, one is convinced that there exists absolutely no unanimity of opinion regarding the effects of the manipulation of the p_H. The fluidextracts prepared by the official process containing 20 cc. of hydrochloric acid per liter show a p_H of approximately 4.5. Apparently this value is subject to a considerable degree of variation. However, as the evidence which is available to show that changing this value to p_H 3 is in no sense convincing, it seems contrary to the policy of the Pharmacopœia to establish such a requirement.

Unless, therefore, prior to the publication of the forthcoming revision of the Pharmacopœia, there is unequivocal evidence produced to show that the manipulation of the p_H is an important factor in controlling the stability, the authors recommend that the acidity of the menstruum and percolate remain unchanged.

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