only, with much expense and labor, be freed from the principle which causes this distinctive color reaction.

6. The modified test, as suggested by the author, is believed to be a distinct improvement over the original test, giving more assured results and a more sensitive test.

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THE ACID-BASE EQUILIBRIUM OF TINCTURE OF DIGITALIS.*

BY JOHN C. KRANTZ, JR.

INTRODUCTION.

Recently a considerable amount of investigation has been carried out to study the effect of changes in hydrogen-ion concentration upon the stability of the various preparations of digitalis. Tainter (1) showed that the physiological activity of freshly prepared infusions of digitalis is independent of their hydrogen-ion concentrations, and that the infusion tended to become acid upon standing. This investigator determined the acidity of the tincture to be the equivalent of an $N/10{,}000$ hydrochloric acid solution, approximately p_H 4.6. Takahashi (2) contrary to the results of Tainter showed that the addition of 0.05 per cent to 0.1 per cent of hydrochloric acid to the infusion of digitalis increases the stability of the infusion as determined by the frog method.

Smith (3) in his studies of 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 5.44 and with 0.2 per cent of the acid 5.13.

With the thought of studying the influence of buffer-salt mixtures in the menstruum upon the stability of the tincture, this investigation was begun.

Buffering the Menstruum of Tincture of Digitalis.—A series of menstrua were prepared using an 80% alcohol and the citrate mixtures described by Sorensen (5). With these a series of tinctures were prepared by the U. S. P. method omitting the extraction of the drug with petroleum ether. The hydrogen-ion concentrations of the menstrua were determined by means of the hydrogen electrode (Wilson type (6)). The values obtained were reproducible within 0.1 unit p_H.

Table I indicates the composition of the buffer mixtures, the p_H of the menstruum and the p_H of the tincture.

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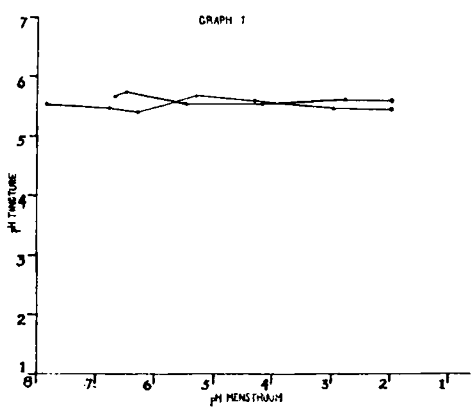
* Scientific Section A. Ph. A., Rapid City meeting, 1929.

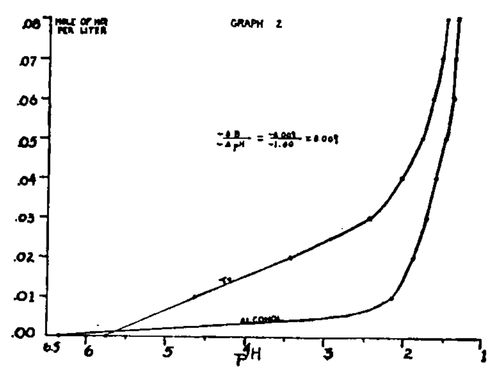
TABLE I.

Number.	Composition of menstruum.	p_H menstruum.	p_H tincture.
1	480 cc. alcohol—120 cc. 0.1 *N.* HCl	2.09	5.46
2	480 cc. alcohol—36 cc. secondary citrate 0.1 *M.*—84 cc. 0.1 *N.* HCl	2.97	5.47
3	480 cc. alcohol—48 cc. secondary citrate 0.1 *M.*—72 cc. 0.1 *N.* HCl	4.30	5.58
4	480 cc. alcohol—66 cc. secondary citrate 0.1 *M.*—54 cc. 0.1 *N.* HCl	5.30	5.61
5*	480 cc. alcohol—27 cc. secondary citrate 0.1 *M.*—3 cc. 0.1 *N.* HCl and 90 cc. distilled water	6.27	5.40
6*	480 cc. alcohol—30 cc. secondary citrate 0.1 *M.* and 90 cc. distilled water	6.71	5.45
7*	480 cc. alcohol—15 cc. secondary citrate 0.1 *M.*—15 cc. 0.1 *N.* NaOH and 90 cc. distilled water	7.75	5.52
8	480 cc. alcohol—120 cc. distilled water—unbuffered control	5.45	5.55

* Nos. 5, 6 and 7 were diluted with water to avoid precipitation of the buffer salts by the alcohol. In other cases this dilution was unnecessary.

The results recorded in Table I indicate that using the buffered menstrua described the extractive material of the digitalis leaves possessed sufficiently buffer





capacity to bring each of the finished tinctures to practically the same hydrogen-ion concentration, *i. e.*, close to the p_H of the unbuffered tincture.

Another sample of digitalis assaying the U. S. P. strength by the one-hour frog method was percolated and prepared into several samples of tinctures. The hydrogen-ion concentrations, the menstrua and tinctures of the second sample of digitalis are shown in Table II.

TABLE II.

No.	p_H menstruum.	p_H tincture.	No.	p_H menstruum.	p_H tincture.
1	1.97	5.63	5	6.44	5.72
2	2.75	5.62	6	6.65	5.57
3	4.17	5.54	7	5.55 control	5.52
4	5.42	5.54			

When the results of these determinations are plotted, using the p_H of the menstruum as abscissæ and the p_H of the tinctures as ordinates, the interesting hydrogen-ion neutralization properties of digitalis is observed.

Buffer Capacity of Tincture Digitalis.—Having observed the power of the extractive matter in the digitalis leaves to absorb acid indicating that only strong acids in relative high concentrations are capable of changing the hydrogen-ion concentration of the tincture, the next step was to determine the buffer capacity of the tincture, quantitatively. The differential ratio of Van Slyke (7) $\frac{dB}{dp_H}$ used by the author (8) in measuring the buffer capacity of acacia solutions was used as a standard for measurement. This ratio expresses the relationship between the increment in gram equivalents of strong base added to the buffer solution and the resultant increment in p_H. If an acid is added the value of each term in the expression is negative, hence the ratio, the Van Slyke "β," has always a positive value.

A sample of tincture of digitalis was prepared and divided into nine portions. To each of these samples a definite gram equivalent of hydrochloric acid was added, and the p_H was determined. This procedure was also employed in case of the pure menstruum. Table III records the results of these measurements and shows definitely the acid-absorbing capacity of tincture of digitalis.

TABLE III.

No.	Cc. 0.1 N. HCl per l.	Mole HCl per l.	p_H tincture, 3 hrs.	p_H tincture, 3 days.	p_H menstruum.
1	00	00	5.75	5.73	6.35
2	100	0.01	4.61	4.55	2.18
3	200	0.02	3.57	3.47	1.89
4	300	0.03	2.43	2.36	1.71
5	400	0.04	2.04	2.04	1.60
6	500	0.05	1.74	1.85	1.49
7	600	0.06	1.62	1.76	1.39
8	700	0.07	1.51	1.67	1.36
9	800	0.08	1.45	1.57	1.33

Table III shows the difference between the hydrogen-ion concentration of the menstrua and that of the tincture of digitalis upon the addition of various quantities of hydrochloric acid. The values obtained after three days' standing were for the most part identical with those obtained soon after the addition of the acid. In Graph 2 this relationship is more clearly shown when the quantities of acid added are plotted as ordinates and the corresponding increments in p_H as abscissæ. From this graph we may obtain the ratio $\frac{-\Delta B}{-\Delta p_H}$ where each of these values is a measurable increment. Thus to change the p_H of a liter of tincture of digitalis from p_H 5.75 to p_H 4.75 approximately 0.009 mole of hydrochloric acid must be added or 90 cc. of 0.1*N*. acid. Then

$$\frac{-\Delta B}{-\Delta p_H} = \frac{-0.009}{-1.00} = 0.009$$

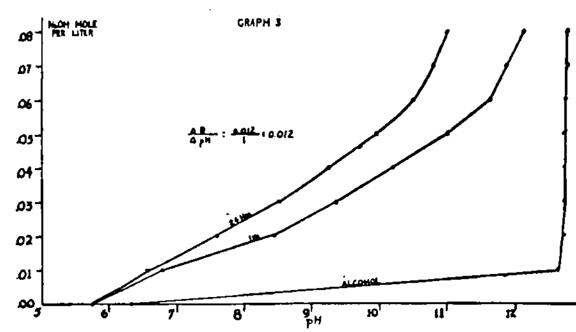
In order to obtain the value "β" the differential ratio, a tangent is drawn to the curve at a definite point, values on the ordinate axis above and below this point are taken and the base line intercepts of the tangent at these points are determined. Between the values p_H 5.75 and p_H 2.5 the curve is a straight line so that the buffer value obtained using the measurable increments is identical with that of the differential ratio $\frac{-dB}{-dp_H} = \beta$, where β equals 0.009 between p_H 5.75 and 2.5. It should be observed that the addition of 0.01 mole hydrochloric acid per liter of the menstruum changed the p_H from 6.35 to 2.2. As Joachimaglu (4) had shown the presence of sodium bicarbonate to be detrimental to the stability of the tincture, and further considering the buffer effect of this drug on acids it was thought interesting to study the influence of the addition of alkali upon the p_H of the tincture.

The procedure followed in this portion of the experiment was the same as that employed in the preceding work. Table IV records these results.

TABLE IV.

No.	Cc. 0.1 *N*. NaOH per l.	Mole NaOH per l.	p_H tincture after 1 hr.	p_H tincture after 24 hrs.	p_H of menstruum.
1	00	00	5.75	5.75	6.35
2	100	0.01	6.83	6.55	12.66
3	200	0.02	8.45	7.60	12.78
4	300	0.03	9.34	8.52	12.79
5	400	0.04	10.20	9.25	12.74
6	500	0.05	11.03	9.97	12.76
7	600	0.06	11.68	10.51	12.77
8	700	0.07	11.87	10.83	12.80
9	800	0.08	12.12	11.00	12.78

Plotting these values as before in Graph 3 we observe the relationship between the action of strongly dissociated alkali upon the tincture of digitalis and its menstruum. After equilibrium is established, it will be observed that the curve to p_H 9.5 is a straight line, hence the buffer capacity of the tincture by direct reading from the curve up to this point.



To change the p_H from 5.75 to 6.75 required the addition of 0.012 mole of sodium hydroxide per liter or

$$\frac{\Delta B}{\Delta p_H} = \frac{0.012}{1} = 0.012$$

Likewise "β" will have the same value.

Nature of the Buffer Material.—In order to determine whether the buffer substance in this tincture was inorganic or organic in nature, the following experiment was performed. Twenty cc. of the tincture was evaporated to dryness and ashed. The ash was dissolved in 20 cc. of 80 per cent alcohol, p_H 6.35—the p_H of the solution was 10.64. Upon the addition of 2 cc. 0.1*N*. hydrochloric acid the p_H of this solution changed to 2.52, upon the addition of 2 cc. 0.1*N*. sodium hydroxide to the original solution the p_H of the solution became 12.82. The amount of acid or alkali added was identical with that added to the tincture in the foregoing experiment, where it will be recalled that it required about 100 cc. per liter or 2 cc. of strong acid or base per 20 cc. to change the p_H of the tincture one unit.

This seems to indicate that buffer influence of the tincture is dependent upon the organic extractive material from the drug.

Effect of Aging upon the p_H of Tincture of Digitalis.—Joachimaglu found that a tincture of digitalis with a p_H of 5.88 changed upon standing at room temperature as follows:

p_H when prepared.	p_H after one year.	p_H after two years.
5.88	5.66	5.38

The author observed a more rapid change of p_H in certain tinctures of digitalis when stored in direct light. The direction of the change was the same as that observed by the previous investigator.

A sample prepared by the U. S. P. method was kept in a flint-glass bottle in direct light in an incompletely filled bottle at room temperature.

p_H when prepared.	p_H after $3^1/_2$ months.
5.67	5.45

Other samples of tincture stored in flint bottles in a closed closet at room temperature showed no significant change in hydrogen-ion concentration over a period of a year.

Several different samples of tincture of digitalis were studied and the following p_H values obtained immediately after preparation.

No. 1—5.88	No. 4—5.67	No. 7—5.61
No. 2—5.67	No. 5—5.68	Average 5.71
No. 3—5.70	No. 6—5.73	

Thus the values obtained by the present author are in close agreement with those obtained by Joachimaglu, but somewhat less acid than those obtained by Tainter.

CONCLUSIONS.

1. The acid-base equilibrium of tincture of digitalis has been studied.

2. The Van Slyke "β" for tincture of digitalis has been determined for strong acids and bases.

3. The influence of aging upon the p_H of the tincture has been determined over a short period of time.

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ABSTRACT OF DISCUSSION.

E. E. Swanson inquired whether Dr. Krantz had tested any of the tinctures for p_H along with the determination of potency.

The author stated that he had attempted to prepare tinctures of different p_H but as these showed about the same p_H this study was abandoned. Dr. Munch had made the physiological tests.

James C. Munch said he had made several of the physiological examinations but was not in position to say which of them, at this time was most potent. He considered that nature had done good work in protecting digitalis and suggested that the change in hydrogen-ion concentration would not be helpful.