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Potentiometric Titration in Two-phase Systems

Determination of Bitter Acids in Hops

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If an aqueous buffer solution is shaken with a water immescible solvent, which is able to extract the free acid, the pH of the solution increases. This increase, determined with a glass electrode, is shown in the following table for some buffer solutions of acids, neutralized to 50 % with sodium hydroxide.

The buffer solution, 20 ml, was shaken with 5 ml of organic solvent, the pH was taken before the procedure and after the separation of the solvent. The separation was not always complete, a light fog could sometimes be observed in the water solution. The glass electrode gives less reliable results in emulsions, hence the small increases, some hundredths in pH, with acetic and propionic acid.

Table 1. Increases in pH by the extraction of buffer solutions.

Acid	Solvent						
	1	2	3	4	5	6	7
0.02 *M* Acetic	0.04	0.04	0.20	0.02	0.06	0.02	0.02
0.02 *M* Propionic	0.05	0.00	0.27	0.00	0.00	0.00	0.00
0.02 *M* *iso*-Valeric	0.04	0.03	0.81	0.10	0.30	0.11	0.03
0.01 *M* Caprylic	1.25	—	2.40	1.61	1.86	1.49	1.35

The solvents are: 1 — n-C_6H_{14}, 2 — Petroleum ether b. p. 45—55° C, 3 — Ether, 4 — C_6H_6, 5 — $CHCl_3$, 6 — CCl_4, 7 — CS_2.

The procedure is a suitable expedient for selecting solvents for extraction, but it can also be used for analytical purposes. Observation of the changes in pH during the extraction of an acid might furthermore be very helpful in preparative and manufacturing practise in order to get the best possible separation as will be shown in regard to bitter acids in hops. (Quoted by Lundin [1]).

THEORY

The theory for the distribution of a substance between immiscible liquids has long been common knowledge [2]. Below follows an application of the theory to a case where an acid is successively neutralized in a two-phase water-oil system with continous determination of the pH.

In the water solution of a partially neutralized acid the pH is determined by the relation

$$\mathrm{pH} = \mathrm{p}k + \log C_{\mathrm{salt}} - \log C_{\mathrm{acid}}$$

where C_{salt} and C_{acid} represent the concentrations of salt and acid respectively. If such a water solution is shaken with a solvent wherein the acid but not the salt is soluble, the factor C_{acid} decreases to a degree determined by the distribution coefficient of the acid and the ratio between the volumes of the water and oil phases. This decrease in the concentration of free acid causes a raise in the pH.

Assuming that the distribution coefficient f is independent of the concentration — a condition seldom valid for acids, but might be accepted as practical in routine work — and if C_{oil} and C_{water} designate concentrations of free acid in an oil phase of volume o, and in a water phase of volume v respectively, and if equilibrium has been established, this equation holds:

$$C_{\mathrm{oil}} = f \cdot C_{\mathrm{water}} \text{ and } v \cdot C_{\mathrm{acid}} = v \cdot C_{\mathrm{water}} + o \cdot C_{\mathrm{oil}}$$

$$\text{or } v \cdot C_{\mathrm{acid}} = (v + o \cdot f)\ C_{\mathrm{water}}$$

From this latter relation and the simultaneously valid equation:

$$\mathrm{pH} = \mathrm{p}k + \log C_{\mathrm{salt}} - \log C_{\mathrm{water}} \tag{1}$$

we get

$$\mathrm{pH} = \mathrm{p}k + \log C_{\mathrm{salt}} - \log C_{\mathrm{acid}} + \log \left(\frac{o}{v} \cdot f + 1\right) \tag{2}$$

In this formula applied to a two-phase system, C_{acid} thus denotes the concentration which the free acid should have had if the oil phase had not been present.

The formula implies that the titration curve in a two-phase system is constantly being displaced towards a higher pH by a factor that depends partly on the volume ratio of the phases, and partly on the value of f, a coefficient characteristic of the acid.

For acids, that mainly go into the oil phase, the last term in equation (2) can without major error be written:

$$\log\left(\frac{o}{v}\cdot f+1\right)=\log\frac{o}{v}+\log f$$

If we introduce m for the total amount of non-neutralized acid, *i. e.* $v \cdot C_{\text{acid}}$, the equation becomes:

$$\text{pH}=\text{p}k+\log f+\log C_{\text{salt}}-\log m+\log o \tag{3}$$

But as a rule the coefficient f is unfortunately not independent of the concentration. In organic solvent acids are often associated into double molecules. The equilibrium between oil and water phases can then approximately be represented by the relation:

$$C_{\text{oil}}=\psi^2\cdot(C_{\text{water}})^2 \text{ where } \psi \text{ is a constant.} \tag{4}$$

For the total amount of non-neutralized acid we get the expression:

$$v\cdot C_{\text{acid}}=o\cdot\psi^2\cdot(C_{\text{water}})^2+v\cdot C_{\text{water}}$$

When the oil-solubility of the acid prevails strongly the last term in this analogy may be omitted and from equation (1) we obtain:

$$\text{pH}=\text{p}k+\log\psi+\log C_{\text{salt}}-\tfrac{1}{2}\log m+\tfrac{1}{2}\log o \tag{5}$$

The pH curve of a two-phase titration in such a case becomes somewhat flatter than without association into double molecules, but it still is on a higher level than the corresponding curve for neutralization in pure water. With increasing volume of the oil phase the pH of the buffer increases to a smaller degree for associating than for non-associating molecules.

The values of $\log f$ or $\log \psi$ can be calculated from the pH increase on the addition of an oil phase to a partially neutralized solution of acids, whose dissociation constants in water are known. But it is more convenient to merge pk and $\log f$, pk and $\log \psi$ respectively into a new term, especially as the distribution coefficients often are unknown. For nonassociating acids the term »apparent p*k*» is suggested, denoted ap*k*. From equation (3) we then derive:

$$\mathrm{pH} = \mathrm{ap}k + \log C_{\mathrm{salt}} - \log m + \log o \tag{6}$$

It is easy to see that ap*k* = the pH of a semineutralized solution when water and oil phases are of equal volume.

This simple definition cannot be used for associating acids, because in that case the pH of a semineutralized solution depends on the absolute concentrations as well. We introduce the term »normal p*k*» = np*k*.

According to equation (5) we then get:

$$\mathrm{pH} = \mathrm{np}k + \log C_{\mathrm{salt}} - \tfrac{1}{2} \log m + \tfrac{1}{2} \log o \tag{7}$$

Here np*k* denotes the pH in a system where the water phase is 1 *N* as regards salt, and the oil phase is 1 *N* as regards acid. Deviations caused by activity coefficints have not been taken into consideration.

EXPERIMENTAL TECHNIQUE

The two-phase titration can be used for analytical characterization of an acid. It is then sufficient to study the change in the pH when an oil phase is added to a partially neutralized water solution. Mixed acids can be separated even if they have identical dissociation constants in water under the premise that their distribution coefficients differ rather widely.

Two different techniques can be used for the titration: either the volume of the water phase is kept constant by titrating with rather concentrated alkali, which gives a curve, entirely (equation 6) or closely (equation 7) resembling ordinary titration curves, or otherwise a standard alkaline solution is added to *e. g.* a petroleum ether solution of one acid or a mixture of acids. If only one acid is present C_{salt} when using the second method, becomes constant and a very flat curve results. The only variable is then log *m*. With either method ap*k* and np*k* can easily be determined.

When using the second technique for a mixture of acids, it is advisable to draw away the water solution before the next portion of alkali is added. If the portions are collected separately when the pH tends to change rapidly, a separation of the acids is attained.

Fig. 1 is a sketch of the apparatus used for the two-phase titration.

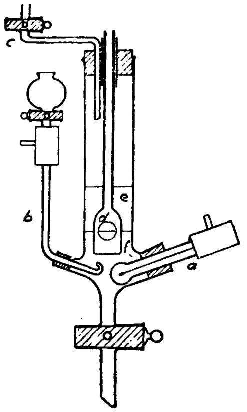

Fig. 1. Apparatus for two-phase titration. a = glass electrode, b = reference electrode, c = burette, d = centrifugal stirrer, e = oil phase, f = water phase.

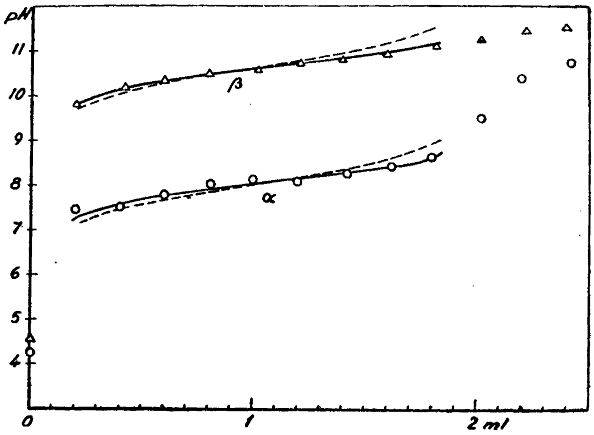

Fig. 2. Two-phase titration in water — petroleum ether on 1 millimole of a-resp. β-acid, with 0.49 N NaOH.

EXPERIMENTS WITH BITTER ACIDS IN HOPS

Hops contain several bittering substances or bitter acids, two of them, humulon and lupulon, also called α-acid and β-acid, have known chemical structures[3, 4] and are soluble in hexane. As possible degradation products from these, hops also contains so-called soft resins and hard resins. The former are soluble in hexane, the latter are not. Humulon is easily determined as an insoluble lead salt[5—7], for lupulon, on the contrary, no specific reagent is known and it is usually determined together with the soft resin.

In methanol solution humulon and lupulon have practically the same acid strength (pk = 5.5). The solubility in water is very low. If a solution of sodium salts of humulon or lupulon is titrated with hydrochloric acid an emulsion is formed. Windisch, Kolbach and Winter[8] found that such emulsions give neutralization curves, which are the higher situated in the pH scale the higher the concentration. The pH values were higher with lupulon than with humulon. A mixture of these two bitter acids gave a neutralization curve permitting at best a very rough estimate of the two components.

The performance of neutralization in two-phase systems gives more accurate results.

As a rule the titrations have been performed in the following way: 10 ml of a petroleum ether solution of the bitter substance is introduced into the titration vessel. About 9 ml CO_2-free water is added, and the phases are brought to equilibrium through vivid stirring for 2 min. The stirrer is stopped and the pH of the water layer is measured with the glass electrode. Small portions of 0.50 N alkali are added, every portion followed by 2 min stirring and the pH is measured when the phases have separated. Now and then emulsions appear, especially at higher pH; they often need up to 30 minutes to separate. With pure bitter-acids however the titrations can usually be completed in about 2 hours. The amount of bitter-acid was chosen to give a consumption of about 2 ml of titration liquid, which gave petroleum ether- and water-phases of equal volume when the acid was half-neutralized.

Fig. 2 shows the result of a two-phase titration on 1 millimole α-acid and 1 millimole β-acid respectively with 0.49 N NaOH, both systems consisting of 10 ml petroleum ether and about 10 ml water.

The points mark the results of the measurements. The lined curves have been calculated according to equation (7) with $\text{np}k = 8.70$ for α-acid and 11.27 for β-acid. The dashed curves have been calculated according to equation (6) with $\text{ap}k = 8.03$ for α-acid and 10.64 for β-acid. There is no great difference between the curves, but the points fit closely to the lined curve. It is obvious that equation (7) is valid for this two phase system, which means that the bitter-acids are associated to double molecules in the petroleum ether phase. The attention is drawn to the great difference between the two acids, which must mainly depend on different distribution constants. In alcohol *e. g.* the acids are of practically the same strength. As is shown by the bottom curve it is easy to determine the end point of the α-acid titration but more difficult to do so for the β-acid, where the pH is very high. At such a high pH an excess of alkali causes but a slight change of the pH. Besides, the glass electrode exhibits a certain inertia at these higher values of pH.

The association to double molecules appears also in another experiment. One millimole of α-acid, neutralized to 50 % in 10 ml water + 10 ml petroleum ether had a $\text{pH} = 8.03$. A fivefold increase of the petroleum ether phase should — according to equation (7) — increase the pH by 0.35 units ($\frac{1}{2}$ log 5), while for simple molecules — according to equation (6) — the increase should be 0.70 units. Experimentally an increase in the pH-value of 0.28 units was measured. On 27-fold dilution of the petroleum ether the increase was 0.75 units, as compared with 0.72 units which had been calculated for double molecules. A fourfold dilution of the water phase resulted for the sodium salt of the α-acid in a decrease of 0.54 pH-units (0.60 calc.); thus the salt shows a tolerably classical behaviour. Corresponding experiments with β-acid gave

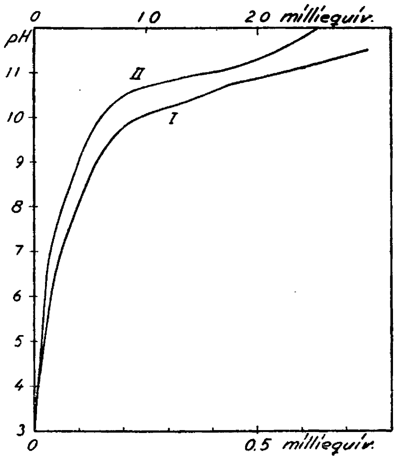

Fig. 3. Two-phase titration (diagram I) and fractionated alkaline extraction (diagram II) of the β-fraction of lupuline in petroleum ether solution.

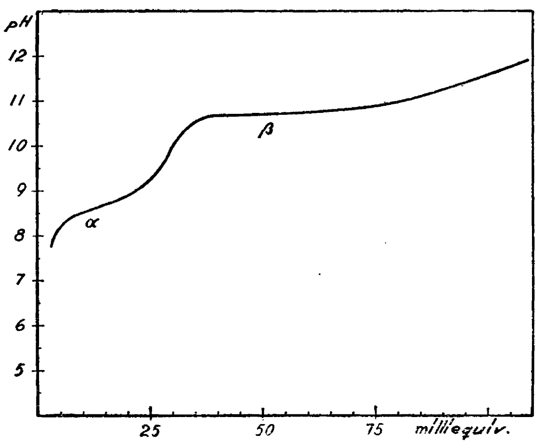

Fig. 4. Two-phase titation of a β-fraction contaminated by some a-acid.

somewhat uncertain results, which is explainable considering the inertia of the glass electrode at higher pH values. But everything indicates that even in this case an association to double molecules occurs.

The two diagrams in Fig. 3 refer to the same preparation of lupulon from lupuline. After precipitation of the α-acid from methanol solution with lead acetate in the usual manner, the solution was diluted with water, acidified with hydrochloric acid and extracted with petroleum ether. We titrated 10 ml of the petroleum ether solution, containing 0.26 g of bitter substances, with 0.50 *N* NaOH in a two-phase system containing 10 ml water, and the diagram I in fig. 3 was obtained. Diagram II shows the results of pH measurements on repeated extractions with 10 ml portions of 0.1 *N* NaOH of 400 ml of the same petroleum ether solution. The scales of the abscisses have been chosen in accordance with the total contents of bitter-acids in the solutions and are given at the bottom resp. at the top of the figure as added milliequivalents of alkali. The deviation in pH between the two diagrams corresponds to the differing concentrations and volumes in the two experiments. Each water phase of 10 ml in the experiment referred to in diagram II was acidified and extracted after the pH-measurement. The extracts were evaporated and dried in vacuum, and the residues of bitter-acids were compared. From the alkaline extracts within the pH-range 10.6—11.8 well crystallized β-acid was obtained. At higher and at lower pH-values we mainly obtained a noncrystalline product besides a small amount of crystallized β-acid. Up to pH 9 mostly soft resins were extracted, possibly contaminated with such α-acid as had been incompletely precipitated by lead acetate. In accordance with the non-crystallization of the products extracted at a lower pH-value, the two-phase titration of the oxidation products from pure α- and β-acids gave values of pH corresponding to the ap*k*:s of the resins from 4 up to 7 or 8. In these cases most curves had no distinct platforms, proving that the products were not homogenous.

An approximate estimation of soft resins in such a β-fraction as is obtained with hitherto prevalent methods of analysis should be rendered possible by a two-phase titration in water-petroleum ether. The alkali needed to reach a pH value of about 8.5 would then correspond to the amount of soft resins. Gravimetrically the analysis could be performed in a simpler manner: by extraction with a buffer of about pH 8.5 which is then acidified and extracted with ether. For comparatively fresh lupuline this latter method seems to give rather reliable results. But as soon as the bitter acids have been exposed to more serious destruction, the values become less reliable.

In preparations of β-acid from lupuline the extraction with small portions of alkali under continuous control of the pH value has been very useful. In the pH-range 10.5—11.7 we have got a comparatively pure β-bitter acid. The acid has been further purified by recrystallization, first from petroleum ether and then from 90 % methanol.

Another example of how the fractionation of an alkaline extract can be surveyed by continuous measurement of the pH value is given in Fig. 4. The diagram represents a preparation from lupuline from which the α-acid, because of incomplete precipitation by lead acetate, had not been entirely removed. This α-acid is revealed by the step at pH 8.5.

This fractionation can be regarded as a development of the method of Windisch, Kolbach and Winter[9], where the acid is purified by successive extractions with ammonium carbonate, sodium carbonate and potassium hydroxide.

How solvents, other than petroleum ether, influence the pH value in a two-phase system, has only been examined in some preliminary experiments. For β-acid we got the following ap*k*-values: in hexane 10.4, in carbon tetrachloride 10.3, in ether 11.1 and in chloroform 12.3. The ap*k*-value of 10.6 in petroleum ether (b. p. 45—55° C) has already been mentioned. Chloroform thus extracts β-acid a hundred times better than do both hexane and petroleum ether.

SUMMARY

Acids, slightly soluble in water, can be titrated with an alkali and characteristic titration curves obtained, provided that the titration is performed in a two-phase system consisting of water and an organic solvent able to dissolve the acids. The distribution equilibrium of the acids must be attained before the determination of the pH.

The composition of lupuline from hops is studied by this method and it is shown that the analytical determination of lupulon is possible by titration in a water-petroleum ether system.

In the extraction of acids from an organic solvent with small successive portions of an alkaline water solution different acids can sometimes be separated. In this way a fairly good separation of the bitter acids and soft resins in hops can be achieved.

This work was performed in 1945. We wish to express our thanks to Professor H. Lundin, to Mr. E. Sandegren at *AB Stockholms Bryggerier* (Stockholm's Brewery Company) and to Dr. S. Brohult, head of *Forskningslaboratoriet LKB* (LKB Research Laboratory) for their stimulating interest.

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