REVIEW ARTICLE

NON-AQUEOUS ACID-BASE TITRATIONS IN PHARMACEUTICAL ANALYSIS

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IN recent years a great number of papers have appeared, dealing with different types of non-aqueous titrations and complete bibliographies on the subject have been published by Riddick[1,2]. This review will treat of the non-aqueous titration of Brönsted acids and bases, a subject which has become of great importance in the analysis of pharmaceutical preparations.

The Brönsted acids and bases are protolytes, proton donors and proton acceptors respectively. An acid A and its corresponding base B are related by the formula:—

$$A \rightleftharpoons B + H^+$$

The strength of an acid or a base is indicated by the K_a value of the dissociation constant of the acid in aqueous solution.

In the titration of an acid A_1 with a base B_2 or *vice versa* a solvent should be used, where the equilibrium

$$A_1 + B_2 \rightleftharpoons B_1 + A_2$$

lies so far to the right, that a large change in the hydrogen ion potential takes place at the end point. The equilibrium constant in a given solution depends on the K_a values and the electrical charge of the protolytes, the ionic strength and the dielectric properties of the solution. The protolytic properties of the solvent have a levelling effect on the strength of dissolved protolytes. Thus perchloric acid and the weaker hydrochloric acid have the same acid strength in aqueous solution because both react completely with the solvent, giving rise to the weaker acid H_30^+. In the same way diethylaniline and the stronger base guanidine in acetic acid solution are of the same strength, both being protolysed to give the base CH_3COO^-. From a practical point of view, the choice of solvent is influenced by the requirements that it should not be too dangerous or too disagreeable to handle, that the solubility of the titrated samples should not be too low, that suitable electrodes or colour indicators should be available for indicating the end-point and that stable titrant solutions can be prepared.

TITRATION OF BASES

The non-aqueous titration of weak bases with perchloric acid is the part of non-aqueous titrimetry that has been until now, most thoroughly dealt with. The method permits a rapid determination of different types of compounds common in the pharmacy of today: amines and heterocyclic nitrogen compounds, amino-acids, alkali and organic salts of weak acids and of hydrogen halides.

Acetic acid is the solvent most commonly used in the non-aqueous titration of bases. The fundamental work on the method was carried out

by Conant, Hall and Werner[3,4,5,6,7] in the years 1927 to 1930. Some years later Kolthoff and Willman[8,9] published important results from studies of the acid strength of different cations in acetic acid.

Titration with acetous perchloric acid in different organic solvents was studied by Fritz[10]. In recent years Pifer and Wollish[11,12,13] have recommended a solution of perchloric acid in *p*-dioxane as being more generally useful than the solution in acetic acid. The levelling effect of acetic acid on the strength of bases can be overcome by titration in aprotic solvents. Fritz[14] performed differential titration of bases of different strength in acetonitrile solution with perchloric acid in dioxane as the titrant.

Spengler and Kaelin[15] applied the acetous perchloric acid titration to many pharmaceutical problems, and gave titration curves for many pharmaceutical compounds. The preparation of the reagents and the role of acetic anhydride were discussed. The application of the method to pharmaceutical preparations has also been discussed by Auerbach[16] and in this journal by Beckett, Camp and Martin[17].

The method is inserted in the Collection of monographs of this laboratory[18]. Details of the technique used have been published[19,20,21].

Reagents

The acetic acid used as solvent in the titration should have a water content not exceeding 0·1 to 0·2 per cent. The perchloric acid solution (usually 0·1 N) is prepared from 70 per cent. aqueous solution, which is diluted with acetic acid. Acetic anhydride is added *after the dilution* to eliminate the water. Excess of acetic anhydride should be avoided if easily acetylated bases are to be titrated.

For back titration Spengler and Kaelin[15] used a 0·1 N sodium acetate solution prepared by dissolving standard sodium carbonate in acetic acid. This solution was also used for the standardisation of the perchloric acid solution. Due to the acidic property of the sodium ion in acetic acid, sodium acetate is a weaker base than the strong organic bases in this solvent. A strong, organic base, not sensitive to acetic anhydride, is recommended for the standard base solution. Guanidine[22] and triethylamine[19] have been used. The former is more easy to handle. It can be used in the form of carbonate or acetate.

Seaman and Allen[23] introduced potassium hydrogen phthalate for standardisation of the acetous perchloric acid. The low ionic strength during the titration, due to the precipitation of potassium perchlorate, gives an extremely sharp end-point.

The standard base solution is best standardised against the perchloric acid. The standard solutions in acetic acid are stable for a long time.

Though many acid-base indicators give colour changes in acetic acid, crystal violet, introduced by the pioneers of the method[6], is still the most commonly used. α-Naphthol-benzein and benzoylauramin were found to have no advantage over this indicator[22]. Crystal violet gives a series of colour changes around the "neutral point" of the acetic acid system and some practice is required if good results are to be obtained with this indicator. In the titration of potassium salts, the end-point is indicated by

the first change from violet to blue, in the titration of strong organic bases the change is from blue to green and in the titration of weaker bases the change is from bluish green to greenish yellow.

A Ciba-dye, Blue BZL, which has a colour change from blue to red at the "neutral point" has been successfully used in our laboratory in the titration of nikethamide and stronger bases. For weaker bases neutral red[19] (colour change red—blue) is recommended. For potentiometric indication of the end-point chloranil[3] or hydroquinone[24] electrodes can be used in cases where the solutions do not contain acetic anhydride or other compounds, which react with the electrode. The glass electrode is most suitable. A calomel electrode or a silver—silver chloride electrode[10] can be used as reference electrode. Reproducible and stable potentials are best obtained with a calomel electrode, connected to the solution by the lithium chloride bridge used by Hall and Conant[3].

Applications

Organic bases: As shown by Hall[7], the basic strength of amines and heterocyclic nitrogen compounds in acetic acid is, in most cases, linear to their strength in aqueous solution. Organic bases with $K_a = 10^{-6}$ or less are strong bases, that is, they give completely ionised acetates in acetic acid solution. Bases with K_a values up to 10^{-3} often are strong enough to be titrated visually. Among the weak bases titratable in acetic acid can be mentioned phenazone and *iso*propylphenazone, nikethamide, nicotinamide and phenylcinchonic acid. Many bases, monovalent by titration in aqueous solution are divalent in acetic acid, for example quinine and procaine.

For the titration of tertiary amines in mixtures with primary and secondary amines, Blumrich and Bandel[25] used acetic anhydride. By boiling with acetic anhydride the primary and secondary amines are acetylated and lose their basic character, and the tertiary amines can be titrated. The method can be used on compounds containing a tertiary and a primary or secondary nitrogen. Examples of this are procaine and tetracaine. On account of the weaker basic properties of the aromatic amino groups in these compounds and the precipitation of diperchlorate during the titration, visual indication of the end-point is not sharp. After boiling with acetic anhydride, giving an acetylated aromatic amine, the strongly basic tertiary aliphatic nitrogen can be titrated in acetic acid. The same procedure has been used for titration of the pyridine nitrogen in isoniazid.

Amino-acids: The potentiometric and visual titration of amino-acids in acetic acid was described by Nadeau and Branchen[22]. The amino-acids are usually very slowly soluble in acetic acid. Heating can cause acetylation and decomposition[20]. Toennis and Callan[26] showed that the amino-acids could be dissolved in a small amount of formic acid and the solution diluted with acetic acid before titration. The final solutions should contain less than 2 per cent. of formic acid. The same procedure can be used for electrolytes, which are difficult to dissolve in acetic acid and sensitive to heat, for example thiamine hydrochloride.

Salts: The low dielectric constant for acetic acid favours the titration of anion bases. Whereas the weakly basic caffeine can hardly be titrated, anion bases of approximately the same strength, e.g., the picrate and the dihydrogen phosphate ion, are titrated as strong bases. Higuchi and Concha[27] titrated salts of such strong acids as nitric acid. Sulphates can be titrated to acid sulphates, but not to sulphuric acid. The halogen ions are too weakly basic to be titrated directly. As a great deal of the pharmaceutically useful organic bases are used as chlorides or bromides, a great widening of the field of non-aqueous acidimetry was made when Pifer, Wollish and Schmall[12] introduced mercuric acetate as a means of removing halogen ions in acetic acid solution. The mercuric acetate reacts with haloid ions giving mercuric halide and an equivalent amount of acetate ions. The mercuric acetate and halide have no basic properties in acetic acid.

In a recently published review article[28], the same authors mention that they have succeeded in titrating sulphates in acetic acid after removing the sulphate ions by heating with mercurous acetate. No details have yet been given.

Today, it is possible to titrate acidimetrically the salts of most of the commonly used acids, except perchloric and sulphonic acids, provided that the acid strength of the cation in acetic acid is not too great. The salts of organic bases, themselves not too weak to be titrated, can be assayed in this way. As to the inorganic cations, it is of special importance to have a rapid method for the assay of the alkali salts.

Titration of Acids

The pioneer in this field was Folin who as early as 1910 estimated fatty acids in non-aqueous solvents by titrating with sodium ethoxide[29]. La Mer and Downs seem to be the first to apply potentiometry to the determination of acids in aprotic systems[30]. In 1948 Moss, Elliot and Hall[31] published an important account of the determination of phenolic compounds in resins. They used sodium aminoethoxide in ethylenediamine as titrant and antimony-antimony electrodes as indicating system. Of fundamental importance to the further analytical development of the method has been the work of Fritz and co-workers who since 1950 have issued a series of papers concerning the non-aqueous titration of both weak acids[32,33,34,35,36], and bases. As titrant Fritz used alkali methoxide in benzene-methanol, while the solvent was varied according to the acid strength of the compound to be titrated.

Solvents

Selection of a proper solvent is essential in non-aqueous titrations. Especially important factors are the basicity and the dielectric properties of the solvent. Increased basicity of the solvent enhances the acidic properties of a dissolved acid and a low dielectric constant of the solvent depresses the ionisation and thereby augments the acid strength or base strength of dissolved protolytes[37].

The solvent power of such a solvent may, however, be poor and the addition of a more polar medium is required. There is evidence that such

mixed solvents (e.g., benzene-methanol) are superior to pure solvents in solubilising effect and sharpness of the indicator change or the potential break at the equivalence point. No solvent with such general applicability for weak acids as glacial acetic acid has for weak bases, has yet appeared. A widely used mixed type solvent is benzene-methanol introduced by Fritz[32]. Dimethylformamide has shown itself to be a very useful solvent[32,34,36], among its advantages is the freedom from odour, and it does not absorb carbon dioxide as eagerly as the more basic solvents. A drawback however is the risk of hydrolysis of the amide linkage, especially in the presence of water, with subsequent liberation of acid. For the most weakly acidic compounds butylamine, pyridine and ethylenediamine seem to be the most suitable solvents.

Titrants

Several alkaline titrants have been used in non-aqueous solvents: alkali hydroxides, alkoxides[1,32], aminoalkoxides[31] and amides[38]; as well as quaternary ammonium bases and—for extremely weak acids—triphenylmethyl sodium[39]. Although none has gained absolute dominance like acetous perchloric acid, sodium methoxide in benzene-methanol has found a rather wide application. There are some interferences however. Water behaves like a weak acid and should be absent. Likewise the need for protection against carbon dioxide is obvious. There is also some risk of atmospheric oxidation of alcoholic alkoxide solutions to acidic compounds[40]. An inert atmosphere in the storage bottle and in the titration vessel tends to minimise the influence of these last mentioned factors. In some instances lithium methoxide may be preferable because of its greater solubility in organic solvents and in other cases the more strongly basic potassium analogue may offer some advantages[28].

Indicator systems

Some acid-base indicators known from aqueous titrimetry have been used with success in non-aqueous media. Here may be mentioned thymol blue, thymolphthalein and phenolphthalein and also azo violet (*p*-nitrobenzene azoresorcinol) and *o*-nitroaniline. Among these thymol blue especially has found a wide acceptance. The colour change is from yellow through green to blue. By comparison with potentiometric determinations the exact colour shade at the equivalence point may be established.

For that purpose several electrode couples have been used, e.g., glass-calomel[35], antimony-antimony[31] antimony-glass, antimony-calomel[32]. In some instances such systems operate successfully but often the electrode response is slow and unstable. Electrode systems in non-aqueous media have been investigated at the National Bureau of Standards[41,42,43], but much work remains to be done in this field. Until now there has appeared no electrode system that functions satisfactorily in solvents of low dielectric constant.

Most of the methods published have employed an alkali methoxide titrant. The procedure used in this laboratory (which follows Fritz

closely) for the determination of barbiturates and other weak acids may serve as a guide to the technique.

Reagents

0·1 M Sodium methoxide

Dissolve 3 g. of freshly cut sodium metal in 50 ml. of dry methanol protecting the vessel from carbon dioxide, add 100 ml. of methanol and then 750 ml. of benzene. The solution is stored in alkali resistant glass and is protected from carbon dioxide.

Thymol blue

0·3 per cent. solution in methanol.

Procedure

To 10 to 20 ml. of a suitable solvent add 2 drops of thymol blue and bubble nitrogen gas through for 10 minutes. Then titrate to a blue colour with the methoxide. Add the sample corresponding to about 1 m-equiv. and titrate to the same colour change. During the titration nitrogen gas is led into the solution whereby the latter is both stirred and protected from carbon dioxide and moisture. The titrant is standardised in the same manner against benzoic acid. The titre is rather sensitive to temperature on account of the high thermal expansion of benzene-methanol and, therefore, frequent restandardisation is advisable.

With this technique it is possible to determine a great many substances of importance in pharmacy. Carboxylic acids and other moderately acidic compounds may be titrated in neutral or basic solvents but the sharpest end-points are obtained in mixed solvents such as benzene-methanol. Thymol blue is a good indicator[31].

In "sulpha" drugs the amide hydrogen is sufficiently acidic to permit of ready titration in dimethylformamide with thymol blue as the indicator[34,36]. The same technique applies to the barbiturates[36] but butylamine and pyridine[44] are also good solvents.

The acidic moiety of ammonium salts and salts of aliphatic amines is amenable to titration in dimethylformamide or ethylenediamine (thymol blue or azo violet)[33]. Likewise negatively substituted phenols possess sufficient acidic character to be titrated in dimethylformamide, azo violet being a suitable indicator. Phenol and alkyl phenols are weaker acids and require a more basic solvent such as ethylenediamine (*o*-nitroaniline)[32,35].

Numerous other compounds of very slight acidity such as enols, mercaptans and imides may be titrated in butylamine or ethylenediamine[32]. In all these cases the water content of the system should be kept at a minimum because of the acidic properties of water. Besides, as the solvents often contain acidic contaminants, it is a good rule always to neutralise the solvent prior to the titration of the sample.

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