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Theoretical problems associated with the use of acetic anhydride as a co-solvent for the non-aqueous titration of hydrohalides of organic bases and quaternary ammonium salts

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ABSTRACT

A potentiometric titration study of organic base hydrohalides and quaternary ammonium salts using perchloric acid as the titrant and a mixture of acetic anhydride and acetic acid as the solvent was carried out and the titration mixture was analysed by NMR in order to clarify the chemistry of the reactions involved. It was found that in contrast to the general belief the formation of acetyl halides and titratable free acetate ion does not take place prior to the titration but NMR spectra proved the formation of acetyl halides in the course of the titration. This observation and the fact that the shape of the titration curves depends on the nature of the hydrohaloic acid bound to the base or of the anion in the quaternary ammonium salts led to the conclusion that the titrating agent is acetyl perchlorate formed *in situ* during the titration. Equations of the reactions involved in the titration process are shown in the paper.

1. Introduction

Although due to their non-selectivity, the importance of titrimetric methods for the assay of bulk drug materials has decreased considerably [1,2], these classical methods are still and will certainly remain for a long time standard parts in their analytical protocols. The share of titrimetry as assay method for bulk drug materials (small molecules) in the European Pharmacopoeia (Ph. Eur. 6) [3] and the United States Pharmacopoeia (USP 32) [4] is about 70% and 40%, respectively.

The introduction and rapid propagation of non-aqueous titrations at the middle of the last century have greatly enhanced the applicability of the volumetric method by enabling weak acids and bases not measurable in aqueous media to be determined [5–8]. Especially the titration of weak basic drugs with perchloric acid in glacial acetic acid medium is widely used. This is applicable to weak bases with pK_a values above 3. This limit can be further decreased by 1–2 units (p$K_a \sim 0.5$–1) in the acetic anhydride/acetic acid mixture enabling as weak bases as caffeine and other xanthenes to be measured. If pure acetic anhydride is used as the solvent even certain carboxamides can be titrated [9].

Another important feature of acetic anhydride as a co-solvent (or reagent) for the titration of pharmaceutical compounds is that it enables the direct non-aqueous titration of halide salts (mainly hydrochlorides) of organic bases and quaternary ammonium salt-type drugs. A widely used former alternative for this is the method where mercury(II) acetate reagent is added to the solution in order to form stable mercury(II) halide complex and free acetate ion (equivalent to the base) which can be titrated with perchloric acid [10,11]. However, due to the toxicity of the reagent, the application of this method shows decreasing tendency. Another alternative for the assay of halide salts of alkaloids and other organic N-bases is alkalimetry in alcohol–water mixtures with potentiometric end-point detection. Here the hydrohaloic acids bound to the bases are titrated by sodium hydroxide. Despite its limitations [12] this is becoming the preferred method of the European Pharmacopoeia [3]. The importance of acetic anhydride as the solvent for the direct titration of pharmaceuticals can be characterised by the following figures: it is prescribed in 33 and 11 monographs in Ph. Eur. 6 and USP 32, respectively, for the measurement of very weak bases and in 70 and 23 cases, respectively, as the co-solvent for the assay of hydrohalides, mainly hydrochlorides.

There is an interesting controversy in the literature regarding the theoretical basis of the possibility of direct titration of base hydrohalides and quaternary ammonium-type drugs in the presence of acetic anhydride. In the majority of papers from the very early literature up to the present time [6,13–18] this is attributed to the reaction of the halogenide ion with acetic anhydride to form acetyl halides and titratable free acetate ion (see Eqs. (1) and (2)).

$$BH^+ X^- + (CH_3CO)_2O = CH_3COX + BH^+ + CH_3COO^- \quad (1)$$

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$$R_4N^+X^- + (CH_3CO)_2O = CH_3COX + R_4N^+ + CH_3COO^- \quad (2)$$

where BH^+ stands for the protonated base, R_4N^+ for quaternary ammonium cation and X^- for halogenide ion. This theory is referred here as "acetyl halide/acetate ion theory".

Another opinion is that a "replacement titration" takes place (see Eq. (3)) [19]:

$$BH^+X^- + H^+ClO_4^- = BH^+ + ClO_4^- + HX \quad (3)$$

It is noteworthy that both theories are hypotheses, based on speculations only: none of them has been supported by experimental evidence. The aim of this study is to settle this open question by creating firm theoretical basis based on experimental evidence for the mechanism of the titration of base hydrohalides and quaternary ammonium-type drugs in the mixture of acetic anhydride and acetic acid.

2. Materials and methods

2.1. Materials

Anhydrous acetic acid (Spektrum 3D, Hungary), acetic anhydride (Reanal, Hungary) and mercury(II) acetate (Sigma) were of analytical grade. D_2O (≥99.9 atom% *D*) was purchased from Sigma. The 0.1 M perchloric acid volumetric solution was prepared by diluting 8.5 ml of 70–73 wt% perchloric acid with 900 ml of anhydrous acetic acid and 30 ml of acetic anhydride and then diluting to 1000 ml with anhydrous acetic acid. Perchloric acid was standardized by titration against potassium hydrogen phthalate [3].

Lidocaine (base), lidocaine hydrochloride, ephedrine hydrochloride, papaverine hydrochloride, homatropine hydrobromide and homatropine methylbromide were of pharmacopoeial grade (Ph. Eur. 6.3) and supplied by Sigma or Aldrich. Lidocaine hydrobromide and hydroiodide were generous gifts from Prof. Ferenc Fülöp, Institute of Medicinal Chemistry, University of Szeged and were of analytical grade. The tetrabutylammonium salts (fluoride trihydrate, chloride, bromide, iodide and acetate) were of analytical grade and purchased from Fluka or Aldrich.

2.2. Apparatus

All the titrations were carried out using a TITRONIC titrator (SCHOTT Instruments, Germany). The pH (mV) was measured by PHM220 LAB pH-meter (Radiometer Analytical, France) fitted with a combined glass electrode (Radiometer Analytical, France). The titrations were performed under efficient stirring with a magnetic stirrer, at room temperature (25 ± 2 °C).

The ^1H NMR spectra were recorded using a 600 MHz Varian VNMRS spectrometer equipped with a dual 5 mm inverse-detection gradient (IDPFG) probe head. Standard pulse sequences and processing routines available in VnmrJ 2.2C/Chempack 4.0 were used. The probe temperature was maintained at 25 °C and standard 5 mm NMR tubes were used.

2.3. Non-aqueous titrations

The non-aqueous titrations were carried out (1) in anhydrous acetic acid, (2) in a mixture of anhydrous acetic acid and acetic anhydride 30:70 (v/v), corresponding to the average composition of the solvent mixtures in pharmacopoeial monographs, and (3) in anhydrous acetic acid in the presence of mercury(II) acetate. In each experiment, 50 ml of an 8–10 mM solution of sample was titrated with 0.1 M perchloric acid using potentiometric endpoint detection. Three parallel titrations were carried out for each substance.

2.4. ^1H NMR analysis

A 30:70 (v/v) mixture of anhydrous acetic acid–acetic anhydride was used as solvent. To lock the spectrometer frequency D_2O was used in an insert tube. Chemical shifts were referenced to the residual HDO signal (4.63 ppm). In the course of the NMR follow-up of the titrations the large methyl signals of the solvents were suppressed using the wet pulse sequence. ^1H spectra were registered acquiring 32 scans. The concentration of papaverine hydrochloride was 8 mM.

3. Results and discussion

Lidocaine as a tertiary amine-type drug material was selected as the model compound for this study. Fig. 1a shows the titra-

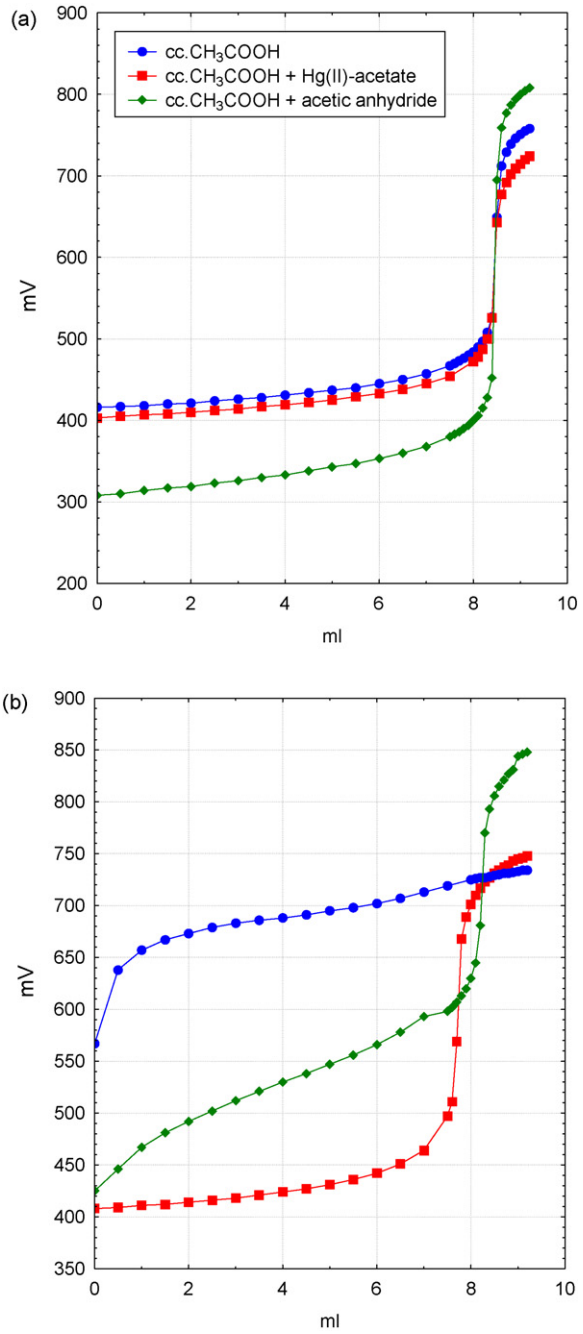

Fig. 1. Potentiometric titration curves of lidocaine base (a) and lidocaine hydrochloride (b) with 0.1 M $HClO_4$ in different solvents.

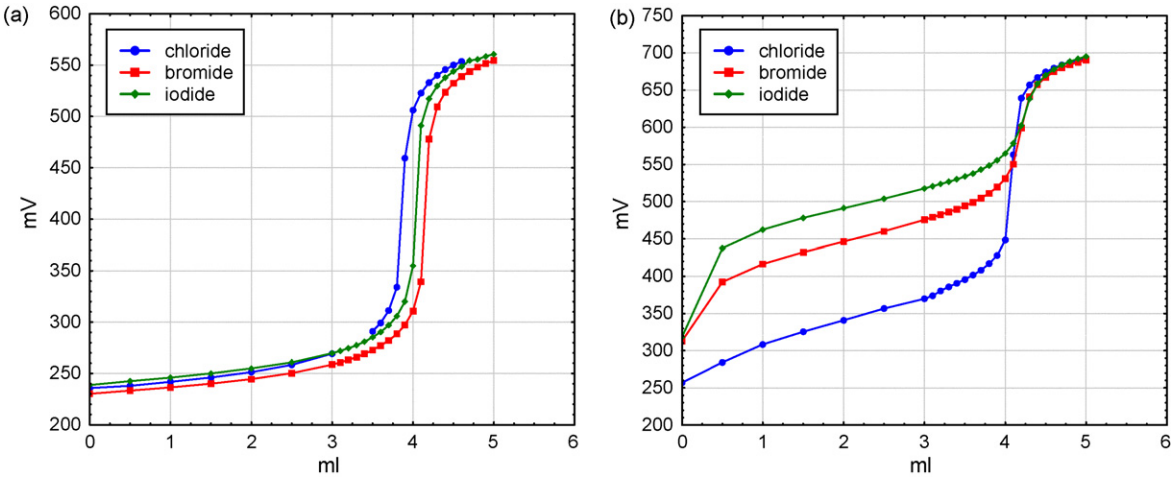

Fig. 2. Potentiometric titration curves of lidocaine hydrohalides with 0.1 M $HClO_4$ in anhydrous acetic acid in the presence of mercury(II) acetate (a) and in a mixture of anhydrous acetic acid and acetic anhydride 30:70 (v/v) (b).

tion curves of lidocaine base. As expectable, as a medium strong base ($pK_a = 7.95$) it can be titrated with perchloric acid in glacial acetic acid with sufficiently sharp endpoint and the shape of titration curve is not influenced by the presence of mercury(II) acetate. The endpoint is much sharper if acetic anhydride is added to the solution. In Fig. 1b the titration curves of lidocaine hydrochloride are shown under the same conditions. As foreseeable, no endpoint can be detected in glacial acetic acid while sufficient sharp endpoint is obtained in the presence of mercury(II) acetate and also in its absence when acetic anhydride is used as co-solvent. In Fig. 2a the titration curves of three different salts (hydrochloride, hydrobromide, hydroiodide) of lidocaine are presented in glacial acetic acid in the presence of mercury(II) acetate. Since in this case the forming free acetate ion is titrated, the shape of the titration curves is independent of the nature of the salt-forming hydrohaloic acid.

Fig. 2b where titration curves of the same salts in the acetic anhydride/acetic acid mixture are depicted plays decisive role in bringing the question of "acetyl halide/acetate ion theory" or "replacement titration theory" to an issue. On the basis of Eqs. (1) and (2) it is evident that in the case of the validity of the "acetyl halide/acetate ion theory" the acetate ion would be titrated in all cases. This means that the shape of the titration curves and the height of the potential jumps at the endpoint would not depend on the nature of the acid bound to the base. As it is seen, this is not the case: the shape of the titration curves depends on the nature of the hydrohaloic acid. The height of the potential jump and thus the sharpness of endpoint increases with the decreasing strength of the acids involved ($HClO_4 \gg HI > HBr > HCl$) furnishing evidence against the validity of the "acetyl halide/acetate ion theory" and seem to support the "replacement titration theory".

The same conclusion regarding quaternary ammonium salts can be drawn from Fig. 3, where the titrations of various tetrabutylammonium salts are shown. Here as a matter of fact the anions are titrated, and the potential jump at the endpoint increases with increasing basicity of the anions in the presence of acetic anhydride (perchlorate $\ll$ iodide < bromide < chloride < fluoride, acetate). This means that the "acetyl halide/acetate ion theory" is not valid in this case either. It has to be mentioned that Streuli [20] came to the same conclusion when titrating alkali halides in pure acetic anhydride but this study was not extended to pharmaceutical compounds.

Fig. 4a shows some practical applications: titration curves of the hydrochlorides of papaverine, lidocaine and ephedrine in the mixture of acetic acid and acetic anhydride with sharp endpoints. The shape of the curves is similar despite the diversity of the pK_a values of the three bases (6.47, 7.95 and 9.56, respectively). (We have to note that here the secondary amine ephedrine. HCl can be titrated because no time was allowed for acylation.) Similarly well evaluable curves were obtained with hydrobromides and quaternary ammonium bromides such as lidocaine hydrobromide, homatropine hydrobromide and homatropine methylbromide (Fig. 4b).

The above potentiometric experiments support the theory that the acetic anhydride/acetic acid solvent is not simply a nivellating solvent (as assumed by the "acetyl halide/acetate ion theory") but this solvent mixture increases the basicity of the bases to be titrated (halogenide ions in our case) in different extent. This can be due to its enhanced acidity caused by the formation of an H-bonded associate between one molecule of acetic anhydride and two molecules of acetic acids, the species called "supramolecule of superacid character" by Buvári-Barcza [18].

After having successfully disproved the "acetyl halide/acetate ion theory" the mechanism of the reactions involved in the titration was further investigated by NMR spectroscopic analysis of the titration mixture. Fig. 5 shows the ^1H NMR spectra of the pure solvent mixture (Fig. 5a), the same in the presence of 8 mM acetyl chloride (Fig. 5b) and in the presence of 8 mM papaver-

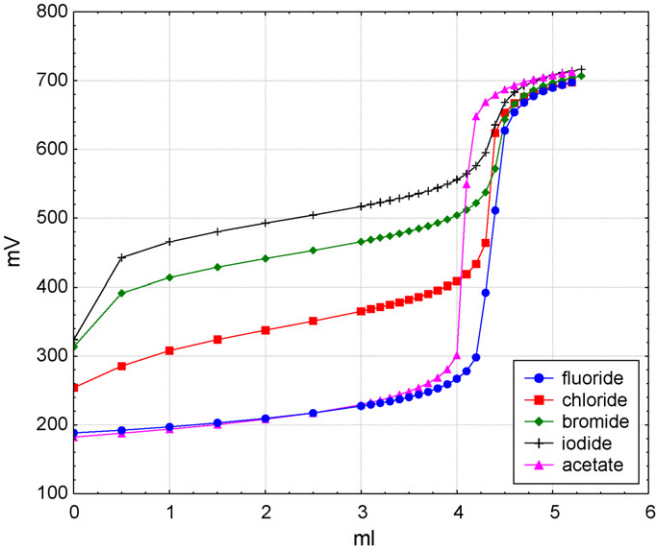

Fig. 3. Potentiometric titration curves of tetrabutylammonium salts with 0.1 M $HClO_4$ in a mixture of anhydrous acetic acid and acetic anhydride 30:70 (v/v).

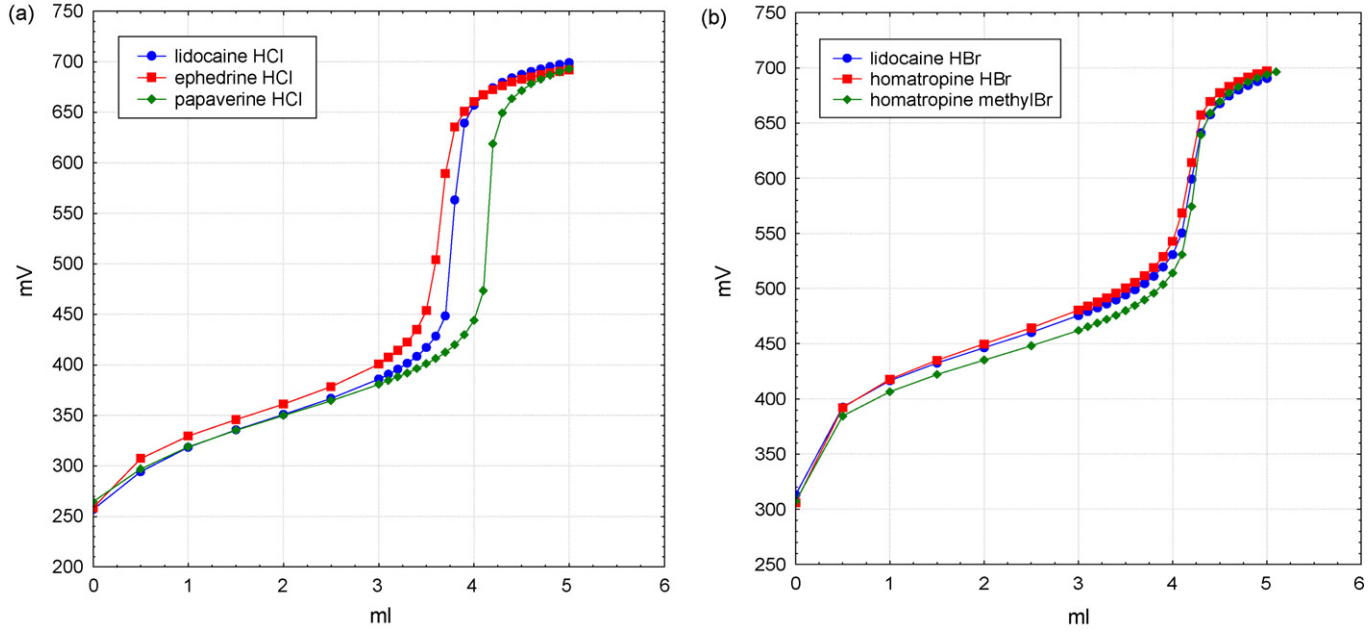

Fig. 4. Potentiometric titration curves with 0.1 M $HClO_4$ in a mixture of anhydrous acetic acid and acetic anhydride 30:70 (v/v); base hydrochlorides (a) and base hydrobromides and a quaternary ammonium bromide (b).

ine hydrochloride (Fig. 5b). As Fig. 5b clearly shows, the methyl signal of acetyl chloride at 3.18 ppm is well separated from the methyl signals of the solvent although no solvent suppression was applied. Acetyl chloride was detectable only at trace level in the 8 mM papaverine hydrochloride solution furnishing further evidence against the "acetyl halide/acetate ion theory". Then the titration of 8 mM papaverine hydrochloride with perchloric acid was followed by scanning ^{1}H NMR spectra after the addition of 0, 0.25, 0.50, 0.75 and 1.0 equiv. of perchloric acid. The spectra in Fig. 6 unambiguously prove the formation of acetyl chloride in the course of the titration process. Its sharp singelet at 3.18 ppm is practically missing in the solution of papaverine hydrochloride dissolved in acetic anhydride/acetic acid mixture but appears when perchloric acid is added to the solution. The quantity of the formed acetyl chloride is stoichiometric to the titrant added.

On the basis of the potentiometric titration curves and the NMR spectra it can be stated that neither the "acetyl halide/acetate ion theory" nor the "replacement titration theory" is valid. The processes taking place during the titration can be interpreted by means of the following equations. Acetyl perchlorate ($CH_3CO^+ \ldots .ClO_4^-$) is instantaneously formed when perchloric acid is added to the solvent mixture (Eq. (4)). In fact this is the titrant which reacts with the base in the course of the titration (Eq. (5)):

$$CH_3CO{-}O{-}COCH_3 + H^+ClO_4^- \rightleftharpoons CH_3CO^+ \ldots .ClO_4^- + CH_3COOH \quad (4)$$

$$BH^+ \ldots .X^- + CH_3CO^+ \ldots .ClO_4^- \rightleftharpoons BH^+ \ldots .ClO_4^- + CH_3COX \quad (5)$$

Both equilibria are shifted in the direction of the upper arrow. The charged species are not dissociated but exist as ion pairs in the low

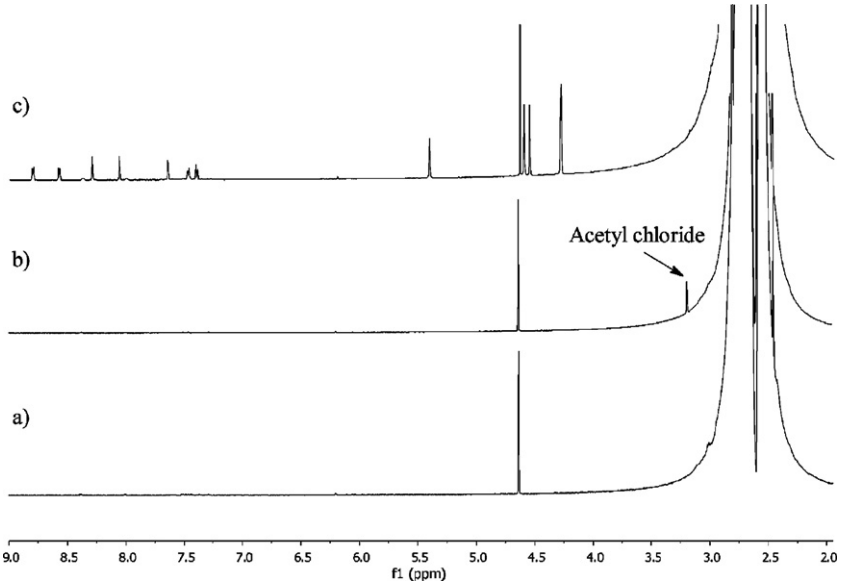

Fig. 5. ^{1}H NMR spectra of acetic anhydride/acetic acid 70:30 (v/v) (a); in the presence of 8 mM acetyl chloride (b); in the presence of 8 mM papaverine hydrochloride (c).

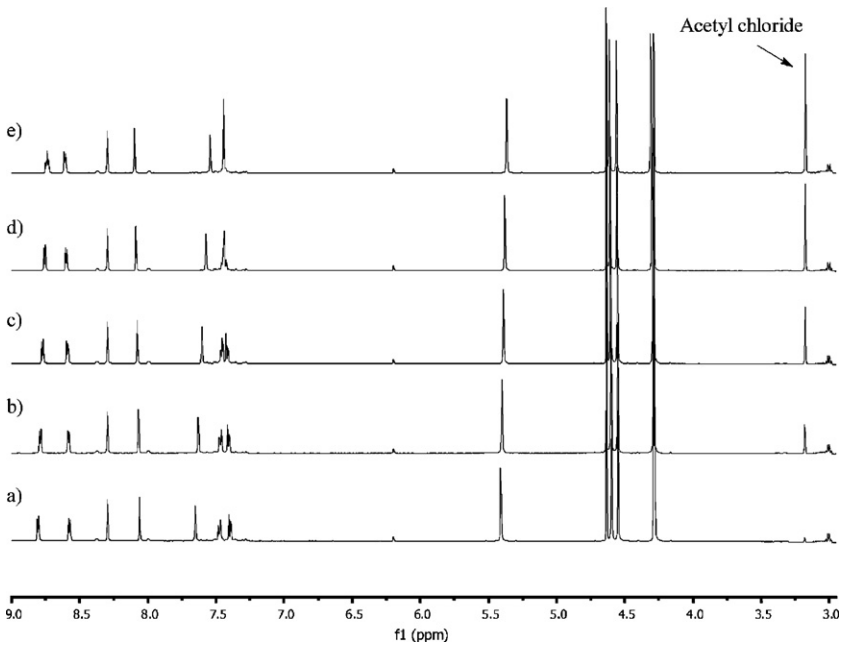

Fig. 6. ^{1}H NMR spectral series of the titration of 8 mM papaverine hydrochloride with 0.1 M $HClO_4$ (a: 0 equiv.; b: 0.25 equiv.; c: 0.50 equiv.; d: 0.75 equiv.; e: 1.0 equiv. $HClO_4$ added).

dielectric constant solvent system. The shape of the potentiometric titration curves is determined by the nucleophilicity of X^- in this medium.

When very weak bases (such as e.g. caffeine) are titrated, an analogous reaction is likely to take place (Eq. (6)):

$$B + CH_3CO^+ \ldots . ClO_4^- + CH_3COOH \rightleftharpoons BH^+ \ldots . ClO_4^- + CH_3CO{-}O{-}COCH_3 \quad (6)$$

4. Conclusions

With this study a misconception, one of the "Myths and legends in analytical chemistry" in pharmaceutical analysis has been cleared up. (This title refers to a lecture held by Professor Ronald Belcher, one of the leading personalities of analytical chemistry at the middle of the last century [21].). The widespread "acetyl halide/acetate ion theory" is originated probably from a misinterpretation of an early paper of Schmidt et al. [22]. In this non-analytical paper the authors describe the reaction between $SiCl_4$, $GeCl_4$ and $SnCl_4$ and acetic anhydride leading to tetraacetates of the semimetals and acetyl chloride. In an unjustified and unfounded manner analogous reactions were later attributed to alkali halogenides, moreover quaternary ammonium halogenides and ammonium hydrohalides and stated that the concomitantly forming free acetate ion is titrated. This is refuted by our potentiometric experiments. On the other hand the "replacement theory" does not take into account the formation of acetyl halides in the course of the titration what was proved here with the NMR study and this theory does not explain the role of the acetic anhydride in the titration either.

In this paper we propose a reasonable interpretation of non-aqueous titration of halide salts of organic bases and quaternary amines and also of very weak free bases in the mixture of acetic anhydride and acetic acid (Eqs. (4)–(6)). Our findings are in agreement with statements in earlier papers [6–7,16–18] about the behaviour of acetic anhydride which has been characterized as an aprotic and very slightly protophilic solvent that is able to dissociate to acetyl and acetate ions. By shifting this equilibrium with adding perchloric acid to the solvent, *in situ* formation of acetyl perchlorate takes place which is as a matter of fact the titrating agent of very weak bases as well as of amine hydrohalides and quaternary ammonium halides.

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