

Effect of water content in perchloric acid on the non-aqueous potentiometric titration of nitrogen-containing compounds¹

Xiaohe S. Qi *, R. Brent Miller, Yoshihiro Namiki, John Zhang, Robert Jacobus

Pharmaceutical Sciences Department, Fujisawa USA, Inc., 2045 N. Cornell Ave., Melrose Park, IL 60160, USA

Received 25 July 1996; received in revised form 1 March 1997

Abstract

In the United States Pharmacopeia (USP), 0.1 N perchloric acid in acetic acid volumetric solution (hereafter HClO₄ VS) used for non-aqueous titration has specified a water content between 0.02 and 0.05%. Preparing this titrant with such a narrow range of water content is very time consuming, precludes the use of commercially available titrants, and, consequently, prompted an investigation to try and expand the range up to 0.5%. In this study, the titrimetric results obtained using HClO₄ VS containing more water were very close to those obtained using the USP specified titrants. A maximum assay difference of 0.7% in the titrations of three selected nitrogen-containing compounds, clonidine hydrochloride, dipyridamole, and adenosine were observed. The titrimetric results obtained using these titrants were also precise with RSDs of not more than 0.4%. Therefore, a wider range of water content in HClO₄ VS between 0.02 and 0.5% is suggested for the USP potentiometric titration of nitrogen-containing compounds. © 1997 Elsevier Science B.V.

Keywords: Perchloric acid; Non-aqueous potentiometric titration; Nitrogen-containing compounds

1. Introduction

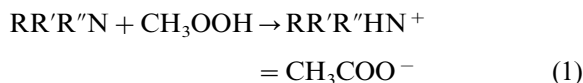
Potentiometric titrations have become well accepted for the titration of many organic and inorganic compounds containing acidic or basic moieties. The advantage of this approach includes its simplicity, good accuracy and precision, and low operating cost. Unlike chromatographic methods, drug substances can be directly titrated without the use of a reference standard. Conse-

quently, titrimetric assay methods have been specified by the USP for many drug substances [1].

Many nitrogen-containing heterocyclic compounds, amines, oxazolin, and quaternary ammonium compounds are important drug substances and can be determined titrimetrically. However, titration of these weak bases in aqueous solution will be neither accurate nor precise because the endpoints generated from weak bases are not significant. The apparent strength of these weak bases can be enhanced if these titrates are dissolved in a weak acid solution such as glacial acetic acid, which results in the formation of acetate, a stronger base.

* Corresponding author.

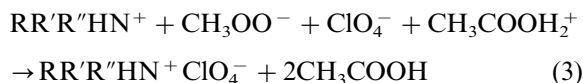
¹ This work was presented, in part, at the Pittsburgh Conference, Chicago, IL, 1996.



Whereas, by using HClO_4 VS titrant, a stronger acid, acetate acidium ($\text{CH}_3\text{COOH}_2^+$), is generated.



The titration of a weak nitrogen-containing base with HClO_4 VS actually becomes the neutralization of acetate acidium and acetate, which is a strong acid-strong base titration, as indicated in Eq. (3):



This results in a significant endpoint, thereby improving the accuracy and precision.

Acetic anhydride contained in acetic acid may interfere by reacting with some easily acetyltable amines to generate corresponding acetylated products, which do not consume acid. Second, too much water in the system including the sample and/or titrant solutions may affect the sharpness of the endpoint of the titration [2]. Third, conductive halide acids (HCl , HBr , or HI) in some amine salts interfere with the electrometric endpoint. To eliminate halide acids, the USP procedure requires the addition of excessive mercuric acetate into the sample solution, thus, producing a mercuric halide that does not interfere with the titration [2]. Generally, an excess of water is added to eliminate anhydride, however, too much water interferes with the endpoint. Since water content is an important factor in the non-aqueous titration system, a suitable amount of water is needed in both the titrate solution and the HClO_4 VS titrant.

The limit of water in the titrate solution has been studied [2,3]. As suggested by Pifer and Wollish [2] in 1952, water content up to 1% could be used for the potentiometric titration of asterol dihydrochloride. Recently, Tsunakawa and Tamura [3] studied 33 drug substances including many USP pharmaceutical substances such as amitriptyline hydrochloride, cyproheptadine hydrochloride, naphazolin hydrochloride, etc. and

found that these titrate solutions containing water content up to 0.5% could be employed due to no obvious interference. The USP procedure does not specify a requirement for water content in the titrate solution, however, it specifies the HClO_4 VS titrant to contain a water content between 0.02 and 0.05% [4]. Similarly, the British Pharmacopoeia (BP) [5] and the Pharmacopoeia of Japan (JP) [6] set limits between 0.1 and 0.2% and less than 0.03%, respectively for water content (Fig. 1). Like the USP, both the BP and JP do not specify a water content in the titrate solution.

The preparation of HClO_4 VS, as per the USP, requires at least two days. First acetic anhydride is mixed with perchloric acid in glacial acetic acid. The mixture is allowed to stand for 1 day to aid in the elimination of water. Then, a sufficient amount of water is added into the mixture and allowed to stand for another day to eliminate the excessive anhydride. The water content is subsequently measured. If the water content is not between 0.02 and 0.05%, then additional water and/or acetic anhydride is added and, again, allowed to stand for another day. The narrow range of water content in HClO_4 VS with the time-consuming adjustment procedure, resulted in our laboratory exploring the possibility of expanding the range of water content up to 0.5%. In doing so, commercially available HClO_4 VS containing about 0.1–0.2% water could be used (Fig. 1). Since it has been proven that water content up to 0.5–1% in the titrate solution does not interfere with the endpoint, this amount of water in HClO_4 VS titrant, which is considered one part of the whole titration system, should not interfere either. Therefore, the effect of water content up to 0.5% in HClO_4 VS was investigated for three nitrogen-containing compounds including clonidine hy-

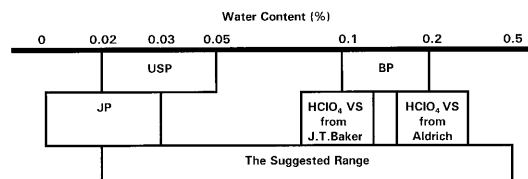


Fig. 1. Water contents (%) in HClO_4 VS.

drochloride, dipyridamole, and adenosine to determine if these commercially available HClO_4 VS's can be employed in the USP potentiometric titration without any treatment.

2. Experimental

2.1. Materials and equipment

HClO_4 VS (0.1 N in glacial acetic acid) was purchased from both J.T. Baker (Phillipsburg, NJ, USA) and Aldrich, (Milwaukee, WI, USA), while Hydranal-Composite 2 (Karl Fisher reagent) was purchased from Crescent, (Hauppauge, NY, USA). Clonidine hydrochloride and dipyridamole reference standards were purchased from the United State Pharmacopeial Convention, (Rockville, MD, USA), while adenosine was purchased from Kyowa Hakko Kogyo, (Tokyo, Japan). All other chemicals were purchased from Mallinckrodt, (Paris, KY, USA) and met ACS requirements. The potentiometric titration and water determination (Karl Fischer reaction) were carried out with a Metrohm 682 autotitrator (Herisall, Switzerland). A Metrohm calomel reference electrode, modified with 0.1 N lithium perchlorate in glacial acetic acid, and a Metrohm glass indicator electrode were used for the clonidine hydrochloride titration. A Metrohm Glass/Silver Chloride combination electrode was used for the dipyridamole and adenosine titrations.

2.2. Preparation of solutions

2.2.1. HClO_4 VS (< 0.02% water)

HClO_4 VS containing less than 0.02% water was prepared by adding 20 ml acetic anhydride per 1000 ml of J.T. Baker HClO_4 VS and allowed to stand for 1 day.

2.2.2. HClO_4 VS (0.02–0.05% water)

HClO_4 VS containing 0.02–0.05% water was prepared by adding a suitable amount of water into the HClO_4 VS (< 0.02%) and allowed to stand for 1 day. Additional water or acetic anhydride was added as necessary and allowed to

stand for 1 day to obtain the desired water content throughout the range of 0.02–0.5%.

2.2.3. HClO_4 VS (0.05–1% water)

HClO_4 VS containing 0.05–1% of water was prepared by adding a suitable amount of water into J.T. Baker or Aldrich Perchloric acid and allowed to stand for 1 day.

2.2.4. 0.1 N Mercuric acetate solution

Dissolve 6.0 g of mercuric acetate per 100 ml glacial acetic acid.

2.3. Determination of loss on drying of nitrogen-containing compounds

The USP loss on drying method [7] was used for determining the amount of volatile matter in clonidine hydrochloride, dipyridamole, and adenosine.

2.4. Determination of water content in HClO_4 VS

USP Method Ia (Karl Fisher Volumetric Titration) was used for the determination of water content [8]. About 20–30 mg of water was used for the standardization of the Karl Fischer Reagent, which was employed to determine water content. Three replicate measurements for each HClO_4 VS were conducted on about 10–26 g of HClO_4 VS samples depending on their level of water content and the average was reported as the final result.

2.5. Potentiometric titration of nitrogen-containing compounds

The USP titrimetric method [1] was applied to the potentiometric titration of clonidine hydrochloride, dipyridamole, and adenosine with HClO_4 VS titrants. A glass electrode and a calomel reference electrode were used for the clonidine hydrochloride titration. The standardization of HClO_4 VS was performed on about 700 mg of accurately weighed potassium biphthalate, which had been dried at 105°C for 4 h, dissolved in 50 ml of glacial acetic acid and titrated to the

Table 1
Potentiometric assay results of clonidine hydrochloride

Desired H ₂ O level (%)	Actual H ₂ O level (%)	HClO ₄ VS (N)	Trial (No.)	Assay ^a (%)	Average ^a (%)	RSD	Assay difference
<0.02	0.004	0.09682	1	99.8	99.7	0.1	0.2
			2	99.8			
			3	99.6			
0.02–0.05 ^b	0.047	0.09864	1	99.9	99.9	0.1	— ^c
			2	99.8			
			3	99.9			
0.06–0.15	0.13	0.09884	1	99.8	99.7	0.1	0.2
			2	99.6			
			3	99.8			
0.2–0.3	0.21	0.09901	1	99.8	100.1	0.3	0.2
			2	100.3			
			3	100.3			
0.4–0.6	0.50	0.09951	1	99.5	99.7	0.2	0.2
			2	99.5			
			3	99.8			
0.8–1.2	1.01	0.09979	1	99.3	99.1	0.3	0.6
			2	98.7			
			3	99.3			

^a On anhydrous basis.

^b The USP specified range.

^c This is the reference.

endpoint. The potentiometric standardization of each HClO₄ VS was conducted on three independent potassium biphthalate standardization reagents and the average was used as the titrant concentration. About 200 mg of clonidine hydrochloride was dissolved in 80 ml of glacial acetic acid, mixed with 15 ml 0.1 N mercuric acetate solution, then titrated with the standardized HClO₄ VS. The potentiometric assay employing HClO₄ VS titrant was conducted on three clonidine hydrochloride titrates. Dipyrindamole and adenosine were titrated in a similar manner except different solvents, as described below, and a glass/silver, silver chloride combination electrode were employed. About 450 mg of dipyrindamole was titrated in 50 ml of glacial acid mixed with 75 ml of acetone. Similarly, about 200 mg of adenosine was dissolved in 50 ml of glacial acetic acid and then titrated. Blank titrations were performed for each solvent in the same manner. The

final titrated volume was corrected by subtracting the blank volume. The assay results of each substance were calculated on the anhydrous basis, corrected by its loss on drying result.

3. Results and discussion

First, the interference of water over the range between 0.004 and 1.01% on clonidine hydrochloride titration was investigated. The choice of clonidine hydrochloride as our focus was due to its relatively complicated titrimetric system with potential interference from hydrochloric acid compared with the other two nitrogen-containing compounds, namely, dipyrindamole and adenosine.

HClO₄ VS containing six levels of water contents between 0.004 and 1% were tested and evaluated for their accuracy and precision of clonidine hydrochloride titrations. To compare the titrimetric results obtained from HClO₄ VS containing a

Table 2
Potentiometric assay results of dipyrnidamole and adenosine

Compounds	Actual H ₂ O level (%)	HClO ₄ VS (N)	Trial (No.)	Assay ^a (%)	Average ^a (%)	RSD (%)	Assay difference
Dipyrnidamole	0.045 ^b	0.0992	1	99.7	99.7	0.1	— ^c
			2	99.8			
			3	99.6			
	0.48	0.0993	1	100.3	100.1	0.4	0.4
			2	100.4			
			3	99.7			
Adenosine	0.045 ^b	0.0992	1	99.3	99.5	0.2	— ^c
			2	99.6			
			3	99.7			
	0.48	0.0993	1	100.1	100.2	0.1	0.7
			2	100.3			
			3	100.3			

^a On anhydrous basis.

^b Within the USP specified range.

^c This is the reference.

wider water content range than the USP, the assay difference was defined as follows:

$$\text{Assay Difference} = \frac{|\text{Assay}_{\text{standard}} - \text{Assay}_{\text{actual}}|}{\text{Assay}_{\text{standard}}} \times 100\%$$

Where $\text{Assay}_{\text{standard}}$ is the titrimetric result using the USP specified HClO₄ VS and $\text{Assay}_{\text{actual}}$ is the titrimetric result using other titrants. An assay difference of not more than 1% was considered acceptable. The titrimetric results are provided in Table 1.

It was found that assay values of clonidine hydrochloride obtained by potentiometric titration were not influenced by the water content in HClO₄ VS since all assay difference values were less than 1%. The resulting RSDs for all assays were not more than 0.3%. Therefore, the precision and accuracy of the titrimetric method are not affected by water content between 0.004 and 1.0%.

Although the experimental results indicated that the water content at the low end (0.004%) may still be usable, it would be not practical to make this titrant, since our objective is to use a commercial HClO₄ VS. Results for our laboratory indicated that commercial HClO₄ VS con-

tains about 0.1–0.2% water. Also, HClO₄ VS water content at the high end of 1% may not be practical, since the acid may not adsorb that much moisture from the atmosphere under normal storage. As a result, HClO₄ VS containing 0.5% water content (actually 0.48%) was examined for the potentiometric titration of dipyrnidamole and adenosine. The results are presented in Table 2. Titrimetric results were accurate with an assay difference of 0.4–0.7% for dipyrnidamole and adenosine, respectively. The RSDs obtained from these titrations were not more than 0.4%. Therefore, it can be concluded that like clonidine hydrochloride, 0.5% water content does not affect the dipyrnidamole or adenosine titrations. Although only three compounds were tested, this results can be extrapolated to other nitrogen-containing compounds, since the non-aqueous titration actually takes place as a neutralization of acetic acidium and acetate as indicated in Eq. (3) and nitrogen-containing compounds are not directly involved in the acid-based reaction. As a result, these commercially available HClO₄ VS's can be employed in the USP potentiometric titration without any treatment.

4. Conclusion

A potentiometric titration of nitrogen-containing weak bases is often specified by the USP as an assay method. However, preparation of HClO_4 VS titrants with the USP requirement of water content between 0.02 and 0.05% is time consuming. Comparing the titration results obtained by the USP specified HClO_4 VS containing 0.02–0.05% water with those containing up to 0.5% demonstrated that there was no interference of water content on the potentiometric assays of clonidine hydrochloride, dipyridamole, or adenosine. Furthermore, accuracy and precision within 0.7% can be achieved easily. Therefore, a wider limit for water content in HClO_4 VS, ranging from 0.02 and 0.5%, can be applied to the USP potentiometric titration of other nitrogen-containing organic compounds.

Acknowledgements

The authors would like to thank Mr Susumu Inada, Fujisawa Pharmaceutical Company (Japan) for his helpful discussions.

References

- [1] Chemical Tests and Assays, < 541 > Titrimetry, in: The United States Pharmacopeia, 23rd revision, The United States Pharmacopeial Convention, Rockville, MD, USA, 1995, pp. 1751–1754.
- [2] C.W. Pifer, E.G. Wollish, *Anal. Chem.* 24 (1952) 300–306.
- [3] N. Tsunakawa, B. Tamura, *Iyakuin Kenkyu* 18 (1987) 756–759.
- [4] Volumetric Solutions, Perchloric Acid, Tenth-Normal (0.1 N) (in Glacial Acetic Acid), in: The United States Pharmacopeia, 23rd revision, The United States Pharmacopeial Convention, Rockville, MD, USA 1995, p. 2060.
- [5] Volumetric Reagents and Solutions, Perchloric Acid VS, in *British Pharmacopoeia*, HMSO, London, UK, 1993, Vol II, p. A72.
- [6] Standard Solutions for Volumetric Analysis, Perchloric Acid, Tenth-Normal (0.1 N) in: *The Pharmacopoeia of Japan*, 12th ed., Yakuji Nippo, Tokyo, Japan, 1991, p. 127.
- [7] Physical Tests and Determinations, < 721 > Loss on Drying, in *The United States Pharmacopeia*, 23rd revision, The United States Pharmacopeial Convention, Rockville, MD, USA, 1995, p. 1801.
- [8] Physical Tests and Determination, < 921 > Water Determination, in: *The United States Pharmacopeia*, 23rd revision. The United States Pharmacopeial Convention, Rockville, MD, USA, 1995, pp. 1840–1841.