WHITE PAPER

Recommendations for converting a manual titration procedure into an automated titration procedure

While several titration methods for assays in compendial monographs are being converted to chromatographic methods or other quantitative procedures, titration still plays an important role in pharmaceutical analytical procedures and processes. Several applications, such as distinguishing between carbonate and bicarbonate or monobasic and dibasic phosphate salts, are only feasible by titration, making titration a fit for purpose method. For example, water determination by Karl Fischer (KF) titration is highly selective for water and sensitive enough to reach to the mg/L (ppm) level.

While the industry is already utilizing modern KF titration instruments for selective and reliable water content determination, many USP monographs still refer to the manual visual endpoint titration methods for other applications. Visual indication with color indicators is the oldest method of determining the equivalence point of a titration, and it is still frequently used and proposed in different guidance documents. It is inexpensive and requires few pieces of equipment. However, it can be tedious to determine the endpoint by adding a titrant dropwise with a manual buret until the color change is stable.

Metrohm

A further drawback of the method with visual indication is that the color perception of individual operators differs and can depend on the lighting conditions. Furthermore, visual endpoint detection is hampered in colored and/or turbid solutions. These factors reduce the reliability of the results as they become more prone to human error. An even bigger drawback is that the visual method cannot be automated and is therefore difficult to validate and it lacks data integrity.

This paper summarizes the steps involved in converting an existing manual titration procedure to semi-automated or automated titration procedures. It discusses topics such as selecting the right electrode and titration mode. For a better understanding, the discussion topics are illustrated with three examples.

EXAMPLE OF TITRATIONS

Three titration examples are used to illustrate possible changes between the existing manual titration procedure and a suitable semi-automated or automated titration procedure. These examples are:

1. POTASSIUM CITRATE

The assay of potassium citrate is done by a non-aqueous acid-base titration using perchloric acid in glacial acetic acid as the titrant and crystal violet as the indicator [**1**].

2. CALCIUM HYDROXIDE

The assay of calcium hydroxide is done by a complexometric titration using disodium edetate (Na_2EDTA) as the titrant and hydroxy naphthol blue as the indicator [**2**].

3. POTASSIUM BROMIDE

The limit of chlorine for potassium bromide is done by a residual precipitation titration using silver nitrate as the titrant, ammonium thiocyanate as the back-titrant and ferric ammonium sulfate as the indicator [**3**].

These examples were selected in such a way to cover different titration reactions as well as type of analysis (e.g., assay or impurities).

WHICH ELECTRODE SHOULD BE USED?

The first and most critical step in converting a manual titration to an automated or semi-automated procedure is the choice of the sensor for indicating the equivalence point. By replacing the visual endpoint detection with a sensor, subjective visual human perception is replaced by an objective sensor. Furthermore, this kind of indication can be easily automated and validated.

The choice of the sensor depends on the titration type, the sample matrix, and the titrant. Acid-base titrations require different sensors than redox titrations or precipitation titrations. Additionally, the sample matrix can have an influence on the sensor. For example, a different combined pH electrode is required for non-aqueous titrations than for aqueous titrations. **Table 1** lists suggestions for respective sensors depending on the titrant and currently used indicator. Using **Table 1** as a reference, the following electrodes were selected for the three examples:

1. Potassium citrate uses perchloric acid as the titrant [**1**]. For this titrant, a combined pH electrode suitable for non-aqueous titration is suggested, regardless of the indicator.

2. Calcium hydroxide uses disodium edetate as the titrant [**2**]. For this titrant, two sensors are suggested depending on the indicator. Hydroxy naphthol blue is used as the indicator for calcium hydroxide [**2**], and therefore a combined calcium electrode should be used in an automated or semi-automated titration.

3. Potassium bromide uses ammonium thiocyanate as back-titrant for the limit of chlorine test [**3**]. Usually only ferric ammonium sulfate is used as the indicator for this titration. However, the choice of the electrode is influenced by the sample itself. In this example, a residual titration with silver nitrate is done [**3**], and a combined silver electrode is the electrode of choice.

Table 1 lists the most common titrants and indicators. If your combination of titrant and indicator are missing from the table, contact the vendor of electrodes and equipment, as they can support you in choosing the right electrode for your titration.

Table 1. Summary of frequently used titrant and indicator combinations with the recommended sensors for replacing those indicators.

Titrants	Indicators	Recommended Sensors
Ammonium thiocyanate, potassium thiocyanate, tetramethylammonium bromide	Ferric ammonium sulfate	Combined silver electrode (silver salts, residual titration with silver nitrate)
		Combined gold electrode (mercury salts)
Bromine, ceric ammonium sulfate, iodine, potassium bromate, potassium ferricyanide, sodium thiosulfate	Starch	Combined platinum electrode
Ceric sulfate	Diphenylamine	Combined platinum electrode
Dichlorophenol–indophenol	None (self-indicating titrant)	Polarizable gold or platinum electrode
Edetate disodium, zinc sulfate	Hydroxy naphthol blue	Combined calcium electrode
	Eriochrome black T, Dithiozione, Xylenol orange	Photometric sensor
Ferric ammonium sulfate	Starch, ammonium thiocyanate	Combined platinum electrode
Ferrous ammonium sulfate	Ferroin, orthophenanthroline	Combined platinum electrode
Hydrochloric acid, sulfuric acid	Phenolphthalein, bromocresol green, methyl red, methyl orange	Combined pH electrode suitable for aqueous titration (solvent is water)
		Combined pH electrode suitable for non-aqueous titration (non-aqueous solvent)
Lead nitrate, lead perchlorate	Xylenol orange, dithizone	Lead ion selective electrode
Perchloric acid	Crystal violet, p-naphtholbenzein	Combined pH electrode suitable for non-aqueous titration
Potassium hydroxide	Phenolphthalein, bromocresol green	Combined pH electrode suitable for aqueous titration (solvent is water)
		Combined pH electrode suitable for non-aqueous titration (non-aqueous solvent)
Potassium permanganate	None (self-indicating titrant)	Combined platinum electrode
Silver nitrate	Eosin Y, ferric ammonium sulfate, potassium chromate	Combined silver electrode
Sodium hydroxide, tetrabutylammonium hydroxide	Phenolphthalein, methyl red, methyl orange, bromophenol blue, bromothymol blue, thymolphthalein	Combined pH electrode suitable for aqueous titration (solvent is water)
		Combined pH electrode suitable for non-aqueous titration (non-aqueous solvent)

OTHER ADJUSTMENTS NECESSARY FOR A METHOD CONVERSION

– VOLUME OF DILUENT

With the electrode selected, the most crucial step of the transfer to a semi-automated or automated titration is complete. However, there are a few other adjustments, which might be necessary. One point that needs to be considered is the amount of diluent (water or solvent) used in the titration. In order to obtain accurate results, it is imperative that the sensor is immersed deep enough into the solution so that both the measuring part and the reference part are immersed in the solution. See **Figure 1** for an example of a combined pH electrode. Sensor manufacturers generally specify a minimal immersion depth required for accurate titrations.

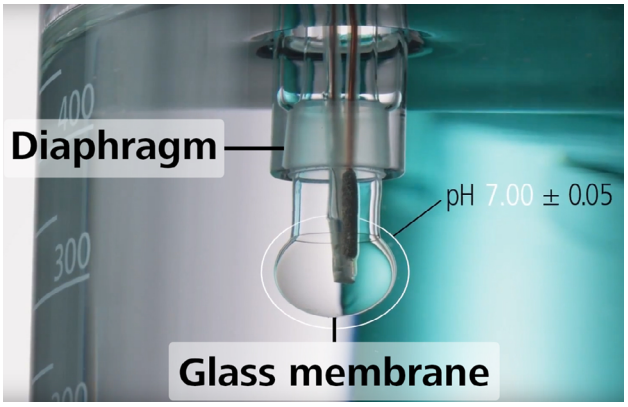

Figure 1. Example showing the optimal immersion depth for a combined pH electrode. Both the glass membrane (measuring part) and diaphragm (reference part) are fully immersed in the sample solution.

Looking at the three examples:

1. For potassium citrate, 25 mL of glacial acetic acid is used as solvent and no beaker size is specified [**1**]. If a 100 mL glass beaker is used, then the 25 mL glacial acetic acid is not enough to immerse the electrode. It is therefore recommended to increase the volume of solvent to, for example, 50 mL, which of course also needs to be performed for the blank.

2. For calcium hydroxide, a total volume of 165 mL is in the titration beaker before the start of the titration and again no beaker size is specified [**2**]. If a 250 mL tall beaker glass is used, no adjustment of the diluent volume is needed, as the volume is sufficient to immerse the electrode.

3. For potassium bromide, a 56 mL solution is in a conical flask after the sample preparation for the limit of chlorine test [**3**]. For the titration, a wide-neck conical flask is required to be able to fit the electrode in the conical flask. If a 100 mL wide-neck conical flask is used, the electrode will not be able to immerse sufficiently. It is therefore necessary to increase the amount of water used in the titration to, for example, 75 mL.

As illustrated in the examples above, the vessel used for the titration also has an important role in determining the amount of diluent required. The selection of the titration vessel is also influenced by the amount of titrant added during the titration. This leads to another point, the sample size, which needs to be considered when converting a manual titration to a semi-automated or automated titration.

– SAMPLE SIZE

Automated or semi-automated titrators are typically equipped with 10 mL or 20 mL burets. However, many manual titration methods have endpoints above 30 mL or even 40 mL. As refilling of the buret will lead to a systematic error, a reduction of the sample size is required. A reduction of the sample size has the additional benefit of producing less waste because less titrant will be consumed.

In general, it is recommended that the equivalence point of a titration lies between 10% and 90% of the buret volume. An optimal sample size leads to a titrant consumption at around 50% of the buret volume. If a 20 mL buret is used for the semi-automated or automated titration, the optimal titrant consumption is 10 mL. Again, looking at the three examples from above:

1. For the potassium citrate assay, a 200 mg sample and 0.1 N perchloric acid are used [**1**]. This combination leads an endpoint volume of approximately 19–20 mL, assuming the assay yields a 100% result. For a semi-automated or automated titration with a 20 mL buret, 100 mg potassium citrate would be an ideal sample size.

2. For the calcium hydroxide assay, a 1.5 g sample is used to prepare a stock solution of 500 mL. From this stock solution, 50 mL is used for the titration, corresponding to sample size of 0.15 g sample within the aliquot [**2**]. For a 0.05 M disodium edetate titrant and a purity of 100%, this corresponds to an endpoint at

approximately 40 mL. The sample size used for the stock solution should therefore be reduced to 0.375 g in order to obtain an endpoint at around 10 mL.

3. For the limit of chlorine test for potassium bromide, it is specified that not more than 1.7 mL of the silver nitrate titrant should be used to pass the test [**3**]. An adjustment of the sample size is therefore unnecessary. However, a 10 mL buret instead of a 20 mL buret should be considered, as 1.7 mL is below the recommended 10% of the equivalence point volume.

With the above considerations addressed, there remains one last steps in the transfer process—the selection of the titration mode and titration parameters.

SELECTION OF THE TITRATION MODE

Similar to how different titrations require specific titrants and sensors, the titration mode and titration parameter settings can influence the result. They can have a particularly strong influence on the precision and accuracy of a result. Therefore, suppliers of automated and semi-automated titrators offer default titration methods for the various combinations of titration type, titrant and sensor.

The titration mode defines the titrant dispensing principle (e.g., monotonous or dynamic addition) and titration curve recording (at the endpoint or whole titration curve). The most common titration modes are endpoint titration, monotonic titration, and dynamic titration. Following these three titration modes are explained briefly. More information on the different titration modes and basic examples of semi-automated and automated titrations can be found in the literature provided by suppliers of titrators.

– ENDPOINT TITRATION

Endpoint titrations are equivalent to manual titrations. Titrant is added until the indicator changes its color, signaling the endpoint of the titration. The basis for an endpoint titration is that the indicator changes reliably and reproducibly at the same endpoint, for example at a defined pH value. For automatic titrations the same principle applies, the titrant is added until the sensor detects the endpoint. Acid-base titrations are easily converted into endpoint titrations. An example is the acid-neutralizing capacity according to USP General Chapter <301> Acid-Neutralizing Capacity [**4**]. Due to their simplicity and speed, endpoint titrations are usually carried out for routine determinations.

If the indication signal is not stable enough for a reliable and reproducible endpoint titration, if more information about the sample should be obtained or if multiple analytes should be determined with the same titration, then recording the whole titration curve is required. When recording the whole titration curve, two different dosing principles can be applied—monotonic or dynamic addition of the titrant. **Figure 2** illustrates the two dosing principles.

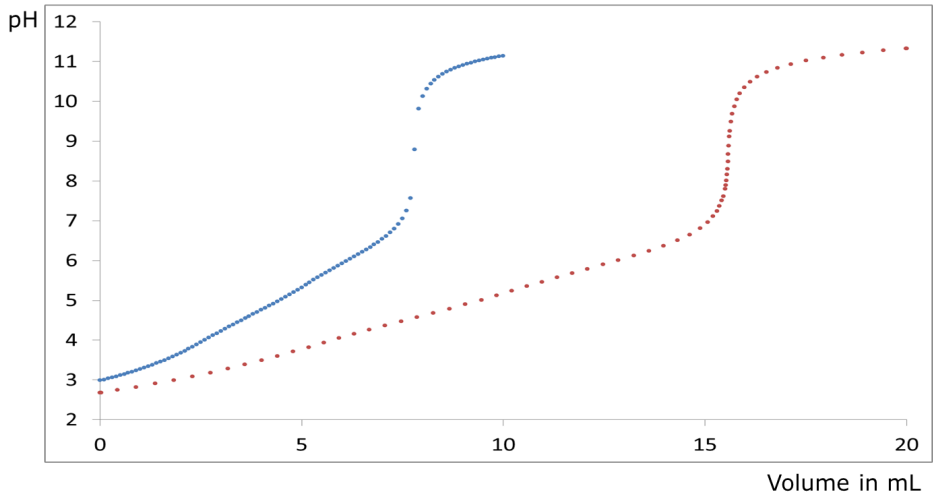

Figure 2. Titration curve of citric acid carried out as monotonic titration (blue) and dynamic titration (red). For an easier display, different sample sizes were used.

– MONOTONIC TITRATION

The titration is called a monotonic titration if constant volume increments are used for the addition of titrant. Monotonic titration is recommended for titrations that have slow reaction kinetics (e.g., slow reacting complexometric titrations). It is also recommended if a small titration consumption is expected (e.g., for blank determinations). Monotonic titration is required if the titration curve does not have an *S*-shape, such as redox titrations or titrations using a photometric sensor for indication. For these kinds of titrations, a dynamic addition of titrant would lead to an over-titration.

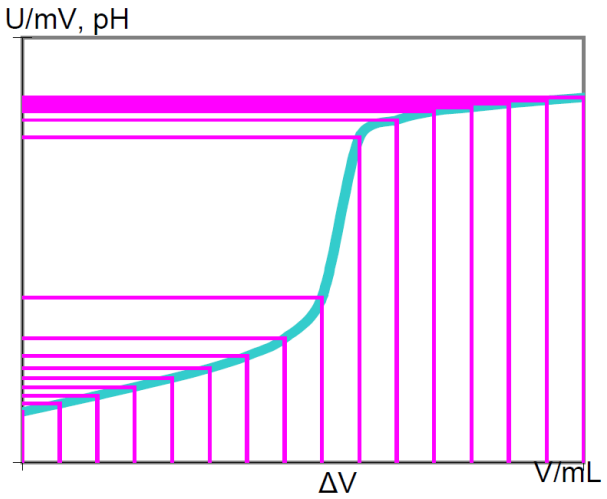

Figure 3. Illustration of the monotonic titration mode. Each pink line corresponds to the addition of a defined fixed volume (ΔV) of the titrant.

The disadvantages of the monotonic titration are the low density of data around the equivalence point (see **Figure 2** and **Figure 3**) as well as the long duration.

– DYNAMIC TITRATION

In a dynamic titration, as the name implies, the titrant is added dynamically depending on the slope of the titration curve. For example, if the signal changes only slightly over several additions, the volume of the next increments will be increased and vice versa. Dynamic titration is similar to manual titration as the analyst will speed up or slow down the titrant addition speed as the color change begins to appear.

The advantage of this method is a high data density around the equivalence point (see **Figure 4**), leading to high resolution, better reproducibility, and a faster titration.

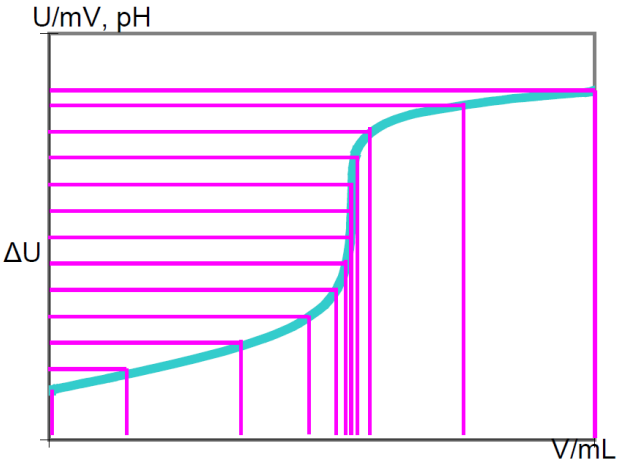

Figure 4.Illustration of the dynamic titration mode, each pink line corresponds to an addition of titrant, which is optimized to achieve approximately constant potential differences ΔU.

Typically, acid-base titrations, precipitation titrations and complexometric titrations are carried out in this mode

Table 2 provides an overview the most common titration modes and their principle. Furthermore, it lists the most common uses for the different titration modes.

The three examples, potassium citrate, calcium hydroxide and potassium bromide, can all be titrated in the dynamic titration mode. However, it is recommended to do the blank determination for the potassium citrate and calcium hydroxide in monotonic titration mode, as low blank values are expected. Because the limit of chlorine test for potassium bromide is a residual titration, the dynamic titration mode can be used for the blank determination.

CONCLUSION

Conversion of a manual titration method to a semi-automated or automated titration method can be achieved successfully, when considering important points such as electrode and titration mode selection. Furthermore, the translation of the titration method provides an opportunity to consider method optimization regarding titrant consumption and thus waste production.

Looking at the three examples, the following changes are necessary for a method conversion from manual titration to semi-automated or automated titration.

Table 2. Overview on the different common titration modes, their principles, and their uses.

Mode	Principle	Uses
Endpoint titration	Titration to a defined, fixed endpoint	Titration to fixed pH value
Monotonic titration	Constant volume increments	Slow reaction kinetics (complexometric titrations)
		Small equivalence point volumes (blank determinations)
		Sudden signal change at equivalence point (titration with polarized electrodes, photometric titrations)
Dynamic titration	Volume increment depends on signal change	Fast reaction kinetics (acid-base titration)
		S-shaped titration curves (redox-titration, precipitation titration)

1. FOR THE POTASSIUM CITRATE ASSAY

- Use of a combined pH electrode suitable for non-aqueous titration instead of the crystal violet indicator.
- Increase glacial acetic acid solvent volume to immerse electrode.
- Reduce sample size from 200 mg to 100 mg.
- Use of the dynamic titration mode.

2. FOR THE CALCIUM HYDROXIDE ASSAY

- Use of a combined Ca ion-selective electrode instead of the hydroxy naphthol blue indicator.
- Reduce sample size from 1.5 g to 0.375 g.
- Use of the dynamic titration mode.

3. FOR THE LIMIT OF CHLORINE TEST OF POTASSIUM BROMIDE

- Use of a combined silver electrode instead of the ferric ammonium sulfate indicator.
- Increase water volume to immerse electrode.
- Use of the dynamic titration mode.

These changes make a validation of the semi-automated or automated titration necessary. USP General Chapter <1225> Validation of Compendial Procedures provides an outline for which parameters need to be tested during a method validation [**5**]. For previously established general procedures, such as titration, a verification of the suitability for use should be done by determining the accuracy, precision, and specificity (absence of possible interference). If the sample size is changed, the linearity should be determined as well.

References

[1] USP. Potassium Citrate. In: USP 42–NF 37. Rockville, MD: USP; 2020:3613.

[2] USP. Calcium Hydroxide. In: USP 42–NF 37. Rockville, MD: USP; 2020:701.

[3] USP. Potassium Bromide. In: USP 42–NF 37. Rockville, MD: USP; 2020:3600.

[4] USP. <301> Acid-neutralizing Capacity. In: USP–NF. Rockville, MD: USP; May 1, 2019.

[5] USP. <1225> Validation of Compendial Procedures. In: USP–NF. Rockville, MD: USP; May 1, 2019.

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