Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

GENERAL PHARMACEUTICAL CHEMISTRY

Titrimetric methods of analysis

Lesson 12

V term

Volgograd,2022

QUESTIONSFORTHELESSON

1. Terms and definition sinthetitrimetric method of analysis.
2. Classification of titrimetric methods of analysis.
3. Depending on the titration method.
4. Depending on the approach of parallel determinations.
5. Depending on the type of chemical reactions.
6. Methods for the preparation of standard solutions.
7. Titration curves. Equipment.

INTRODUCTION

Titration is a quantitative technique and methods based on titration provide high accuracy (99.5–100.5%) and precision (<0.5% relative standard deviation (RSD)). Titration is an official method in the European Pharmacopoeia (Ph. Eur.) and in the United States Pharmacopoeia (USP), State Pharmacopoeia of the Russian Federation.

In pharmaceutical analysis, titration is mainly used for quantitative analysis (assay) of active pharmaceutical ingredients (APIs) and excipients with the purpose of assessing the purity of a given substance (analyte).

Titrimetry, titrimetric analysis (titration) is a method of quantitative analysis that is based on measuring the volume of a reagent solution of exactly known concentration consumed in reaction with the determined substance.

More generally, a titration can be described by the following titration equation:

$$x(Analyte)+y(Titrant)\rightarrow Products$$

Here ***x*** and ***y*** are the number of mol of analyte (pharmaceutical compound) and titrant involved to complete the titration. The titration is completed when all the analyte has reacted and transformed toproducts. The titration has then reached the equivalence point.

The titrant is added either manually from a burette or automatically from an automatic titration apparatus termed an automatic burette or titrator.

Detection of the equivalence point can be based on visual inspection of colour change (Indicator detection) or based on electrochemical measurements (Potentiometric end point detection).In the latter case,

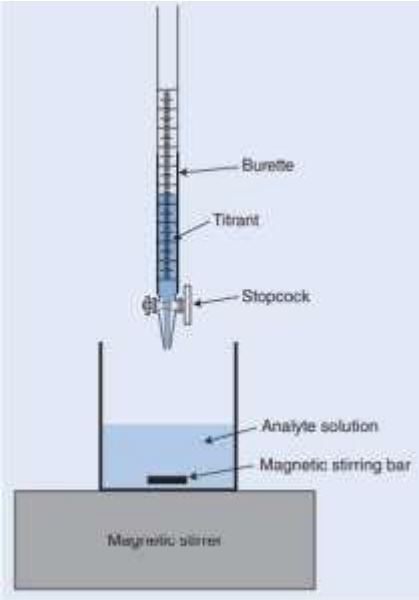


Figure1.Schematicviewofthe burette used for titration

the titration is often termed Potentiometric titration. The latter is preferred in the pharmaceutical industry because it can be automated.

The volume of titrant consumed is read from the burette/titrator and the quantity of analyte in the sample solution can be calculated on the basis of the underlying titration equation and the exact concentration of reagent in the titrant.

Control(blank)experiment

In many cases in titrimetry, regardless of the titration method, a control (blank) experiment is required. This is necessary to eliminate the influence of impurities, reagents used, inaccuracies associated with the use of measuring vessels, conditions of determination due to side reactions and other reasons. All reagents (titrated solutions, indicators, etc.) except the analysed substance, or reagents and the analysed substance without a characteristic reagent for the analysed substance, are involved in the control experiment.

The amount of titrated solution used for the control experiment is then taken from the total amount of titrated solution used for the titration of the test solution.

A number of requirements should befulfil led for asuccess fultitration:

- ✓ Thetitrationreactionmustbewell-definedandwithoutanysidereactions.
- ✓ There action must bevirtually complete (≈100%ofanalytemustbe converted to product).
- ✓ Other substances in thesampleshouldnotreactwiththetitrant.
- ✓ Theequivalencepointshouldbeclearlydetected.
- ✓ Theexactconcentrationoftitrantmustbeknown(standardizedsolution).

TERMSANDDEFINITIONSINTITRIMETRICANALYSIS

Standardisation is the process of finding the concentration of an active reagent in a solution (most often by titrating it with a standard solution).

A standard or titrated solution is a reagent solution of exactly known concentration intended for titrimetric analysis.

Standard substances (reference substances) are used to prepare solutions of precisely known concentration used in titrimetric analysis.

A primary standard substance (primary standard) is a substance of high purity that is used to determine the concentration (standardisation) of a titrant, or canitself be used to prepare a titrant solution of exactly known concentration.

A primary standard solution is a standard solution prepared from a primary standard substance whose concentration is known.

Requirementsforprimarystandardsubstances:

- ➢ highpurity;

- stabilityintheair;
- absenceofhygroscopicmoisture(substancesmustbehygroscopic),
- highermolarmassequivalent(whichreducesrelativeweighingerror),
- availability,
- notoxicity.

Table1showsthemostcommonprimarystandardsforthetitrimetricmethods used.

Table1

Titrant	**Primarystandard**
Acid-basetitration	
HCl,H_2SO_4	SodiumcarbonateNa_2CO_3
	Sodiumtetraborate$Na_2B_4O_7 \cdot 10H_2O$
NaOH,KOH	Potassiumbiphthalate$KHC_8H_4O_4$
	Oxalicacid$H_2C_2O_4 \cdot 2H_2O$
	BenzoicacidC_6H_5COOH
Precipitationtitration	
$AgNO_3$	notneed
Redoxtitration	
$KMnO_4$	Sodiumoxalate$Na_2C_2O_4$
$Na_2S_2O_3 \cdot 5H_2O$	Potassiumbichromate$K_2Cr_2O_7$
Complexometrictitration	
$Na_2C_{10}H_{14}N_2O_8$(TrilonB, disodium salt ethylenediaminetetetraacetic acid)	notneed

A secondary standard solution is a solution whose concentration has been determined by standardisation or prepared from a known mass of a secondary standard substance.

A secondary standard substance (secondary standard) is a substance used for standardisation; the content of the active ingredient is found using the primary standard.

Titration is the process of adding a titrated solution to a test solution of a determined substance until the equivalence point is reached.

The equivalence point is the state of the system when the quantity of the titrated solution added is equivalent to the quantity of the determined substance.

Thetitrationend-pointisthepointatwhichthetitrationends,whichisdetermined using indicators or physical parameters of instrumental methods.

CLASSIFICATIONOFTITRIMETRICMETHODSOFANALYSIS

Titrametricmethodsaredividedinto:

1. Dependingonthemethodoftitration.
2. Dependingontheapproachtomakingparalleldeterminations.
3. Dependingon thetype ofchemical reactionoccurring between the substances of the test solution and the titrated solution.

1. Dependingonthetitrationmethod:

- directtitration,
- reversetitration,
- indirect(substitution)titration.

In***directtitration***,thesolutioncontainingthetestsubstanceistitratedwiththe standard (titrated) solution until the equivalence point.

A+T(titrant)=product Direct

titration is carried out in the following cases:

1. the reaction of the interaction of the test substance with the reagent must be specific;
2. the interaction between the substance and the titrant must be stoichiometric, with the titration end-point clearly fixed;
3. thereactionmusttakeplaceatasufficientspeedandbepracticallyirreversible;
4. thereactionmustgothroughtotheend;
5. theremustbenosubstancesinthesolutionthatcouldinterferewiththemain reaction or the fixation of the titration endpoint.

In ***reverse titration***, a test solution and two standard (titrated) solutions are used. One standard solution is an auxiliary solution and the second (main) solution is used directly for the titration.

In the test, to the precisely measured volume of the test solution, add a knowingly excess, but precisely measured volume of the first titrated solution. As a result of the chemical reaction, the detectable substance (A) present in the test solution is completely consumed, interacting with the first titrated solution (T_1). The second (basic) titrated solution (T_2) then titrates an excess of the first auxiliary titrated solution which has not reacted with the detectable substance:

$$A+T_1(excess)=product1+T_1(residue)$$

$$T_1(residue) + T_2 = product\ 2$$

The quantity of the determining substance shall be calculated from the differencein volume of the titrated solutions, corrected for the control experiment taking into account the molarity of the solutions and the titer of the determining substance as specified in the pharmacopoeia and/or the regulatory documentation.

Reversetitrationisusuallyusedinthefollowingcases:

1. thespeedofthedirectreactionisslow;
2. thereisnosuitableindicator;
3. thedeterminedsubstancemaybelostduetoitsvolatility.

In ***indirect or substitution titration***, a test solution and two titrated solutions are also used.

During the test, an excess of the first (auxiliary) titrated solution (B) is added to a precisely measured volume of the test solution containing the substance (A). As a result of the reaction, the substance of the test solution is completely consumed to form an equivalent amount of the corresponding reaction product (A1), which is then titrated with a second (basic) titrated solution until the equivalence point is reached.

$$A+B(reagent)=A_1(substitute)$$

$$A_1(substitute) + T = product$$

Indirecttitrationisusuallyusedinthefollowingcases:

1. thedeterminedsubstancedoesnotinteractwiththetitrantinquestion;
2. the interaction of the determined substance and the titrant leads to the formation of a mixture of several products, the quantitative ratio of which is not constant;
3. thetitrationreactionisnon-stoichiometric;
4. thesuitableindicatorisnot available;
5. thesubstancetobedeterminedis unstable.

2. Dependingontheapproachtomakingparallel determinations:

- Single-samplemethod
- Pipettingmethod

Single-samplemethod

1. Asampleweightofthesubstancetobeanalysedis calculated.
2. The individual, closely related samples are weighed on the analytical scales. and then weighed on an analytical balance.
3. Dissolve it in a suitable volume of solvent and and titrated with a standardsolution.

Pipettingmethod

1. Calculateasampleweightoftheanalysedsubstance.
2. Weighthesampleonananalyticalbalance.
3. The quantityis transferred to avolumetricflask,dissolved in solvent and the volume of the solution is adjusted to the mark with the same solvent.
4. Pipetteanaliquotofthepreparedsolutionintoaconicalflaskandtitrate with a standard solution.

3. Depending on the type of chemical reaction occurring between the substances of the test solution and the titrated solution.

- acid-base,
- redox,
- complexometric,
- precipitation.

WAYSOFPREPARINGSTANDARDSOLUTIONS

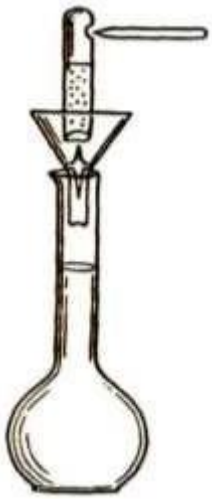
1. From an accurate weighing of the starting substance (primary standard solution).

Themainstepsofthework:

1. calculatetherequiredweighingweighttothenearest0,0001г;
2. weightheexactsampleonananalyticalbalance;
3. the weighed sample is transferred quantitatively to a volumetric flask of the same volume as the solution, the substance is dissolved, topped up to the mark with purified water and stirred;
4. if the sample taken differs from the theoretical calculation, then the concentration of the solution is recalculated.

2. Withthehelpoffixanals.

Fixanals (standard-titres, primary standards) are substances in strictly defined amounts contained in ampoules.



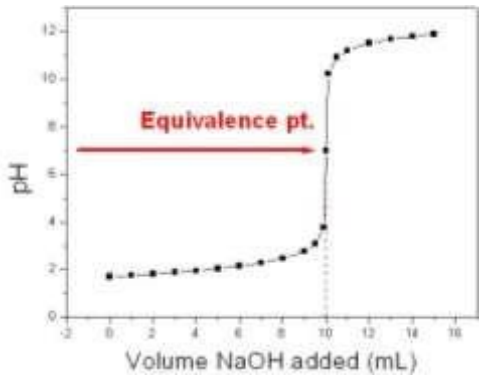
1. The ampoule is broken with a special striker in the funnel.
2. The contents are transferred quantitatively to a volumetric flask and diluted to the requiredvolume.

The resulting solution is often used in titrimetry as a titrant. As a fixanal that can be used as a fixanal are $KMnO_4$, $K_2Cr_2O_7$, HCl, $AgNO_3$, etc.

Thecompoundsusedasf fixanalsmustbeextremelypure,stable at room temperature and must not adsorb H_2O and CO_2 from the air.

TITRATIONCURVES

A titration curve is a graphical illustration of the relationship between the value determinedinthetitration(concentrationofthetitratedsubstance)andthevolume of the added standard solution of titrant.



Therearefoursectionsofthetitration curve:

1. Thestartingpoint;
2. Thesectionbeforethetitrationjump;
3. The titration jump including the equivalence point;
4. Thesectionafterthetitrationjump.

A titration jump is a section of the titration curve that corresponds to an extreme change in the properties of the system near the equivalence point. For example, in acid-base titration, an extreme change inpH.

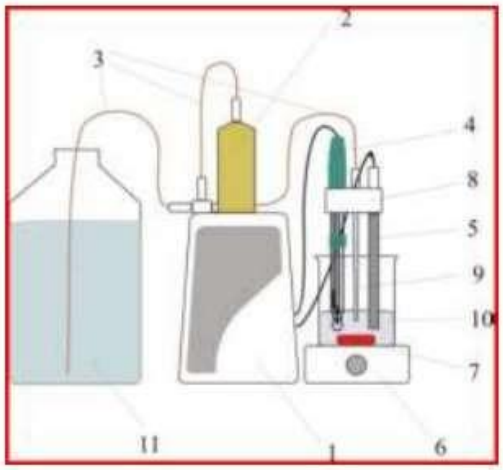
EQUIPMENTFORTITRIMETRICMETHODS

Burettes, pipettes and other utensils are used for ***manual titrimetric analysis***. In addition to traditional glass pipettes, modern dispensing devices such as pipettes and electronic pipettes can be used to accurately measure solution volumes.

For ***automated titrimetric analysis***, various automatic titrators (autotitrators) are used, which have an electronic control system that allows any type of titration and automatic processing of the results obtained.

Titrators are instruments designed for partially or fully automated automated measurements.

Thesoftwaresuppliedwiththetitratorallowsthetitrationcurvetobedrawn automatically from the data obtained, the titration end-point to be determined and the concentration of the solution tested to be calculated.



The titrator consists of a titration block (1), burette (2), liquid tract (3), combined electrode for pH-metry (4), resistance thermometer (5), magnetic stir bar (6), magnetic stir bar armature (7), and stand (8). Electrode, thermometer and spout of the liquid path (9) is submerged in the beaker sample (10). The titrant is in a bottle(11).

Dependingonthetype oftitrimetric analysisused,different***instrumentalmethods*** areusedtorecordthetitrationendpoint:

- potentiometric;
- photometric;
- conductometric;
- amperometric;

- coulometric

CALCULATIONSINTITRIMETRICA NALYSIS

In order to save the analysed dosage forms and titrant, it is necessary to calculatethe optimum amount of dosage forms and titrant before carrying out the analysis.

The weight of the dosage form for quantification is calculated according to the formula:

$$a = \frac{V * K * T * P}{b}$$

a-thesampleweight,gorml; V -

volume of titrant, ml;

T-titeroftheworkingsolutionfortheanalyzedingredient,g/ml;

b-quantityoftheanalysedingredientasprescribedintheprescription,g;

P - mass or volume of the dosage form according to the prescriptionrespectively g, ml;

K-correctionfactorforthetitratedsolution.

The titrant volume (V) in the case of individually prepared dosage forms must be between 0.5-2 ml. This is due to the express nature of intra-pharmacy quality control of medicines. The analyst should use a micro-, or semi-micro pipettes or micro-, semi-microburettes.The concentration of of the titrated solutions can vary from 0.1 mol/l to 0.01 mol/l.

The correction factor (K) of the prepared titrated solution is the ratio of the true (experimentally determined) and the theoretical concentration of the prepared titrated solution (or its true and theoretical titre):

$$K = \frac{M_E}{M_T} = \frac{T_E}{T_T}$$

M_E, M_T - experimentally determined and theoretical concentration of the titratedstandardised solution, respectively, mol/l;

T_E,T_T-actualandtheoreticalconcentrationofthedissolvedsubstanceinthe standardized titrated solution, g/ml.

- Whendeterminingthecorrectionfactor,atleastthreeparalleltitrations.
- If the titration results do not differ by more than 0,05 mL, the arithmetic mean of the results is taken and is calculated by K.
- If the discrepancy between the individual titrations exceeds 0.05 mL, the titration is repeated until the results are convergent.
- Therelativeerrorindeterminingthecorrectionfactorshouldnotexceed ±0.1%. To do this, titrate at least 20-30 ml of solution and use measuring flasks and pipettes previously tested for accuracy.
- The Pharmacopoeia correction factor should fall within the range 0.98-1.02. In cases where the correction factor values do not fall within these limits, solutions should be strengthened or diluted.

    - In the case of ***diluted*** titrated solutions, subtract 1.0 from the calculated K value and multiply the resulting difference by thevolume of solution prepared in ml.The result of multiplication shows the amount of solvent in ml that needs to be added to the prepared solution to bring K to the required value.
    - To ***strengthen***atitratedsolution (Klessthan1.0),subtract Kfrom1.0 and multiply the resulting difference by the mass of the initial substance weight taken to prepare the given volume of titrated solution.
    - After addition of the calculated quantity of solvent or starting substance,the correction factor is determined again (three times). If K complies with the Pharmacopoeia, the titrated solution is ready foruse.

Aftertitration,thesubstancecontentofthedosageformis calculated.

DIRECTTITRATIONCALCULATIONS

Thepercentageconcentrationoftheingredients(inliquiddosageforms,ointments) in direct titration is calculated according to the formula:

$$X(\%) = \frac{V * K * T * 100}{a}$$

X(%)-contentofthedeterminedsubstance,in%;

T-titerofthetitrantforthedeterminedsubstance,ing/ml; V -

volume of titrated solution, in ml;

K-thecorrectionfactoroftheti tratedsolution; a -

sample weight of the test drug (in g or ml).

Calculatetheingredientcontentingramsusingthefollowingformulasthe following formulas:

a) forliquiddosageforms:

$$X(g) = \frac{V * K * T * V_{DF}}{a}$$

b) forsolidandsoftdosageforms:

$$X(g) = \frac{V * K * T * P}{a}$$

X(g)-massofthedeterminedmedicinalsubstance,ing;

V_{DF}-volumeofliquiddosageform(accordingtotheprescription),inml; P - total

weight of powder, ointment according to the prescription, in g;

V-thevolumeoftitratedsolution,inml;

T-titerforthesubstancebeingdetermined,ing/ml; K -

correction factor for the titrated solution;

a-volume,in mlormass,ing,ofthedosageformsampledfor analysis.

Calculationofsubstancecontentingrams,takingdilutionintoaccount

In some cases, in order to reduce weighing errors when taking a sample, it is proposed in the normative documentation to carry out quantification of the active ingredient in an aliquot of solution or filtrate.

In such cases the volume of the volumetric flask (V_f, ml) and the volume of the aliquot (V_a, ml) taken for titration are added to the calculation formulae.

$$X(\%) = \frac{V * K * T * 100 * V_f}{a * Va}$$

$$X(g) = \frac{V * K * T * V_f * V_{DF}}{a * V_a}$$

X(g)-massofthedeterminedmedicinalsubstance,in%org;

V_{DF}-volumeofliquiddosageform(accordingtotheprescription),inml; P - total mass of powder, ointment according to the prescription, in g;

V-volumeoftitratedsolution,inml;

T-titerforthesubstancebeingdetermined,ing/ml; K - correction factor for the titrated solution;

a-volume,inml,ormass,ing,ofthedosageform,sampledforanalysis; V_f - volume of the flask where the dilution was carried out, ml;

V_a-volumeofdilution(aliquot)takenfordetermination,ml.

CALCULATIONSINREVERSETITRATION

When quantification by reverse titration two titrated solutions are used, one of which is added in excess. The calculation of the ingredient content is carried out according to the formulas:

a) inpercentages:

$$X\,(\%) = \frac{(V1K1 - V2K2) * T * 100}{a}$$

b) ingramsinliquiddosageforms:

$$X(g) = \frac{(V1K1 - V2K2) * T * V_{DF}}{a}$$

c) ingramsinpowdersandointments

$$X(g) = \frac{(V1K1 - V2K2) * T * P}{a}$$

V1andV2-thevolumesoftitratedsolutionstakeninexcessandspentontitration, ml, respectively;

V_{DF}-volumeofliquiddosageformaccordingtotheprescription,inml; K1 and K2 - respective correction factors;

T-titerforthesubstancebeingdetermined,ing/ml;

P-totalmassofpowder,ointmentaccordingtotheprescription,ing.

The contents of the ingredients, taking into account the reference test, are calculated according to the formulas:

a) inpercentages:

$$X(\%) = \frac{(V - V_{rt}) * K * T * 100}{a}$$

b) ingrams:

$$X(g) = \frac{(V - V_{rt}) * K * T * V_{DF}}{a}$$

V rt - the volume of the second titrant used for the titration of the reference test, in ml;

V-volumeofthesecondtitrantusedforthetitrationofthemainexperiment,ml; P - weight of powder or ointment, g.

For medicinal products that contain crystallising water or are hygroscopic,the moisture content may be decreased or increased during storage.

However, the quantitative content of the above pharmaceutical substances as required by the Pharmacopoeia must be calculated in the sample to be analysed in terms of anhydrous substance.

In such cases the titre for the substance to be determined ($T_{B/A}$) is calculated according to the formula:

$$T_{B/A} = \frac{N_B * f_{equiv.}(A) * [M(A) - n * M(H_2O)]}{1000}$$

N_B-themolarconcentrationofthetitrantequivalent,g-eq/mol; M (A)

- molar mass of the drug substance, g/mol;

$F_{equiv.}$(A)-drugsubstanceequivalencefactor;

n- number of water molecules in the analyzed medicinal substance according togross formula;

M(H_2O)- molarmassofwater,g/mol.

Theactiveingredientcontent(X,%)iscalculatedintermsofdrysubstance.Calculation formula:

$$X(\%) = \frac{V * K * T * 100 * 100}{a * (100 - B)}$$

B-Actualmoisturecontent.

Calculationoftheapproximateaveragetitre.

The presence in mixtures of substances with similar structures and properties (e.g. sulphonamides, alkaloids, salts of hydrogen-halogenic acids) makes it difficult to determine them separately.

If the two substances in a mixture can be titrated with the same titrated solutionand there is no method for determining one of them separately, the total content of the components may be calculated using an approximate average titre. Its shall be calculated according to the formulas given below:

1) If the determined total ingredients are prescribed in similar quantities and their titres differ very little from each other, then the average approximate titre is calculated according to the formula:

$$T = \frac{T_1 b_1 + T_2 b_2}{b_1 + b_2}$$

2) In the event that the prescribed quantities of both ingredients are the same, the formula is converted to the formula below:

$$T = \frac{T_1 + T_2}{2}$$

T1-titerofthefirstcomponent,ing/ml;

b1-prescribedmassofthefirstcomponent,ing; T2 - titer of

the second component, in g/ml;

b2-prescribedmassofthesecondcomponent,ing.