Addis Ababa University

School of Graduate Studies

Department of Chemistry

Determination of the Antiretroviral Drug Lamivudine by Potentiometric Titration using All Solid State Graphite Based Iodide Selective Electrode

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Acronyms

HIV: Human Immune virus

AIDS: Acquired Immunodeficiency Syndrome

CD4: Cluster Difference 4

WHO: World Health Organization

UNAIDS: United Nations Program on HIV/AIDS

DNA: Deoxyribonucleic Acid

RNA: Ribonucleic Acid

ART: Antiretroviral therapy

ARV: Antiretroviral

HAART: Highly Active Retroviral Therapy

LMV : Lamivudine

NRTIs: Nucleoside Reverse Transcriptase Inhibitors

UV: Ultraviolet

HPTLC: High Performance Thin Layer Chromatography

HPLC: High Performance Liquid Chromatography

RP-HPLC: Reversed Phase High Performance Liquid Chromatographic

AAS: Atomic Absorption Spectrometry

ISE: Ion selective electrode

EMF: Electromotive force

SD: Standared deviation

mv: millilvolt

ml: milliliter

mg: miligram

Abstract

A solid-state iodide selective electrode with Ag_2S-AgI adsorbed on graphite rod, recovered from a dry cell, was prepared and validated for its typical response characteristics with an objective to develop a potentiometric titration system for rapid measurements of Lamuvidine (LMV) in tablets used as an antiretroviral drug for Hepatis B and HIV patients. The partial validation exhibited typical response values of linear range of 10^{-6} to 10^{-1} M iodide and a slope 57.57 mV/decade which reasonably agreed with early data from this Laboratory. The basis of LMV determination is its oxidation by excess bromine generated from bromate-bromide mixture. The unreacted bromine oxidized the added excess iodide to iodine. The amount of bromine consumed by LMV, the basis of its determination, was determined by subtracting the back-titrated iodine with thiosulphate whose end-point was located potentiometrically with the solid state iodide selective electrode against a reference electrode. The iodometric back titration provided for the indirect determination of 1-30 mg LMV in turbid tablet test solutions. There was no interference from the excipients in the tablet and this provided for LMV assays with and without filtration of the turbid (insoluble) pharmaceutical excipients. Acid concentrations between 0.2 – 1.5 M HCl did not have any influence on the titration end-points. The intra-day and inter-day variation of the result was not significant. The system recovery for standard added sample was 99.3 ± 5.3 % and for a reference LMV was 98.4 ± 0.8 %. For quality control checks, the potentiometric back-titration method was favorably compared with an independently applied reference test method (UV spectrophotometry at 280 nm), by quantitating LMV in a blind sample and reference drug material. This inexpensive potentiometric titration system was easy to operate and exhibited good recovery for LMV determinations in the typical drug dose labeled to contain 150 mg per tablet.

Key words: Antiretroviral Drug LMV; ISE method; Potentiometric Titration; Bromate-Bromide Mixture; Iodometric Back Titration.

1. Introduction

Retrovirus is any of a family of ribonucleic acid (RNA) viruses containing the enzyme reverse transcriptase. They are so named because they carry their genetic information in the form of RNA rather than DNA so that the information must be transcribed in "reverse" direction from RNA into DNA. The genetic information of the virus is stored in a molecule of single-stranded ribonucleic acid. After entering the target cell, the virus uses reverse transcriptase to direct the cell to make viral deoxyribonucleic acid (DNA). The DNA becomes integrated into the DNA of the host cell. Retroviruses are enveloped and assemble their capsids in the cytoplasm of the host cell. They are used in laboratory research to import foreign DNA into a cell. They are transmitted by through exposure to infected blood or blood products and from an infected mother to the child. HIV which causes acquired immunodeficiency syndrome is a retrovirus [1].

The HIV is the well known retrovirus however there are others to cause infections and some cancers. AIDS is the disease complex caused by an infection of the HIV retrovirus in the human body which imposes itself on CD4 (Blood cells that play a major role in maintaining the body's immune system) helper lymphocytes which is essential to immune response, weakening the immune system and opening up the human body to opportunistic diseases. The higher the number of CD4 cells the stronger will be the immune system. People without HIV infection have about 700 to 1000 CD4 cells in a drop of blood the size of a pea. HIV infected people are considered to have "normal" CD4 counts if the number is above 500 CD4 cells in that same size drop of blood. If the number of CD4 cells in that drop of blood ever drops below 200 CD4 cells, the person will classified as having AIDS whose body immune system is no longer strong enough to prevent illness and infection [2].

HIV is a virus like other viruses which infect and damage the cells of living organisms by replicating (making new copies of themselves) within those cells. People can become infected with HIV from other people who already have it, and when they are infected they can then go on infecting other people. AIDS is an extremely serious condition and at this stage the body has very little defense against any sort of infection [2].

AIDS and its retrovirus HIV are one of the deadliest disease complexes, taking the lives of millions of children and adults throughout the world every day. As of December 2007, the Joint UNAIDS and WHO estimate that AIDS has killed more than 25 million people since it was first recognized in 1981, making it one of the most destructive epidemics in recorded history. In 2007, 33.2 million people were living with HIV in which 2.5 million children, of whom 2.5 million were newly infected. Generally 2.1 million people worldwide died of AIDS in the same year. It is estimated 38.6 million people now living with the disease worldwide [3].

The Sub-Saharan African countries are particularly and significantly affected by the disease. It strongly affects the economy since it grabs the bread earners of the society. On a macroeconomic level, AIDS affects economic growth by reducing the labor force, increasing healthcare expenditures, and reducing the taxable population [4].

In 2003, Ethiopia was the 16th highest in HIV/AIDS prevalence and the third largest number of people living with the virus next to South Africa and India. Official reports show that 2.2 to 2.6 million people were infected with HIV/AIDS, where around two million are adults and 200 to 250 thousand children. The peak ages for AIDS cases are 25 to 29 for both males and females. In 2006, 1.5 million people were living with HIV. In 2010 it was estimated that 7-10 million Ethiopians would probably be infected [5].

Country	Year	% of people living with HIV who knew their status and received result		
		Women	Men	Overall
Benin	2006	24.9	A	23.5
Cote d'lvoire	2005	13.6	23.6	16.5
D.R of Congo	2007	8.7	A	10.7
Ethiopia	2005	8.4	5.6	7.6
Guinea	2005	5.4	A	5.4
Mali	2006	13.0	A	12.9
Rwanda	2005	31.3	31.6	31.4
Swaziland	2007	44.0	28.8	38.7
Zimbabwe	2005-2006	26.9	19.3	23.7
Haiti	2005	30.7	15.6	24.5
Dominican Republic	2007	72.6	49.1	60.7
India	2005-2006	6.8	12.8	10.3

Table 1. Percentages of women and men living with HIV aged 15–49 years who had received an HIV test for selected countries, 2005–2007. Where "a" is the number of cases is very small (n = 25–49) and cannot be interpreted (UNAIDS and UNICEF 'universal Access' 2008).

The known confirmed transmission paths of the virus can be through sexual intercourse, blood transfusion, birthing process, and through contaminated syringes; those activities that allow infected bodily fluids such as semen, vaginal discharge, blood products and breast milk, to enter the blood or mucous system of the target subject [6]. Prevention is aimed at decreasing risk behaviors for transmission of the virus. These include: promoting awareness of transmission paths through educational programs; promoting "safe" sex through abstinence, monogamy and the use of condoms; decreasing blood transmission through needle exchange programs, blood screening to decrease the transmission rates and initiating a drug prevention programs by use of antiretroviral (ARV) drugs to treat the disease [6].

1.1 Antiretroviral Therapy

Antiretroviral therapy (ART) is the treatment that suppresses or stops a retrovirus. One of the retrovirus is HIV that causes AIDS. ARV drugs are used to combat diseases such as AIDS. Sometimes, these types of drugs are taken in combination cocktails and are called highly active retroviral therapy (HAART). A single ARV cannot suppress the virus effectively; most doctors prescribe three or more ARVs from two different classes. This is known as combination therapy, or ART; when several drugs are taken, it may be known as HAART.

ARVs directly attack HIV. This enables the immune system to continue functioning and to overcome most opportunistic infections. Infected persons receiving first line ARV therapy versus no therapy experience an average increase in life expectancy of 29.1 years. While not all ARV treatments are effective to the same degree, the widespread consensus is that, ART is instrumental in prolonging live in HIV-infected patients [7].

Antiretroviral medication can prolong the time between HIV infection and the onset of AIDS and hence reduces both the mortality and the morbidity of HIV infection. Without drug treatment, HIV infection usually progresses to AIDS in an average of ten years. Modern combination therapy is highly effective and theoretically, someone with HIV can live for a long time before it becomes AIDS [8]. There are over 20 ARVs in different classes, defined according to the method by which they attack the virus. These antiretroviral drugs include Lamivudine, Zidovudine, Zalcitabine, Didanosine, Stavudine, Zidovudine, Abacavir etc. [8].

Nucleoside reverse transcriptase inhibitors (NRTIs) antiretroviral treatment for HIV infection consists of drugs which work against HIV infection in their own way but synergistically slowing down the replication of HIV in the body. Lamivudine (LMV) is a potent antiviral agent used in the treatment of AIDS. It belongs to group of medications known NRTIs. NRTIs medications work by blocking a process that the HIV and hepatitis B viruses need in order to multiply. Conventional oral formulations of LMV are administered multiple times a day (150 mg twice daily) because of its moderate half-life ($t_{1/2}$ = 5-7 hours) [9].

Studies show that using triple combination therapy reduces not only viral load by 99 % but also the rate of mortality due to HIV/AIDS by as much as half. Among those in triple combination therapy, 65 to 81 percent had reduced their level of virus to undetectable levels after six months of treatment [10].

In quality the control of its production and body fluids, lamivudine can be determined by HPLC, HPTLC and UV spectrophotometry either independent or in combination with other drugs. But determination by HPLC and HPTLC takes more time of analysis and are expensive. The UV spectrophotometry may subject to interferences such as color in the sample [11].

Recent development shows that Lamivudine can be determined by ion selective electrodes and iodometrically by a back titration of bromine after an excess of the latter is allowed to react with drug substance which is to improve the time of analysis and cost since it is cheap and easy to operate.

1.2. Analytical Applications of Bromine and Iodine reaction

Iodate and Bromate are used as analytical reagents in titrations for many official and other published methods. Iodine and Bromine are used for the determination of a variety of samples such as iodized salt, foods, water, clinical and biological samples, environmental samples and pharmaceutical formulations. The bromine generated from bromate-bromide solution in acidic medium was applied for spectrophotometric and/or back titration iodometry by Basavaiah and co-workers in India including Lamivudine. This is reviewed in section 2.2.1 (page 11). Several determinations of other pharmaceutical products that react with bromine have also been reported [12].

Two simple methods were described for the determination of gatifloxacin sesqui hydrate (GTF) in bulk drug and in formulations using bromate-bromide as the oxidimetric reagent. The methods were based on the oxidation of GTF by in situ generated bromine followed by determination of unreacted bromine using two different methods. In one procedure, the residual bromine was reduced by an excess of iron(II), and the resulting iron (III) was complexed either with thiocyanate and measured at 470 nm (method A) or with tiron at pH 1.09 and measured at 670 nm (method B). In both methods, the absorbance was found to decrease linearly with GTF concentration. Beer's

law was obeyed over the ranges 0.3-3.0 and 1-15 μg/mL for method A and method B. The reported molar absorptivity values were 1.3×10^5 and 2.5×10^4 L/mol/cm for method A and method B, respectively [13].

Two sensitive spectrophotometric methods were developed for the determination of lansoprazole (LPZ) in bulk drug and in capsule formulation. The methods were based on the oxidation of lansoprazole by insitu generated bromine from acidified bromate-bromide solution followed by determination of unreacted bromine by two different reaction schemes. In one procedure (method A), the residual bromine is treated with excess of iron (II), and the resulting iron (III) was complexed with thiocyanate and measured at 470 nm. The second approach (method B) involves treating the unreacted bromine with a measured excess of iron (II) and remaining iron (II) is complexed with orthophenanthroline at a raised pH, and measured at 510 nm. In both methods, the amount of bromine reacted corresponds to the amount of LPZ [14].

In the reported titrimetric, spectrophotometric and kinetic methods for the assay of atenolol in bulk drug and in tablet formulation using bromate–bromide and methyl orange. The methods were based on the oxidation–bromination reaction of the drug by bromine, generated *in situ* by the action of acid on a bromate–bromide mixture. In the titrimetric method, the drug was treated with a known excess of bromate–bromide mixture in hydrochloric acid medium. The determination of the unreacted bromine was made iodometrically. The spectrophotometric method involves the addition of a measured excess of the acidified bromate–bromide reagent to atenolol. At equilibrium, the unreacted bromine was allowed to react with a fixed amount of methyl orange, and absorbance measured at 520 nm. The kinetic method depends on the existence of a linear relationship between the concentration of the drug and the time of the oxidation–bromination reaction, indicated by the extent bleaching of the acid colour of methyl orange [15].

One titrimetric and three spectrophotometric methods for the determination of stavudine in bulk drug and in dosage forms were developed using bromate-bromide and three dyes. Stavudine has a similar oxidizable functional group as that of Lamuvidine. In titrimetry, aqueous solution of STV was treated with a known excess of bromate-bromide in HCl medium followed by estimation of unreacted bromine by iodometric back titration which allowed the quantitation 3.5-10 mg STV in the test solution of the tablet. The spectrophotometric methods involved the addition of a measured

excess of bromate-bromide in HCl medium and the subsequent estimation of the residual bromine by reacting with a fixed amount of methyl orange, indigocarmine or thymol blue followed by measurement of absorbance at 520 nm (method A), 610 nm (method B) or 550 nm (method C). In all the methods, the amount of bromate that reacted with STV corresponded to the amount of STV [16].

This report also illustrates the determination of the amount of lamivudine in pharmaceutical tablets by subtracting potentiometricaly back-titrated iodine formed from the total (excessive) bromate-bromide mixture that generates the oxidant, bromine.

1.3. Lamivudine

Lamivudine (3TC, LMV) has a molecular formula of $C_8H_{11}N_3O_3S$, a molecular weight of 229.3g and the following structural formula:

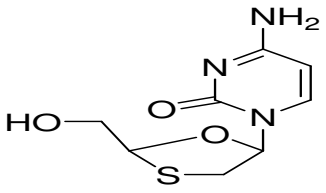


Fig.1 structure of Lamivudine

(2R, cis)-4-amino-1-(2-hydroxymethyl-1, 3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. It is a white to off-white crystalline solid with a solubility of approximately 70 mg/ml in water at 20°C. LMV inhibit viral reverse transcriptase which is an enzyme that HIV requires in order to replicate itself by incorporating into viral DNA and terminating the viral DNA chain [17].

1.3.1. Application of Lamivudine

LMV is a synthetic nucleoside analogue with activity against HIV and HBV. Since HIV and HBV convert their genetic material into DNA by using a special protein called the reverse transcriptase enzyme. To create DNA, this enzyme uses several different molecular building blocks. LMV works by tricking reverse transcriptase into thinking it is one of these molecular building blocks.

However, it is just different enough that when used to create DNA, LMV actually stops the DNA from being made. Without DNA, HIV and HBV cannot multiply [17].

It is important to understand that lamivudine is not a cure for HIV, AIDS, or hepatitis B.

LMV is not only used for treating these diseases but also it can use for treating

- Inflammation of the pancreas (known as pancreatitis)
- Liver disease, such as liver failure, cirrhosis, or hepatitis
- Kidney disease, including kidney failure (renal failure)
- Any allergies, including allergies to food, dyes, or preservatives [17].

1.3.2. Lamivudine Dosing for HIV or AIDS

The recommended LMV dose for treating HIV or AIDS in adults is 150 mg twice daily or 300 mg once daily. For children of ages between 3 months to 16 years old, the recommended dose is based on weight, about 1.8 mg per pound twice daily (up to a maximum of 150 mg twice daily). A lower LMV dosage may be recommended for people with kidney problems [18].

1.3.3. Lamivudine Dosing for Hepatitis B

The recommended LMV dose for treating hepatitis B in adults is LMV (Epivir-HBV) 100 mg once daily. For children 2 to 17 years old, LMV dosing is based on weight, about 1.36 mg per pound once daily (up to a maximum of 100 mg per Day) [19].

1.3.4. Common Side Effects of Lamivudine

LMV side effects that occur in a group of people taking the drug are documented and are then compared to side effects that occur in another group of people not taking the medicine. This way, it is possible to see what side effects occur, how often they appear, and how they compare to the group not taking the medicine. The most common side effects of LMV included:

- Ear, nose, or throat infections -- up to 25 % of people
- Fatigue and a general ill feeling (malaise) -- up to 24 %
- Headaches -- up to 21%
- Abdominal pain (stomach pain) -- up to 16 % and Nausea and vomiting -- up to 15 %

Other common lamivudine side effects, occurring in 5 to 14 percent of people, included fever or Chills, Sore throat, diarrhea, muscle pain and Joint pain [19].

2. Literature Review on the Determination Methods of Lamivudine

Several analytical methods that have been reported for the estimation of LMV in biological fluids or pharmaceutical formulations include HPLC, Titrimetry and UV-visible spectrophotometry. There is still a need for simple, rapid and valid test method for LMV in drugs and biological fluids that would save time and cost of analysis [19].

2.1 Official Analytical Methods

One of the International Pharmacopeia procedures for single dose Lamuvidine per tablet was based on its absorbance at 280 nm. This involves addition of 5 ml of a tablet solution containing 50 mg of Lamivudine to a 50 ml volumetric flask and diluting to volume with 0.1 M sulfuric acid. The absorbance of this solution was taken in a 1 cm cuvette at 280 nm against a solvent cell containing a blank. The mass or the percentage of lamivudine ($C_8H_{11}N_3O_3S$) in the original tablet samples is calculated back from the absorbance using the absorptivity value of 60.7 ($A^{1\%}_{1cm}$= 607) [20].

2.2. Non-Standard Methods

2.2.1. Methods for Lamivudine

Two simple and sensitive spectrophotometric methods (Methods A and B) have been developed for the estimation of LMV in both pure and tablet dosage form. Methods A and B were based on the condensation reaction of LMV with carbonyl reagents such as p-dimethylaminobenzaldehyde (PDAB) and vanillin in acidic condition to form yellow colored chromogen with absorption maxima at 476 nm and 474 nm respectively [21].

The electrochemical reduction and adsorption of LMV a systemic antiviral drug, were studied in a phosphate buffer medium at a hanging mercury drop electrode (HMDE). Cyclic voltammetry studies showed one well-defined reduction peak in the potential range from −1.2 to −1.8 V under different pH conditions, but the best results were obtained at pH 3.4 [22].

Application of UV spectrophotometric measurements for estimation of LMV in tablet was reported involving two new, simple, cost effective and sensitive spectrophotometric methods (A and B) for the determination of lamivudine in dosage and bulk forms were described. LMV was estimated at 279.6 nm in 0.1 N HCl by method A and at 269.8 nm in 0.1 N NaOH by method B. In both the methods linearity was found to be in the range of 0-6 µg /mL for method A and 0-10 µg / mL for method B [23].

One titrimetric and two spectrophotometric methods for the assay of LMV in bulk drug and in tablet are have been reported on the basis of bromate-bromide redox mixture and two dyes, methyl orange and indigocarmine as reagent. In titrimetry aqueous solution of LMV was treated with measured excess of bromated-bromide mixture in HCl medium, followed by iodometric determination of unreacted bromine. Spectrophotometric methods involve the addition of a known of excess of bromated-bromide mixture to LMV in acid medium, followed by the determination of residual bromine by reacting with either a fixed amount of methyl orange and measuring the absorbance at 520 nm (method A) or indigocarmine and measuring the absorbance at 610 nm (method B). In all the methods the amount of bromated reacted corresponds to the amount of LMV. Ttitrimetric method was applicable over 2.5-7.5 mg range and the reaction stoichiometry was found to be 1:1 (LMV: bromine) [24].

2.2.2. Methods for LMV with Combination Drugs

A UV spectrophotometric method for the simultaneous estimation of LMV, Nevirapine and zidovudine in combined pure bulk drug and in tablet dosage form in acidic medium. The λ max for LMV, nevirapine and zidovudine were 280.2 nm, 312 nm and 266.8 nm respectively and linearity for the drugs was in the range of 5-25 µg/ mL, 5-50 µg/ mL and 5-40 µg/mL respectively [25].

Determination of LMV and Zidovudine in Human Plasma using isocratic HPLC-UV method was developed to evaluate the bioequivalence of fixed-dose combination drug products. The method was applied for LMV /zidovudine in human plasma using a liquid-liquid extraction method. Analysis was carried out using methanol/25 mM KH_2PO_4 buffer (pH=6.5):35/65 % v/v as a mobile phase, in Luna C_{18} column (150 X 4.6mm) with UV detection at 245 nm [26].

Uslu and Ozkan described a method for simultaneous determination of LMV and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio spectra and HPLC–UV methods. This depends on the first derivative of the ratio spectra by measurements of the amplitudes at 239.5 and 245.3 nm for LMV and 225.1 and 251.5 nm for zidovudine [27].

Simultaneous spectrophotometric estimation of LMV and Silymarin in mixture was developed which was claimed to be simple, rapid and sensitive. The method employed formation and solving of simultaneous equation using the analytical wavelengths of 270.9 nm and 326.4 nm, respectively [28].

Habte developed a simultaneous determination of LMV and Zidovudine in Pharmaceutical Formulations by HPTLC-Densitometric Method. The method developed was based on HPTLC separation of the two drugs followed by densitometric measurements of the spots at 276 and 271 nm for LMV and zidovudine respectively [29]. Very recently an MSc research work is reported at Addis Ababa University using a new HPTLC densitometric method for the simultaneous determination of Abacavir Sulphate and Lamivudine in fixed dose combination tablets [30].

Simultaneous determination of LMV and stavudine in antiretroviral fixed dose combinations was done by first derivative spectrophotometry and high performance liquid chromatography. The first method depends on the first derivative UV-spectrophotometry with zero-crossing measurement technique [31].

Simultaneous determination of LMV, stavudine and nevirapine in antiretroviral fixed dose combinations by high performance liquid chromatography method. RP-HPLC method for the simultaneous quantitative determination of the nucleoside reverse transcriptase inhibitors lamivudine, stavudine with the non-nucleoside reverse transcriptase inhibitor nevirapine in pharmaceutical fixed dose combinations was reported [32].

Among the above reported methods, the HPLC method described that was used to determine LMV in pharmaceutical formulations required more time to equilibrate the column from one analytical run to another. In addition to that the internal standard (Finasteride) used to assay the drug is not easily available [29].

3. Ion Selective Electrodes

An ISE is defined as a potentiometric sensor with a membrane whose potential indicates the activity of the ion to be determined in the solution. The membranes of ISEs consist of (i) liquid water immiscible electrolyte solutions (ii) precipitated salts (single crystals and glasses that used to sense pH and sodium. Direct measurements with ISEs have certain undoubted advantages: (a) they are non-destructive i.e. in direct potentiometery, they do not affect identity of the test solution; (b) they are portable; (c) they are suitable both for direct determinations and as sensors for titrations; and (d) they are not expensive [32]. Further, they may be used in measuring ions or in direct potentiometery or titrations while the test sample exhibits turbidity, e.g. insoluble excipients from pharmaceutical tablets.

ISE are membrane electrodes that respond selectively to ions in the presence of others. These include probes that measure specific ions and gasses in solution. Other ions that can be measured include fluoride, bromide, iodide, cadmium and gasses in solution such as ammonia, carbon dioxide and nitrogen oxide [33].

The widely used fluoride ion selective electrode is based on single crystal, $LaF_{3,}$ as membrane. The silver halide precipitate electrodes are sensitive to light causes composition changes in E_{lSE}, Such electrode type is the iodide ISE which is suitable for measurement of iodide and silver ion activity [34]. Typically, the solid state ion selective electrode devices can be immersed into a sample solution of unknown ion concentration. A standard reference electrode is also placed in contact with the sample solution. The voltage of the electrode device can be measured with respect to the external reference electrode. For example, both the electrode device and the reference electrode can be connected electrically to a reference source such as a potentiometer or voltmeter to display the voltage or potential difference in millivolts (mV) or concentration units of the ion being measured [35].

The basic ISE setup includes a pH/mV meter (capable of reading millivolts), the ion-selective probe (selective for the ion of interest) and a reference electrode immersed in a cell containing ion and a pH buffer and/or ionic strength adjustment solution. The expense is considerably less than other methods, such as Atomic Adsorption Spectrophotometry or Ion Chromatography. ISE determinations are not subject to interferences such as color or turbidity in the sample. There are few matrix modifications needed to conduct these analyses. This makes them ideal for clinical use (blood gas analysis) where they are most popular. They have also found practical application in the analysis of environmental samples, often where in-situ determinations are needed and not practical with other methods [34, 35].

Standard solutions of known concentrations must be prepared with a good precision balance (0.1 mg or better readability). These solutions are then measured with the pH/mV meter after adjustment, if necessary, with pH and buffer/ionic strength. The mV reading of each solution is noted and a plot of log (concentration) Vs the potential in mV recorded. The unknown solution is be measured using the calibration graph or on the basis of the slope within the linear range [34].

Ion Selective Electrodes, including the most common pH electrode, work on the basic principal of the galvanic cell. By measuring the electric potential generated across a membrane by "selected" ions, and comparing it to a reference electrode.

The basic formula is given for the galvanic cell:

$$E_{cell} = E_{ise} - E_{ref}$$

The potentiometric response is based on the Nernst equation which is:

$$E = E^0 - 0.059/n \log a$$

Where E is the reading potential, E^0 is constant potential for slope 0.059 V at 25 ^{o}C, n is charge of the ion and a is the activity of the analyte. Although the electrode responds to the log (activity) of the ion sensed, proportionality of the potential to the log (concentration) is valid only when all the standards and samples have the same ionic strength. This entails that the activity coefficient must be maintained constant. The following relationship holds true for the relation between concentration of iodide and its activity.

$$\alpha_I^- = \gamma_I^- C_I^-$$

Where γ is the activity coefficient and C_I^- is the concentration of the analyte iodide. An ion-selective membrane is the key component of all potentiometric ion sensors. When it is selective, it establishes the preference with which the sensor responds to the analyte in the presence of various interfering ions from the sample. However, an ISE that is not selective to the analyte ion may respond to ion(s) other than itself. The interference of other ion(s) is expressed by Nikolsky equation, which is extension of the Nernst equation which relates the electrode potential to the activity of all the contributing ions in the sample, including any interfering ions. It is the same as the Nernst equation but with **log a** replaced by

$$\mathbf{\log [a_x + k_{x,y}(a_y)^{(Zx/Zy)} + k_{x,z}(a_z)^{(Zx/Zz)} ...etc.]}$$

Where $k_{x,y}$ = selectivity coefficient for ion y of an electrode sensitive to primary ion x

$k_{x,z}$ = selectivity coefficient for ion z of an electrode sensitive to primary ion x

a_x = activity of primary ion x , a_y and a_z = activities of interfering ions y and z

Z_x = an integer with sign and magnitude corresponding to the charge on the primary ion x

Z_y and Z_z = integer with same sign as Zx charge on interfering ions y and z [35]

3.1. Applications of Ion Selective Electrodes

ISEs are produced by a number of industrial companies all over the world. ISEs other than glass electrodes find their principal applications in the fields of environmental analysis (in which they are used to determine the concentration of fluoride, iodide and other halides, nitrate, cyanide, and sulfide species), metallurgy (used to monitor pickling and electroplating baths), geological survey (halides), food analysis (halides), and in clinical assays (Na^+, K^+, Ca^{2+}, Cl^-, and F^- ions). ISEs are frequently used in research, mainly to measure ionic activities in the study of electrolyte equilibria [36-38].

ISEs are generally unaffected by color or turbidity which makes them useful in industrial and pharmaceutical applications. They have widespread applications in the fields of biology, chemistry and medicine. These electrodes provide a useful analytical technique for detecting and measuring the concentration of a particular ion species in solution. The applications of ISE's include biomedical research, clinical testing, industrial pollution testing, pharmaceutical and chemical-process control [37]. The work described in this report illustrates the case for Lamuvidine determination in tablets by back titrations using iodide ISE as endpoint indicator.

3.1.1. Design of ISEs

A) Commercial ISEs

Typical designs of ISE's, including the commercial ones, comprise an ion selective membrane affixed to the lower opening of a plastic electrode body. The electrode body has an inner electrolyte solution and a reversible internal reference electrode sealed within. This design has several disadvantages including durability and better reproducibility [39]

B) Adsorbed or Coated Conductor Electrodes

ISEs can also be prepared without internal reference electrodes by coating or adsorption of the active material on a conductive surface (silver, platinum and graphite rods). This obvious simplicity allows reuse of the conductor after cleaning. The ISE active material may be a coating of an ion-ion association salt or adsorbed sparingly soluble inorganic salts such as silver halides.

The latter types are all solid state ISEs as the salts are directly adsorbed on the conductor surface, graphite rod decreases the porosity of the electrode and acts as a binder for the solid sensor composition [36-39].

Solid state electrodes have good physical contact between the ion selective membrane and the element to insure direct electrochemical interaction with test solution. However, these types of electrodes tend to drift and may require more frequent calibration of electrode response [39, 40].

Laboratory designed solid state ion selective electrodes are inexpensive and simple ion sensors equally applicable as the expensive commercial ISEs for detecting and measuring the concentration of ions in solution including potentiometric titrations. In the results and discussion of this work the application of an iodide electrode used in end-point detection of redox titrimetry involving the conversion of iodine to iodide.

3.2. Iodide Ion Selective Electrode

Iodide ion-selective electrodes consist of compressed pellet of silver sulfide and silver iodide. These selective electrodes are generally prepared by co-precipitation of Ag-iodide and Ag_2S. To an equal molar solution of potassium halide and sodium sulfide excess silver nitrate solution is added till complete precipitation is produced [41].

$$KI\,(aq) + Na_2S\,(aq) + 3AgNO_3\,(aq) \longrightarrow AgI(s) + Ag_2S(s) + KNO_3\,(aq) + NaNO_3\,(aq)$$

Due to its large compressibility, silver sulfide serves as a base material for halide as well as for metal electrodes. Silver sulfide is an ion conducting material and exhibits larger silver ion conductivity than the corresponding halide compounds and can be easily pressed into thick disks. Because of its silver ion conductivity, this material shows a Nernestian response to silver ions in solution and is thus well suited for use in ion-selective electrodes. Such semi-conductor like materials do not respond so strongly to redox systems because of their larger forbidden energy bands, which cannot accommodate any electrons. Chloride and bromide do not interfere even if present at higher concentrations. Sulfide, cyanide and ammonia typically interfere. Silver, mercury (II), sulphide and, cyanide ions interfere as they are directly sensed by the electrode.

Ammonia interference arises from its complex formation with the silver on the electrode. Strong reducing solutions may damage the membrane by forming a layer of metallic silver on the surface [42].

3.3. Iodometric Potentiometric Titration

ISE-based potentiometric redox titration is a technique similar to direct titration of a redox reaction with color-changing indicator. No indicator is used in the former; instead the potential response due to the changing sensed analyte ion concentration during the titration reaction shows abrupt change at the end point. To do this, two electrodes are used, the ISE, as indicator electrode and reference electrode. The potential is recorded at intervals as the titrant is added. A plot of the potential in mV against volume of the added titrant will a give typical titration curve where the end point of the reaction is half-way between the jump in potential [43].

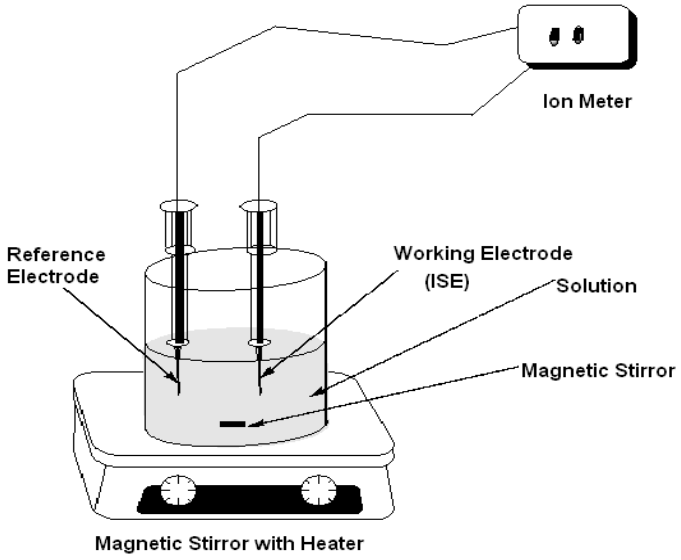


Fig.2. A Potentiometric titration cell

Potentiometric titration response depends on the concentration of iodide as follows:

$$E = E^0 - 0.059\ V/n \log [I^-]$$

Where E terms have their usual significance, the potential changes with changing concentration of iodide.

The determination methods mentioned in the review literature have certain drawback, some of them use unstable reagents and expensive chemicals, low sensitivity and some of them liquid-liquid extraction step or heating step and the others affected by color or turbidity with large time of analysis [23, 43].

This report relates to the application of inexpensive potentiometric iodometric titrations with iodide solid state ISE for the determination of LMV in tablets instead chemical indicators e.g. starch-iodide complex as indicator.

Several papers on the applications potentiometric ISE were reported with the use some of them are the following;

Determination of thiosulphate was made at trace levels using an iodide ion-selective electrode. Aqueous thiosulphate was shaken with a solution of iodine in carbon tetrachloride. The organic phase was separated; the excess of iodine extracted into an aqueous sulphite solution and the iodide liberated was measured with an iodide-selective electrode. It was possible to determine thiosulphate in various concentration ranges by changing only the concentration of the standard iodine in carbon tetrachloride solution. A linear calibration graph was obtained over the concentration range 4 x 10^{-7} – 8 x 10^{-6} M thiosulphate with a relative standard deviation was 0.92 % at the 6 x 10^{-6} M thiosulphate level. Good recoveries of thiosulphate from natural water samples were achieved using ISE method [44].

A simple and sensitive micro method for chloral hydrate determination based on oxidation with iodine in chloroform solution is described. The produced iodide ion in the extract is determined using the iodide ion-selective electrode by either a direct measurement, standard addition technique or potentiometric titration with standard silver nitrate solution. Samples containing 0.1 - 4.0 mg chloral hydrate are analyzed with an average recovery of 99-9% and standard deviation of 0.1 % [45].

Iodide-selective electrodes based on bis (2-mercaptobenzothiazolato) mercury(II) [Hg $(MBT)_2$] and bis (4-chlorothiophenolato) mercury(II) [$Hg(CTP)_2$] carriers were described [45]. The electrodes were prepared by incorporating the ionophores into plasticized PVC membranes, which were directly coated on the surface of graphite disk electrodes.

The electrodes displayed high selectivity for iodide with respect to a number of inorganic and organic anions. The influence of the membrane composition and pH, and the effect of lipophilic cationic and anionic additives on the response properties of the electrodes were investigated. The electrodes exhibited near-Nernstian slopes of -57.6 ± 0.8 and -58.4 ± 1.4 mV/decade of iodide concentration over the range $1 \times 10^{-6} - 1 \times 10^{-1}$ M, with detection limits of ~4×10^{-7} and 6×10^{-7} M for the electrodes based on $[Hg(MBT)_2]$ and $[Hg(CTP)_2]$, respectively [46].

Iodide ISE application from this Laboratory for potentiometric titration was reported for the determination of iodine in iodized salt and ascorbic acid by iodide selective electrode using iodate-iodide mixture for the generation of iodine which would react with ascorbic acid [47].

Basavaiah and co-worker established an indirect titrimetric for the assay of LMV in tablets on the basis of its oxidation with bromine (bromate-bromide redox system in acid) using a colorimetric end-point indicator. This project is aims at replacing the colorimetric indicator system with a potentiometric titration using graphite-based iodide selective electrode for the indirect determination of the antiretroviral drug Lamivudine using the same titrimetric reactants employed by Basavaiah and co-worker [23].

4. Objectives

4.1. General Objectives

The main objective of this project is to assess the application of iodide ion selective electrode for the determination of Lamivudine.

4.2. Specific Objectives

1. To validate electrode response properties
2. To study basic back titration parameters for the bromate-bromide mixture with LMV.
3. To determine the amount of Lamivudine per tablet.

5. Experimental

5.1. Reagents and Chemicals

Sodium sulfide 32-38 % (BDH, England), Potassium iodide 99-100.5 % (Scharlau chem. S.A European Union), Silver nitrate 99.8-100.5 % (Riedel-de Haen Europe) were used for coating of electrode, Potassium iodate 99.5 % purity (BDH, England), sulphuric acid 97-99 % (Techno Pharm. Chem. Bahadurgarh India) and potassium iodide were used to prepare iodine and water solution, Potassium chloride 99 % (Riedel-de Haen, AG) were used inside the reference electrode, Potassium nitrate (Research Chemicals Ltd) was used to prepare (0.1M) to prepare the standard solutions, Sodium carbonate 99 % (Hopkin & Williams Ltd.), Sodium thiosulfate 99.9 % (SISCO Chem. Industries, Mumbai, India) were used for thiosulphate solution preparation, Lamivudine 99.5 % purity (APL Research Center Analytical Research Department). Other reagents used include Hydrochloric acid 37 % (Riedel-de Haen AG), potassium bromide 99.5 % (BDH, England) and potassium bromate 99.8 % (BDH, England). Distilled or de-ionized water was used throughout the experiment.

Bromate–bromide mixture (5 mM $KBrO_3$ – 50 mM KBr) was prepared by dissolving 0.835 g of $KBrO_3$ and 6 g of KBr in water and diluting to one liter in a volumetric flask and used in the titrimetric analysis. A solution of 0.02 M $KBrO_3$ – 0.02 M KBr was also prepared and used for the titrimetric procedure. Hydrochloric acid solution (5M) was prepared by diluting the appropriate volume of concentrated acid with water. Sodium thiosulphate solution (0.03 M) was prepared by dissolving 7.44 g of the chemical in 1 litre of water for standardizing with iodine generated from iodate-iodide solution in acidic medium. Potassium iodide (10 %) was prepared by dissolving 10 g of the chemical with water. Similarly Iodate-Iodide mixture (5 mM KIO3-50 mM KI) was prepared by dissolving 1.07 g KIO3 and 8.3 KI in water and diluting to one liter calibrated flask.

5.2. Apparatus and Instruments

Potentiometric measurements were made at room temperature using Jenway 3345 Model Ion meter with graphite coated electrode (GCE) Ag_2S-AgI sensor for iodide against a Saturated Calomel external reference $Hg/HgCl_2$ electrode (Hanna Model HI5412). Magnetic stirrer was used to mix the solution, Filter paper was used to removed the most of the excipients in the lamivudine tablets, mortar and pestle were used to grind lamivudine tablets.

5.3. Electrode Preparation

The electrode was prepared by using the same procedure earlier reported by Mihretie and Mekuannt and Moges [47]. A 2 cm long graphite rod taken from dry cell was cleaned and inserted into a ball point pen older so that 1.5 cm length of the rod was protruding out, and from this part, 0.5 cm of it (just after the pen) was covered by paraformaldydye film (American Can Company) to avoid contact of the test solution with the copper wire used to connect the electrode with the pH/mV. The electrode was dip-coated by keeping it into freshly prepared saturated solution of sodium sulfide, and silver nitrate respectively for 30 minutes in each solution. The electrode was washed several times with water. The same procedure was repeated using saturated solution of potassium iodide and silver nitrate to precipitate the electroactive species (Ag_2S-AgI) on to the graphite electrode. The electrode was rinsed using small drops (5 drops) of distilled water. 2-3 drops of mercury metal was added from the top position of the pen holder to insure electrical conductivity between the electrode and the copper wire. The electrode was allowed to stay for 2 hours and finally conditioned for at least an hour before use. Stable potential was recorded within 1-5 minutes after conditioning by using the Teflon coated magnetic stirring bar [41].

5.4. Solution Preparation

5.4.1. Preparation of Calibration Standards

0.1 M stock solution of iodide was prepared by dissolving 4.15 g of KI in 250 ml volumetric flask. $1x10^{-2}$ M, $1x10^{-3}$ M, $1x10^{-4}$ M, $1x10^{-5}$ M, $1x10^{-6}$ M solutions were prepared by tenfold serial dilution of the stock using 0.1 M KNO_3 to keep the ionic strength constant at 0.1.

5.4.2. Solution Preparation for Standardization of Sodium Thiosulphate

A 0.07 M $Na_2S_2O_3$ was prepared by dissolving 8.7 g of $Na_2S_2O_3$ in 500 ml of freshly boiled distilled water containing 0.05 g of Na_2CO3. This solution was kept in a tightly capped amber bottle. Similarly, 0.01 M KIO_3 was prepared by weighing 1.07 g of solid reagent and dissolving it in a 500 ml volumetric flask. 50.00 ml of 0.01 M KIO_3 solution was added into a flask. To this solution were added 2 g of solid KI and 10 ml of 0.5 M H_2SO_4. This solution was titrated potentiometrically against the sodium thiosulphate using the iodide electrode-reference electrode system.

5.4.3. Preparation of Lamivudine Sample Solutions

Ten tablets each containing 150 mg LMV (according to manufacturer's certificate) were crushed into powder with mortar and pestle). The mass of the powder containing 150 mg (the mean mass per tablet was 312.26 mg) 0.75 mg/ml LMV was transferred into 200 ml calibrated flask then dissolved with water, the residue was filtered by using Whattman filter paper No 41 and the bottle filled up to the mark. The tablet solution still remained turbid which was used directly for analysis. For comparison of test results, single tablet solutions were also prepared in 200-ml volumetric flasks. The nominal concentration of LMV was 0.75 mg/ml. For reference drug substance 75 mg LMV was taken and directly dissolved in 100-ml volumetric flask (concentration 0.75 mg/ml LMV).

5.4.4. Potentiometric Titration Procedure for LMV

A measured volume (2-40 ml) of LMV tablet (or the reference drug solution) containing 0.75 mg/ml was acidified to 1 M HCl in a 50 beaker and mixed with 10 ml of Bromate–bromide mixture (initially 5 mM $KBrO_3$ – 50 mM KBr) and kept for 15 min with occasional swirling. A solution of potassium iodide (10 % w/v) was added. The iodine produced from any LMV-unreacted bromine from the Bromate–bromide system was titrated with thiosulphate using the iodide ISE for monitoring the end point. Stepwise addition of the thiosulphate from a burette was made and the potentials recorded at equilibration.

5.4.5. Reference Method Procedure

For inter-comparison with the developed potentiometric iodomteric titration method for LMV determination, the following spectrophotometric procedure was adopted [20]. One tablet (instead of 20 tablets used in ref [20] was initially weighed and powdered in a clean mortar and pestle. A quantity of the powder containing 50 mg of LMV was transferred to a 500 ml volumetric flask and about 400 ml of de-ionized water was added. The content in the flask was sonicated for about 5 minutes before dilution to the 500-ml mark. A portion of this cloudy solution was filtered through a 0.45 μm filter, discarding the first few ml of the filtrate. Five ml of this solution was taken in a 50-ml volumetric flask was diluted with 0.1 M sulphuric acid. The absorbance of this solution was recorded in a 1 cm cuvette at 280 nm against a cell containing a blank. The blank solution (50 ml) was prepared by diluting 5 ml of water with 0.1 M sulphuric acid. The mass or the percentage of LMV ($C_8H_{11}N_3O_3S$) per tablet was calculated using the absorptivity value of 60.7 ($A_{1cm}^{1\%}$ = 607). Additionally, absorbance calibration at 280 nm was made for 0.01 mg/ml – 0.1 mg/ml of standard LMV in 0.1 M sulphuric acid was also made to cross check the data validity of the tablet solutions. The UV method was also applied to assay the drug in a blind sample and the lamivudine reference material, the latter after direct dissolution in water. The blind sample was prepared by dilution to about 5 times of the powdered tablet in 200 ml of water which was originally with a label value of 150 mg of lamivudine per tablet.

6. Result and Discussion

6.1. Calibrtaion of the Iodide Selective Electrode

The iodide selective electrode was calibrated by preparing different solutions which differ in concentration from 0.1 M to 10^{-6} M KI solutions. The solution was prepared by ten fold dilution of succsesive solution with 0.1 M KNO_3. A plot of potential (mV) vs the logarithm of the concentaton of iodide was found linear for 10^{-1} M to 10^{-6} M iodide and exhibited a nernestian behavior with a slope of 57.57 mV/decade.

The EMF of the cell for the Ag_2S–AgI membrane electrode coupled with saturated calomel reference electrode plotted against –log [I⁻] is shown in Fig. 4. The EMF of the cell was found to follow the following equation.

$$E = -778.1 + 57.57 \log [I^-]$$

The electrode response showed linearity in the EMF vs. –log [I⁻] plot over the concentration range of 10^{-1} M to 10^{-6} M with a slope of 57.57 mV per decade change in concentration of iodide ion at 25^0 C (R = 0.99989). The slope is near to the expected value from Nernst equation and is quite acceptable for a well behaved solid state membrane electrode. Detection limit of the electrode was $6x10^{-6}$ M with standard deviation of 1.80871 (n = 10) at $2x10^{-4}$ M iodide. The sensors have response time of 1-5 minutes. Due to potential drifts of electrode responses from time to time, the electrode was frequently calibrated (once day) within the desired working range before measuring the unknown sample.

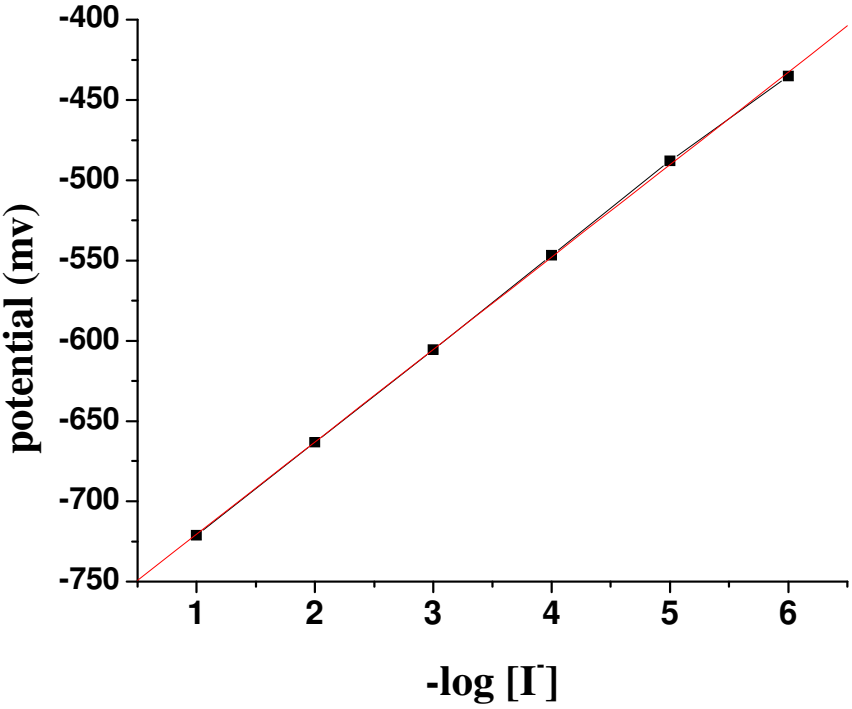


Fig.3. EMF response for calibration graph of the iodide selective electrode titrated with 10^{-1} to 10^{-6} M KI (Slope 57.57 mV/decade).

6.2. Standardization of Thiosulphate

Standardization of sodium $Na_2S_2O_3$, which was used as a titrant in all the experimental results described below, was done by using iodide selective electrode. The solution prepared as in 5.4.2 was immediately titrated with $Na_2S_2O_3$ solution. The concentration of the $Na_2S_2O_3$ was calculated from the potentiometric titration curve shown in Fig. 5. Using this method, the mean volume of $Na_2S_2O_3$ solution (n = 3) equivalent to 50.00 ml of the 0.010 M KIO_3 solution from three titrations was 42.75 ml. The concentration of the thiosulphate solution was calculated to be 0.0702 M.

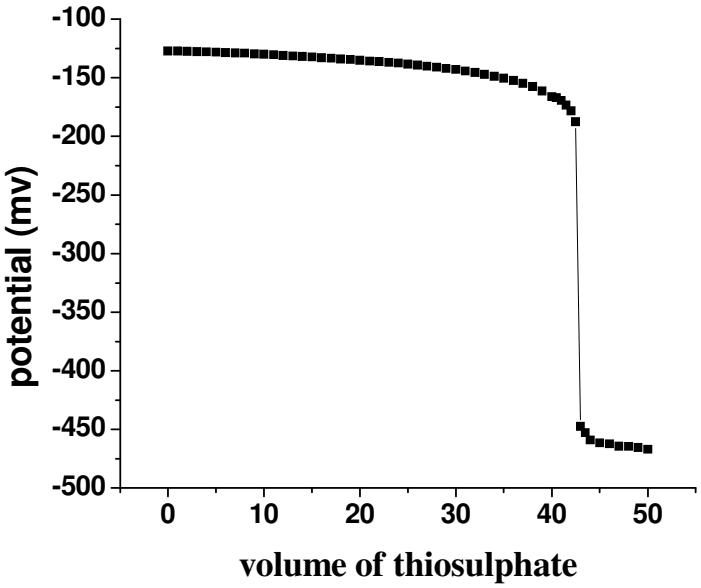


Fig.4. Titration curve for the standardization of thiosulphate by potentiometric titration of 50 ml of 0.01 M KIO_3.

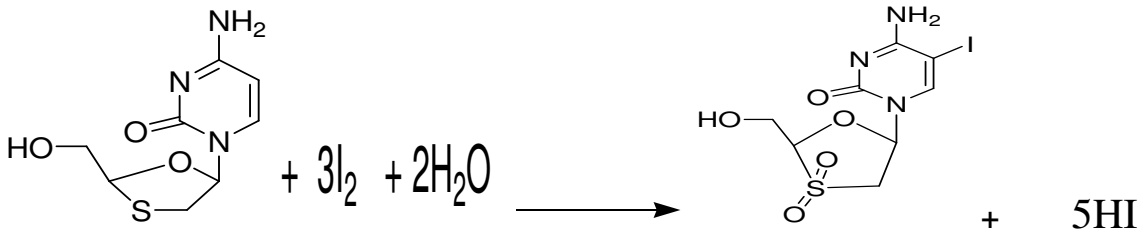
6.3. Possible Test Methods Studied

The possible reaction of iodine with LMV was studied, that would make the reaction route shorter if feasible. Further, the possibility of direct potentiometric endpoint detection of any iodine (iodide oxidized) by bromine left-over from the *in situ* generation of the oxidant (bromate-bromide system) that reacted with LMV was also investigated.

I) Testing a Possible Reaction of LMV with Iodine

The possible reaction of LMV with iodine was tested initially which would obviously shorten titration reaction steps if feasible.

$$LMV + 3I_2 + 2H_2O \longrightarrow LMV\text{-}I(o_2) + 5I^- + 5H^+$$



Testing this possible LMV-iodine reaction would have an advantage over the reagent cost and the time needed to determine LMV. However, iodate-iodide system that generate tri-iodide titration response with and without LMV showed that similar to the titration of iodine with thiosulphate due the absence of reaction between iodine and LMV. LMV was tested with tri iodide. In this experiment tri iodide was generated by the reaction of iodate with excess iodide in the presence of sulfuric acid (Eq. 1 & 3) and back titrated with standard thiosulphate solution (Eq 4 & 5). The expected reaction of LMV with iodine would be as follows:

$$IO_3^- + 5\,I^- + 6\,H^+ \longrightarrow 3\,I_2 + 3\,H_2O \quad \text{.....(1)}$$

$$LMV + 3I_2 + 2H_2O \longrightarrow LMV\text{-}I(ox) + 5H^+ + 5I^- \quad \text{.....(2)}$$

$$2I_2 + I^- \longrightarrow 2I_3^- \quad \text{.....(3)}$$

Back titration of excess triodide (iodine complex) with Iodide ISE

$$I_3^- + 2S_2O_3^{2-} \longrightarrow 3I^- + S_4O_6^{2-} \quad \text{.....(4)}$$

If there was a reaction the net reaction for the oxidation of LMV with iodate would be

$$IO_3^- + H^+ + LMV \longrightarrow LMV\text{-}I(o_2) + H_2O \quad \text{.....(5)}$$

The experiment result showed that at the specified condition the reaction between LMV and iodine did not occur at all (Fig. 6).

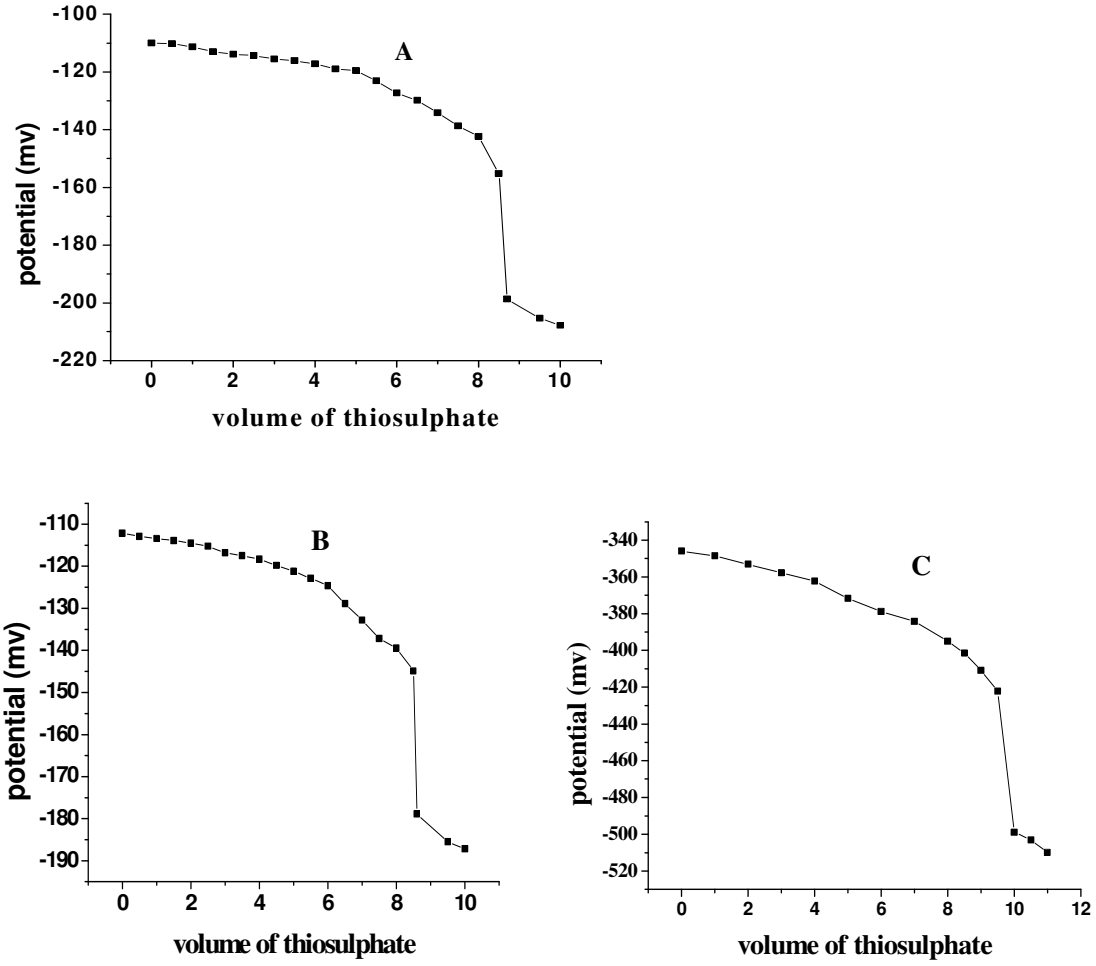


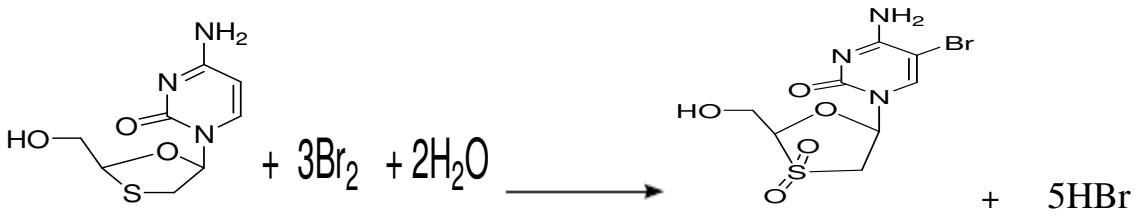
Fig. 5. Titration of iodine only with thiosulphate A) (end point 8.6 ml), Titration of iodine with thiosulphate in presence of B) 0.75 mg LMV (end point 8.55) C) 7.5 mg LMV (end point 9.75 ml)

If there was reaction, the expected volume of thiosulphate at 10 ml 0.01 M iodate, 0.75 mg LMV, and 0.07 M thiosulphate would have been 8.3 ml. The mean end point volume (n = 2) for the titration was 8.6 ml. All the iodine from the iodate added in presence of LMV was identical the iodidate solution alone. The amount in meq of iodate recovered from the LMV-treated test solution was 0.602 relative to the iodate without LMV 0.6 meq. Similarly the expected volume at 10 ml 0.005 M iodate, 7.5 mg LMV and 0.03 M thiosulphate would be 3.46 ml, but it provides the average of 9.75 ± 0.25 ml, the iodate was consumed only by thiosulphate excluding LMV. The results clearly show that the iodate did not react with LMV within the studied period of 5-20 min.

II) Monitoring the Reaction of LMV with Bromine via Direct Titration with Iodide

An aqueous solution of LMV was treated with a known excess of bromate-bromide in HCl medium followed by the estimation of unreacted bromine by iodometric back titration.

$$LMV + 3Br_2 + 2H_2O \longrightarrow LMV\text{-}Br(o_2) + 5Br^- + 5H^+ \ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$



The iodometric determination of the excess bromine from the first reaction is back titration as reported by Basavaiah and co-worker [24]. The attempt here is to monitor the unreacted bromine by direct titration with iodine though the appearance of iodide leading to a potentiometric end-point detection with iodide ion-selective sensor instead of monitoring the bromine formed by titration with thiosulphate using a bromide ion selective electrode. No attempt was made to construct a bromide electrode as it was reportedly unstable for long time applications.

$$Br_2 + 2I^- \longrightarrow I_2 + 2Br^- \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

About six experiments was done by taking 5 ml-50 ml (bromate-bromide), 10 ml 7.5 mg LMV containing solution, 5 ml 5 M HCl and keeping it for 15 minutes with continues shaking, then titration with 0.001 M potassium iodide. During titration the yellow color of bromine changed to brown color indicating that the presence of iodine. This titration was expected to be easier to calculate the amount of LMV from the curve, but the response time of the electrode was very large about 12-15 minutes for one addition of iodide volume which is not acceptable for monitoring titration reactions.

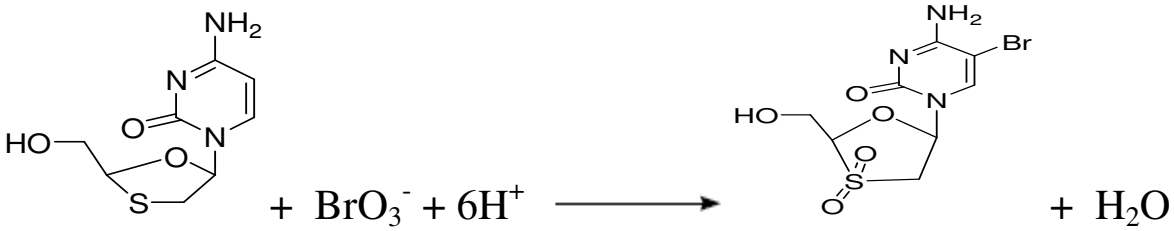
III) Modifiying the Basavaiah Method: **Potentiometric Iodometric End point Detection**

The disappearance of iodine generated could still be followed by the generation of iodide using the same end-point indicating electrode in its titration with thiosulphate.

$$BrO_3^- + 5Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O \quad \text{.....(8)}$$

$$LMV + 3Br_2 + 2H_2O \longrightarrow LMV\text{-}Br(o_2) + 5Br^- + 5H^+ \quad \text{.....(9)}$$

$$LMV + BrO_3^- + 6H^+ \longrightarrow LMV\text{-}Br(o_2) + H_2O \quad \text{.....(10)}$$



The Back titration reactions are:

$$I^- + 3Br_2 + 3H_2O \longrightarrow IO_3^- + 6Br^- + 6H^+ \quad \text{.....(11)}$$

$$IO_3^- + 6H^+ + 5I^- \longrightarrow 3I_2 + 3H_2O \quad \text{.....(12)}$$

$$5I_2 + 2S_2O_3^{2-} \longrightarrow 10I^- + S_4O_6^{2-} \quad \text{.....(13)}$$

The Basavaiah method used is based on the iodometric back titration which was done by treating aqueous solution of LMV with a measured excess bromate- bromide mixture to provide bromine molecule. The liberated bromine reacted with excess iodide to produce iodine for color indicators. In this project the iodometric titration was titrated with thiosulphate solution by potentiometric via iodide ISE. During the titration as the volume of thiosulphate increases the potential decreases more and more indicating that the reaction between thiosulphate and iodine would produce iodide solution which is sensed by the electrode. Since in the Nernst equation the electrode potential and concentration was inversely related as it follows; $E = E^0 - 0.059V/n \log [I^-]$ where E is electrode potential,E^0 is the constant potential at standard condition , $[I^-]$ is concentration of iodide ion. As the iodide ion in the solution increases the potential would decreases.

6.4. Back Titration of Lamivudine Tablet Solution

LMV calculation in meq and mg

Principle: LMV is determined by back tritration of the Bromate-Bromide mixture (bromine solution) after addition of exess iodide which is convetrted to iodine which was determined with thiosulphate. Hence the equation for the quantitation of LMV from the back titration would simply be: **meq (LMV) = meq BrO_3 – meq $S_2O_3^{2-}$** and

$$\text{meq of LMV} = \frac{\text{mass of LMV taken}}{\text{No. of eq of LMV}}$$

$$\text{No. of eq of LMV} = \frac{\text{Molecular mass of LMV}}{\text{No. of electrons involved in the rxn}}$$

From the net equation is 1 mole of I_2 equivalent to 1 mol $S_2O_3^{2-}$. Hence, the equivalent weight of thiosulphate is its formula weight. The eqn. below for bromate BrO_3^- = $3Br_2$ (6 Br^o), has 6 equivalents per mole BrO_3^-. one deduces that the mass of 1 eq of LMV in mg is its FWT/6 , i.e 229.3 mg/6, which is 38.22 mg/meq, This easily allows one to calculate the mass of LMV in titration test solutions.

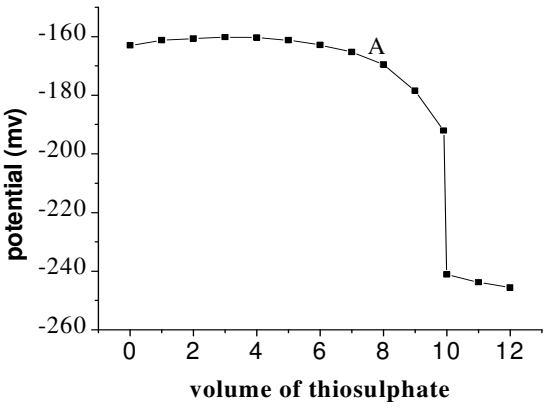
$$BrO_3^- + 6I^- + 6H^+ \longrightarrow Br^- + 3I_2 + 3H_2O \quad \text{……..(14)}$$

The iodine in this reaction was titrated with thiosulphate which used for the indirect determination of LMV from its solution.

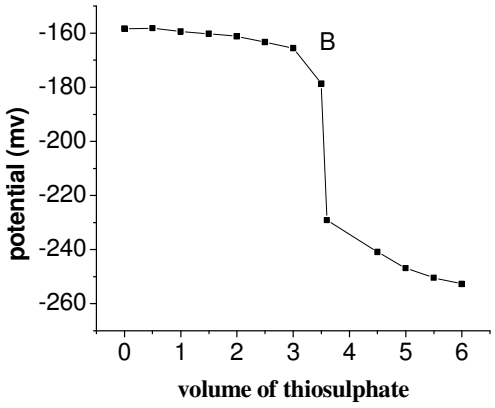
6.5. Study of Possible Interference from Excipients in LMV Tablets

The comparison of the result of sample solutions taken from single tablet, reference drug substance, filtered and unfiltered test mixtures from the ten-tablet bulk sample and the blank bromate solution was done as shown in Fig.7 A-E. The titration curves B, C, D and E clearly indicate that the excipients in the in the tables did not exhibit any interference on the system. Actually, the filtered tablets had still little turbidity in routine titrations. The performance of

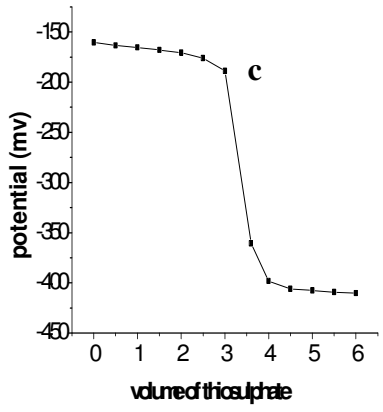
electrode response was not affected due to possible poisoning or adsorption of the excipients on sensing layer. The blank value obtained from curve A is used to assay the total bromate without LMV.



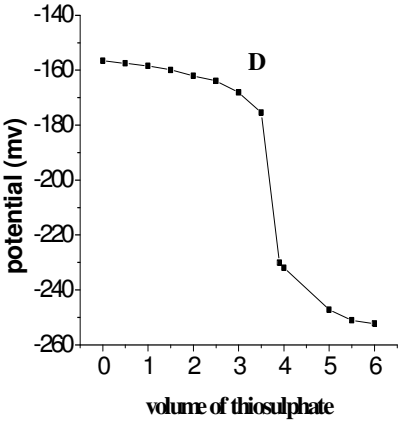
Graph of Blank (bromate)
End point 9.95 ml



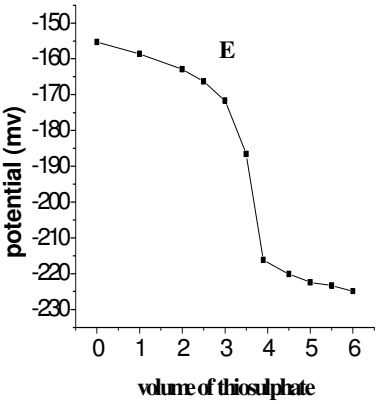
Graph of Reference Drug substance
End pint 3.55



Graph of single tablet filtered
End point 3.3 ml



Graph of filtered ten tablet
Powder end point 3.7 ml



unfiltered ten tablet Powder
End point 3.68 ml

Fig.6. Titration curve of potential *vs.* volume of thiosuphate for blank and different lamivudine samples having 0.75 mg/ml.

The amount of Bromate (in meq) found by the iodide ISE from the blank (without LMV) from the blank titration using (10 ml of 5 mM-50 mM Bromate-bromide, 5 ml 5 M HCl and excess KI (5 ml of 10 %) was found to be 0.2985 meq which will be used as total bromate. This is equivalent to standardizing the Bromate solution. The results in Fig. 7 (B – E) are also summarized in Table 2. The whole LMV amount in this work was calculated using the difference in meq between the blank value the $Na_2S_2O_3$.

LMV aliquot (7.5 mg)	**Mean end point Volume of $Na_2S_2O_3$ (ml) (n=3)**	**Mean LMV found (mg)**	**SD (n=3)**	**RSD (%)**
Reference	3.55	7.34	0.06	0.78
Single tablet	3.3	7.59	0.44	5.80
Ten tablet filtered	3.7	7.17	0.11	1.59
Unfiltered ten tablet	3.68	7.20	0.21	2.92

Table 2. Comparison of the amount of LMV found for LMV test samples taken (reference value and tablet label value 7.5. mg LMV).

6.5.1. System Titratability for LMV Determination

Titratability of the redox system with in presence of with different amounts of LMV (1.5 - 30 mg) using both tablets and the drug substance was investigated. For amounts higher than 7.5 mg LMV, the bromate-bromide mixture was adjusted to is 0.02 M BrO_3^- and 0.2 M Br^- but with the same titrant , 0.03 M $S_2O_3^{2-}$, The concentation of $S_2O_3^{2-}$ can be doubled to reduce the resulting titration volume.

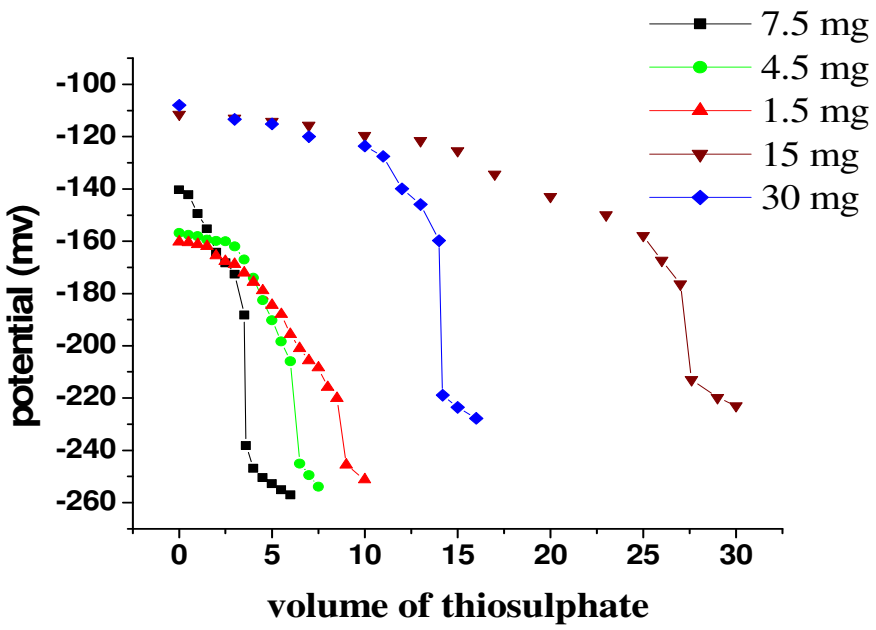


Fig.7. Titration curves using 0.03 M $Na_2S_2O_3$ for 1.5 -7.5 mg LMV in tablets with 5 mM BrO_3^-- 0.05 M Br^- , for 15 and 30 mg of LMV in 0.02 M BrO_3^-- 0.2 M Br^- .

Potentiometric titration for LMV determination was feasible for 1.5-30 mg of the dug in Tablets and reference drug substance.

LMV taken (mg)	**LMV found (mg) from Reference ± SD (n=3)**	**LMV found from single tablet ± SD**	**LMV found from Ten tablet filtered ± SD**
1.5	1.47 ± 0.06	1.44 ± 0.02	1.40 ± 0.05
4.5	4.45 ± 0.04	4.41 ± 0.01	4.41 ± 0.178
7.5	7.34 ± 0.06	7.59 ± 0.44	7.20 ± 0.21
15	14.32 ± 0.48	14.28 ± 0.15	14.56 ± 0.35
30	29.48 ± 0.23	29.20 ± 0.18	28.97 ± 0.35

Table 3. Amount of LMV found during the titritability study for different amount in mg taken.

This result used to estimate the titritability of LMV for which range of LMV amount could be determined at the specified condition. In all the cases, the amount of bromate reacted corresponds to the amount of LMV. The recent published method of LMV determination using bromate-bromide mixture excess iodide titrated with 0.03 M $Na_2S_2O_3$ and starch indicator showed that the application was from 2.5-7.5 mg of LMV [24] and 3.5-10 mg range as reported by [48] using excess chloramine-T in sulphuric acid medium for iodometric determination. The potentiometric ISE method was, sensitive for smaller and larger amount of analyte, found to be applicable effectively over 1.5-30 mg range which was very wider than the recent reported determination methods for LMV.

6.5.2. Determination of Lamivudine in Tablets and Reference Drug

The determination was studied by taking the 7.5 mg LMV for single tablets, powdered samples, and the reference drug substance as prescribed in the procedure using the direct back titration. To test of the results check the validity. Standard solution containing 3.75 mg LMV was added to titrate the tablet test solution. The results of all the tests for triplicate measurements are shown in Table 4. The reference drug recovery was 98.4 ± 0.8 % for the for the supplied 99.5 % purity. For the tablet samples with standard addition the recovery was 98.84 ± 5.3 %.

LMV sample	**LMV found in mg(n = 3)**	**LMV amount per tablet (mg)**
Single tablet	7.59 ± 0.44[a]	151.8 ± 8.8 [a]
Ten tablet filtered	7.17 ± 0.11[a]	143.4 ± 2.3 [a]
Ten tablet unfiltered	7.19 ± 0.21[a]	143.8 ± 4.2 [a]
Standared addition for ten filtered	7.56 ± 0.19[a]	151.2 ± 3.8 [a]
Reference drug substance	7.34 ± 0.06[a]	146.8 ± 1.14 [a]

"a" is no significant difference between the result

Table 4. Statitistical comparison of back titration method providing LMV amount per tablet

The label value of the manufacturer was 150 mg per tablet. The International Pharmacopiea accepts variation of ± 10% of the label value. The one way ANNOVA test (Duncan multiple test) by SPSS showed that there was no significant different between the result of both LMV amount found in mg and amount of LMV per tablet. Therefore ISE result have a good agreement with the labeled LMV amount per tablet and the experimental values lie within the range recommended by the International Pharmacopea.

6.5.3. Interference Studies

As in table 2 mentioned the effect of interference was studied by comparing filtered LMV and without filtraion how the residue of the LMV tablet change the result. In this experiment the result of filtered LMV and without filtration was not changed since the electrode sensitivity was only for the concentration of iodide present in the mixture. The behaviour of the titration curve of the unfiltered tablet test samples with the the turbid substances and the residues was not affected. Further, electrode performance was still the same despite the presence of the pharmaceutical excipients used for taste, binding, coloring. For 7.5 mg LMV per sample from a filtered tablet solution of the titration data was found 7.17 ± 0.11 mg and 7.20 ± 0.21 mg were the result of unfiltered LMV tablet dissolved in water. One sample t-test precision indicate there was no significant different between the mean value of the filtered and unfiltered LMV sample at 95 % confidence interval.

6.5.4. Effect of the Acid

The effect of concentration was studied keping the other parameters within the optimal conditions. For the hydochloric acid concentration within 0.2 M -1.5 M the shape of the titration curve and the endpoint volume of thiosulphate were not significantly different. Hence a 1 M HCl was used in the general procedure for the titrimetric determination of LMV in tablets. For test solutions with 7.5 mg LMV the endpoint volume of 0.03 M $Na_2S_2O_3$ varied within 3.3 ml - 3.65 ml which is similar to 1 M HCl.

6.5.5. Precision of the Method

The amount of LMV found from the titration curves containing 0.005 M-0.05 M Bromide, 5 ml 5 M HCl and 10 % (M/V) excess iodide solution titrated with 0.03 M $Na_2S_2O_3$ solution was studied. The mean mass and intra-day repeatability standard deviation for 7.5 mg reference LMV was found to be 7.304 ± 0.3 (n = 6) and inter-day reproducibility was 7.272 ± 0.3 (n = 6). The precision of the result compared by using SPSS version 17 analysis software, one sample t-test at 95 % (P = 0.005) showed that there was no significant difference between the inter-day and intra-day measurements.

6.6. Laboratory Developed ISE Result Analysis for LMV Tablet Solutions

In this work the determination of LMV amount from singly added tablets (n = 3) the mean value was 7.59 of 7.5 mg added per tablet with SD of 0.44. The sample taken from the ten tablet powdered samples and reference LMV sample was studied by doing triplicate analysis for each. The mean mass per tablet was 312.26 mg (from which the label value was 150 mg was LMV). The reference drug substance taken was 150 mg for comparison. The amount of LMV found from reference substance LMV was 146.8 ± 1.14 mg (% recovery of 98.4 ± 0.8) while the LMV found per tablet was, based on a single tablet was 151.8 ± 8.8 mg, 143.4 ± 2.3 mg, for the filtered and 143.8 ± 4.2 for unfiltered powdered sample. The results for recorded LMV tests in the tablets lay within the WHO International Pharmacopeia which states ± 10 % of the label values, namely 135 to 165 mg. It is established that the actual availability of LMV from single tablet and ten tablets at the consumer level can vary for certain range because of uneven distribution of LMV in the product. This loss of LMV might be due to the stability factor, impurities, packing and environmental conditions during storage and distribution. Moreover, even though one tablet & ten tablets sample were taken from the same packing, the amount of LMV found (calculated) in these samples is different. Since LMV tablet has the binder and certain ingredients, this variation might be due to their in-homogeneity and un equal distribution of LMV during analysis and in addition to this some amount of LMV might be discarded with the filtrate (residue or ingredients).

6.7. Blind Sample: Comprarison with Spectrophometer

The result of unkown LMV sample determined by iodometric titration and independently conducted spectrophotometry was compared as shown in Table 5. the The concentration of LMV obtained by direct back iodometric titration (1.43 ± 0.18) was in a good agreement with the result of direct measurement of spectrophotometry (1.37 ± 0.10). The recoveries for the official spectrophotometric method and the iodometric back titration for in the reference drug substance were 99.9 ± 0.2 and 98.4 ± 0.8, respectively. The two independent test methods and the comparisons made for recoveries between the methods are important quality control information for the validity of the developed method.

Back-titration Iodometry		**UV Spectrophotometry**
Direct back titration	1.433 ± 0.18*	1.37 ± 0.10*
Standard addition	1.372 ± 0.12 *	-
Reference drug recovery	98.4 ± 0.8**	99.9 ± 0.2 **

* LMV found in mg from unknown (n=3); ** Recovery of reference drug in percent

Table 5. Comparison of independently conducted measurements for the blind sample (nominal value 1.5 mg/ml) including standard addition to the blind sample in the back-titration mode and recoveries of the two methods for the reference drug substance

The result of direct back titration compared with the titration after standard (3.75 mg LMV) added to the blind sample by t-test. The statistical comparison showed that there was no significant difference between the means at 95 % (p = 0.005) confidence interval. Direct back titration result for the blind sample was also compared with the result of spectrophotometric method which exhibited that there was no significant difference between the two methods at 95 % confidence interval.

7. Conclusion and Recommendation

The reported determination of LMV in tablets resulted from the well-behaved titration curves using the solid state iodide ISE as an endpoint indicator; the titration system was applicable for tablet test solutions containing 1.5-30 mg LMV. Direct dissolution of the single tablets, or powdered samples equivalent to one tablet, showed that the electrode endpoint indication can be used without interference from the excipients without affecting the normal behavior of the electrode. Comparison of the mean values for LMV determinations, recoveries and precisions between the back titration system and the official spectrophotometric method exhibited statistical agreement at 95 % confidence levels. Hence, the inexpensive electrode designed in the laboratory can be reliably applied to determine the anti-retroviral drug Lamivudine in tablets even in presence of its exipients used for binding, coloring and taste.

The potentiometric method can possibly be extended to other pharmaceutical drugs which are known to be oxidized by iodine or bromine Stavudine is a similar to LMV with anti-retroviral drug, also with similar structure containing identical oxidzable group found in LMV. This drug therefore deserves investigation with the titration system for testing single or combination drugs. It is expected that the total drug (stavudine and lamivudine) in combination drugs can simultaneously be determined using this bromate-bromide system and the iodide ISE-based iodometric titration.

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Declaration

This undersigned confirm that the results reported in this work were obtained by research carried out by me under the supervision of my advisor in the Faculty of science, Department of Chemistry, Addis Ababa University in the academic year 2010. No part of this work shall be published in scientific journals or reported in the media or presented at a conference without the knowledge and consent of my advisor, who is the principal scientist responsible for my publication. Furthermore if the work is published the international address given should be that of the Chemistry Department Addis Ababa university.

Name: Shemsia Mohammed

Signature ……..

This project work has been submitted for examination with my approval as a university advisor.

Advisor: Dr. Ghirma Moges

Signature ……

Place of Submission: School of Graduate Studies Addis Ababa University

Date: / 07 / 2010