

APPLICATION OF GRAPHITE BASED SOLID STATE IODIDE
SELECTIVE ELECTRODE FOR THE DETERMINATION OF IODINE
IN IODIZED SALTS AND ASCORBIC ACID

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Abbreviations

IDD - Iodine Deficiency Disorder

AI – Adequate Intake

TSH – Thyroid Stimulating Hormones

TGR - Total Goiter Rate

RDA - Recommended Dietary Allowance

WHO – World Health Organization

UNICEF - United Nations International Children's Emergency Fund

ICCIDD . International Counsel for Control of Iodine Deficiency Disorder

CIVD - Cold-induced Vasodilator

DCIP - 2, 6-Dichlorophenolindophenol

EVA - Ethylene- co-Vinyl Acetate

BFEFMCPE - Bis (Ferrocenyl Ethyl) Fluoren-9-one Modified Carbon Paste Electrode

ISEs – Ion Selective Electrodes

CuPc - Copper Phthalocyanine

THF – Tetra Hydrofurane

GCE - Graphite Coated Electrode

DCPIP - Dichlorophenol Indophenol

SD - Standard Deviation

Abstract

A laboratory-made iodide selective solid-state electrode ($\text{Ag}_2\text{S-AgI}$) was prepared. The electrode response was linear in the iodide concentration range of 10^{-6} M to 10^{-1} M, with a slope of 56.85 mV/decade and detection limit of 6×10^{-7} M iodide. The application of the laboratory-made iodide electrode (vs. a saturated calomel reference electrode) was investigated for direct potentiometric titration of iodized salts and for potentiometric back titration of vitamin C in pharmaceutical products and orange juices. The electrode was used as an endpoint indicator system in place of the traditional starch-triiodide complex. Vitamin C with I_3^- was back titrated against $\text{Na}_2\text{S}_2\text{O}_3$ in acidic media while acidified samples of table salt iodized with iodate (in presence of excess concentration of iodide) was directly titrated with $\text{Na}_2\text{S}_2\text{O}_3$. The curves of both titrations exhibited distinct endpoints down to 10 ppm as potassium iodate.

1. Introduction

1.1. Iodine

Iodine is the heaviest member of the halogen group. It has the greatest affinity for oxygen and least for hydrogen. Iodine is a vital element for us and it has specific influence as a catalyser. Although it is found everywhere in our body, yet the thyroid gland is by far the richest in iodine contents, as it contains 1/6 to 1/7 of the total iodine. The thyroid gland is the “central laboratory” in the body, which is responsible for the movement of iodine in the body, its absorption and organic combination. Thyroxin is the compound to which is ascribed to the chief action of iodine in the human body and no other drug can take its place where it is constitutionally indicated. Iodine, as a constituent of thyroxin, plays an important role in the efficient conversion of beta-carotene to vitamin A, with a subsequent increase in the efficacy of protein synthesis and cholesterol absorption. Iodine is readily absorbed (almost 100%) from foodstuffs and is excreted in the urine if not needed by the body [1].

Iodine is used to treat cuts and scrapes on the skin as a tincture of iodine, which is a dilute mixture of alcohol and iodine. Iodine is also used in photography and lasers as silver iodide, in dyes, and as a nutrient added to table salt. Iodine-131 is used for a number of medical procedures, including monitoring and tracing the flow of thyroxin from the thyroid. With its short half-life of 8 days, it is essentially gone in less than three months. Iodine-129 has no important commercial uses [2].

Iodine is an essential nutritional factor with important biochemical functions such as mental development, and basic metabolisms [1]. It is required for maintenance of cell growth in human and animals. In some areas where waters and soils are deficient in the amount of iodine required by the normal diet, iodine supplements as salts are administered. The most important application is the use of iodized table salt in the form of iodate or iodide. Iodine compounds are also used for the preparation of some pharmaceutical products used to compensate iodine deficiency or used as antiseptic and disinfecting agents. Therefore, the determination of iodine and its related

compounds is important in a variety of samples such as iodized salt, foods, fodder, water, clinical and biological samples, pharmaceutical preparation and environmental samples [3].

1.2. Iodine deficiency diseases

1.2.1. Goiter

Iodine is one of the essential trace elements and is of much interest in nutritional research. It is required for the production of thyroxine and tri-iodothyronine hormones for proper growth and development of the human body. In addition to thyroid hormones, iodine appears to be related to other hormones. It is related to estrogen and progesterone and, probably, testosterone, insulin, and other hormones. The most familiar iodine deficiency disorder (IDD) is goiter. However, its effects go beyond goiter to all the effects of iodine deficiency on growth and development. The consequences of IDD are many such as abortions, stillbirths, congenital anomalies, deaf mutism, psychomotor defects, impaired mental function, retarded physical development and goiter with its complications [4].

1.2.2. Neuropsychological Development

The major features of the IDD at the different stages of life are varying effects on brain function, which are characteristic of the effect of any environmental stimulus on a population. These states of altered brain function arise from fetal damage or hypothyroidism at various stages of life, i.e., fetus, neonate, juvenile and adult. There is much anecdotal evidence coming from long-standing observations in Europe supported by recent reports from China and India, indicating that iodine-deficient village populations suffer from general lethargy, poor work performance and defective school performance in children. These effects are due to hypothyroidism, particularly cerebral hypothyroidism. Iodine Deficiency Disorders (IDD), the world's greatest single cause of preventable brain damage and mental retardation, are estimated to affect 30% of the world's population [5].

1.2.3. Iodine and the Breast

All cells in the body need iodine for proper functioning. Iodine deficiency is a major cause of breast cancer and other diseases of the reproductive organs such as ovarian, uterus and prostate cysts and cancers. Iodine supplementation has been quite successful in treating fibrocystic breast disease (cysts in the breast). In one case a 37 year-old woman with severe fibrocystic breast disease was completely cured after supplementing with 50 mg of iodine a day for two months. Women with larger breasts need more iodine than women with smaller breasts. Other medical authorities agree that iodine deficiency can lead to fibrocystic breast disease and/or ovarian cysts [6].

Geographical variations in the incidence of breast cancer have been attributed to differences in dietary iodine intake, and an effect of iodine on the breast has been postulated. The possible interactions between thyroid gland and breast tissue are based on the common property of the mammary and thyroid epithelial cell to concentrate iodine by a membrane active transport mechanism as well as on the presence of thyroid stimulating hormones (TSH) receptors in fatty tissue, which is abundant in mammary gland. The present study found a high prevalence of goiter as well as a high prevalence of autoimmune thyroiditis, confirmed mainly by antibody positivity, in breast cancer patients [7].

1.3. Iodine deficiency in Ethiopia

Studies have demonstrated that goiter is one of the nutritional problems with public health significance in Ethiopia. Subsequently one out of every 1,000 people is a cretin, and about 50,000 prenatal deaths are occurring annually due to iodine deficiency disorders. 26% of the total populations have goiter and 62% of the populations are at risk of IDD [8].

A stratified goiter survey conducted in Ethiopia (with the exception of Tigray) on 36,635 school children and 19,128 household members revealed that the prevalence was 30.6% and 18.7% respectively. It was also found that the prevalence is higher in high altitudes for both school children and household members. A baseline survey of goiter prevalence conducted among five

endemic regions (Shoa-Majetie; Gamu Gofa-Sawla; Shoa-Gohatsion; Bale-Adaba; Gojam-Bure) and four non-endemic regions (Harergie-Alemaya; Shoa-Mojo; Sidamo-Yabello; Arsi-Hurruta) from 1988 to 1991 revealed that both the endemic and non-endemic regions were found to have a higher goiter prevalence rate than previously reported. In this study, it was found that the non-endemic sites were also found to be endemic due to exposure over time. A mean goiter prevalence rate of 21.80 ± 5.4 was found for both the endemic and non-endemic sites [9]. This prevalence rate was regarded as severe according to the classification of WHO/UNICEF/ICCIDD. The prevalence rate was higher in females (56.1%) than in males (50.1%) [8].

A mother with IDD suffers from a high risk of spontaneous abortion and/or stillbirth. The survival of her child after birth is put in jeopardy. Iodine deficiency is also the world's leading cause of negative and irreversible effects on brain development. This is particularly true in early childhood years, resulting in potential IQ reduction of up to 15 points and in extreme cases may result in cretinism and mental retardation. The highland populations of Ethiopia are particularly susceptible to the disorder because iodine is missing from the soil. Run-off from the highlands causes the earth to lose minerals. Soil degradation from floods and over farming also puts local populations at risk [9].

A study in Shebe town (South West Ethiopia) on iodine concentration in salt at household and retail shop levels shows that 81% of household salt samples and 82 % of shop salt samples have iodine levels below the standard (60- 80 ppm as potassium iodate) set by the Quality and Standard Authority of Ethiopia. Information, education and communication on the importance consuming iodized salt and its proper handling in the house and regular monitoring of the salt iodine level at consumer level is essential for elimination of IDD [10].



Figure 1. Goiter prevalence in Northern Ethiopia, taken from humanitarian trends and activities in Ethiopia [9].

1.4. Recommended daily intake of iodine

As per WHO recommendation, the safe and adequate dietary intake of iodine for infants to adults ranges from 50 to 200 microgram per day [2]. In many countries, regulations envisage the control of the level of daily iodine intake through diet. Thus, a database of total iodine contents in food products will be helpful in recommending a controlled diet.

Table 1. Recommended daily intake of iodine based on sex and life stage [2].

Life stage	Amount
Infants	50-60 μg
Children	70-150 μg
Adult men	150 μg
Adult women	120 μg
Pregnancy	150 μg
Lactation	200 μg

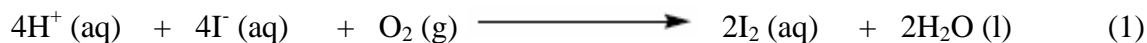
1.5. Salt iodization and its stability

Salt iodization is considered the most effective long-term public health intervention for achieving optimal iodine nutrition. Effective salt iodization is vital for the sustainable elimination of iodine deficiency disorders, e.g. retarded mental and physical development, hypothyroidism, endemic goiter, reproductive failure and childhood mortality. In South Africa, direct and indirect evidence of continued endemic goiter and iodine deficiency led, in 1995, to the introduction of mandatory iodization of table salt at an iodine concentration of 40–60 ppm. Salt iodization had previously been voluntary [11].

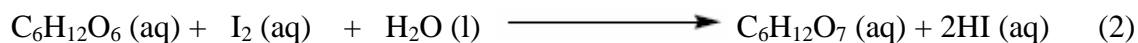
As in other African countries, the regulations in South Africa require potassium iodate to be used for this purpose. After the introduction of mandatory iodization, increased iodine concentrations were reported in retailed salt and the iodine status of primary-school children improved. Nevertheless, there continued to be shortcomings in the accuracy of salt iodization. There was considerable variation in the iodine content of retailed salt, and evidence of endemic goiter and iodine deficiency persisted in some areas. Recent monitoring of the iodine concentration of household salt indicated that 62.4% of households used adequately iodized salt containing at least 15 ppm iodine, considerably below the international goal of 90% coverage. A possible reason for this is that salt is underiodized at the production stage [12].

The effectiveness of iodized salt critically depends on the chemical stability of the fortifying salt that may result from the oxidation of iodide (Eq.1) or reduction of iodate to elemental iodine, whichever is used for salt iodization. Elemental iodine readily sublimates and is then rapidly lost to the atmosphere through diffusion. This results in a loss of the iodine content and variation in its amount due to inhomogeneous distribution in a solid bulk. Consequently, monitoring salt iodization is estimated not only at the point of production, but also at intermediate distribution levels, such as wholesale and retail levels. Therefore, quality assurance and control of iodine levels in iodized salts is of considerable interest all over the world [13].

Potassium iodide is less stable than potassium iodate, as it can be oxidized to elemental iodine by oxygen or other oxidizing agents, especially in the presence of impurities, such as metal ions and moisture, which catalyze the reaction. Iodide ion, in the form of potassium iodide, may be added to table salt to produce iodized salt in order to easily provide the population with a sufficient dietary supply of this essential nutrient. One difficulty with this is that iodide ion is easily oxidized to iodine by atmospheric oxygen (Eq.1).



The reaction equation explains why the accumulation of iodine in a box of table salt would result in yellow to red coloration and the development of a very noticeable bad taste. To avoid this problem, a reducing agent, typically dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$) is added to reduce back any iodine formed to the colorless iodide ion (Eq.2) [12-13].

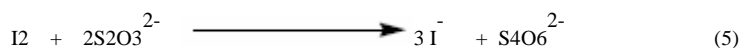
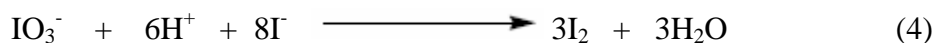
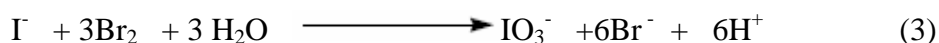


Potassium iodate may be reduced to elemental iodine by a variety of reducing agents in the salt, such as ferrous ions. Moisture is naturally present in the salt, or is abstracted from the air by hygroscopic impurities such as magnesium chloride. The pH of the condensed moisture on the salt is very much influenced by the type and quantity of impurities present, and this affects the stability of the iodine compounds. Elevated temperatures increase the rates of iodine loss [14].

The actual availability of iodine from iodized salt at the consumer level can vary over a wide range as a result of variability in the amount of iodine added during the iodization process. Moreover uneven distribution of iodine in the iodized salt within batches and individual bags, loss of iodine due to salt impurities, loss of iodine due to food processing, washing and cooking processes in the household, packaging, and environmental conditions during storage and distribution [3]. According to the WHO/UNICEF/ICCIDD, the loss of iodine from production to household has been estimated to be upto 20% [14].

1.6. Determination methods for iodine (iodide/iodate) levels in table salt

Some manufacturers use KI for salt iodization, the following procedure is followed to determine the amount of iodine in these salts [15, 16]. A sample of salt (50 g) is dissolved in 250 ml volumetric flask. 50 ml sample solution is taken in 200 ml Erlenmeyer flask, neutralization is made by using methyl orange with 2 N H₂SO₄. Br₂-H₂O is added from the burette equivalent to 20 mg Br₂, the remaining free Br₂ is destroyed by adding 1% Na₂SO₃. Finally, 1 ml of 2 N H₂SO₄ and 5 ml of 10% KI is added and the liberated iodine is titrated with Na₂S₂O₃. However, usually iodization is done by adding KIO₃ in these salts addition of bromine is not required. It can easily be titrated by adding excess KI and acidification with H₂SO₄ (as in reaction 4 and 5) with starch indication of the end point [15, 16].



1.7. Vitamin C

Vitamins are specific organic compounds that are essential for normal metabolism. Many participate as cofactors or coenzymes in mammalian biochemical reactions. The common thread

for the diverse chemical structures of the vitamins is that they are micronutrients. Micronutrients are compounds that are required in only small amounts and are not synthesized by humans, either at all or, at least, in sufficient quantity for metabolic needs are obtained from the diet or as synthetic preparations used in food fortification or supplements [17].

L-Ascorbic acid (vitamin C) is an important compound having a chemical structure that justifies its classification as a carbohydrate. Unlike glucose, it contains an unusual, highly reactive combination of molecules called the ene-diol group. The presence of this group confers upon the ascorbic acid molecule certain unique biochemical characteristics, which may explain its vital importance in the living process. It is widely distributed in plant and animal tissues, and involves the metabolism of various substances in vivo. The reversible oxidation of L-ascorbic acid to dehydro-L ascorbic acid is the basis for its physiological activities, stabilities, and technical applications [18].

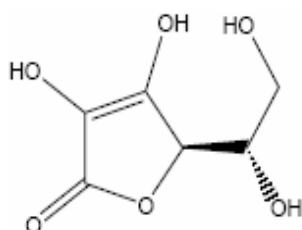


Figure 2. Structure of L-Ascorbic acid

Ascorbic acid is water-soluble vitamin, which can be found in many biological systems and foodstuffs. It is found in citrus fruits, green peppers, red peppers, strawberries, tomatoes, broccoli, brussels sprouts, turnip and other leafy vegetables. Fish and milk also contain small amounts of C. There is a gradual decline in the amount of vitamin C as foods age because of air oxidation [19, 20].

1.7.1. Importance of Vitamin C in the Human Diet

Ascorbic acid plays an important role in collagen biosynthesis, iron absorption, and immune response activation and is involved in wound healing and osteogenesis. It also acts as a powerful antioxidant, which fights against free-radical induced diseases [21].

Vitamin C is a cheap and safe nutrient; several of the suspected side effects of large amounts are unfounded. For example, none of the intervention trials has revealed any significant side effects of vitamin C. Furthermore, in a recent epidemiological study the men, who had been taking vitamin C supplements, on their own initiative, had a standardized mortality rate 30% lower than the control group. Accordingly, there are good reasons for reconsidering the potential role of vitamin C in the treatment of the common cold. The beneficial effects of vitamin C against the common cold may be caused by its antioxidant properties. It appears that neutrophils play a significant role in producing the symptoms of the common cold; in an infection they release large amounts of oxidizing compounds that are toxic to other cells. (Activation of neutrophils promotes an efficient consumption of extracellular vitamin C, which suggests that high concentrations of the vitamin C may provide protection against the harmful effects of the oxidants released [22].

Ascorbic acid is a potent detoxicant which counteracts and neutralizes the harmful effects of many poisons in the body. It will combat various inorganic poisons, such as mercury and arsenic, and it neutralizes the bad reactions of many organic poisons, drugs, and bacterial and animal toxins. Ascorbic acid detoxifies carbon monoxide, sulfur dioxide, and carcinogens, so it is the only immediate protection we have against the bad effects of air pollution and smoking [21]. It has also been shown that ascorbic acid increases the therapeutic effect of different drugs and medicines by making them more effective. Thus, less of a drug is required if it is taken in combination with large amounts of ascorbic acid. Diabetics could reduce their insulin requirements if this were practiced. It only does this, however, at relatively high doses, not a nutritional level. Even an aspirin should be accompanied by a large dose of ascorbic acid to heighten its analgesic (painkiller) effect and lessen its toxic action on the body. One of the body's defenses against bacterial infections is the mobilization of white blood cells into the affected tissues. The white blood cells then devour (consume) and digest the invading bacteria. This process is known as phagocytosis and is controlled by ascorbic acid. The number of bacteria that each white blood cell digests is directly related to the ascorbic acid content of the blood. This is

one of the reasons why a lack of ascorbic acid in the body produces lowered resistance to infectious diseases. [23].

Vitamin C is essential for the development and maintenance of connective tissues. It plays an important role in bone formation, wound healing and the maintenance of healthy gums. Vitamin C plays an important role in a number of metabolic functions including the activation of the vitamin B , folic acid, the conversion of cholesterol to bile acids and the conversion of the amino acid, tryptophan, to the neurotransmitter, serotonin. It is an antioxidant that protects body from free radicals' damages. It is used as therapeutic agent in many diseases and disorders. Vitamin C protects the immune system, reduces the severity of allergic reactions and helps fight off infections [19].

In stressful situations, adrenal glands react by releasing hormones that trigger the fight or flight reaction. It has been indicated that 200 mg of vitamin C a day may reduce the levels of stress hormones. Stress suppresses the immune system. Mega doses of vitamin C increase the levels of antibody that fights against germs and viruses in both stressed and unstressed rats, with greater antibody increase in the unstressed rats. Stressed animals may need larger vitamin C doses for proper function of the immune system [23].

In the first controlled human experiment results showed that, regular amounts of vitamin C can keep you warmer in winter. Specifically, they administered 200 mg of ascorbic acid daily for 17 days to 20 healthy medical students whose diets furnished about 80 mg of vitamin C daily during the testing period. By measuring the temperature of their skin at room temperature (20°C, 68°F) and 40 minutes after exposure to cold (5°C, 41°F), the authors demonstrated conclusively that skin temperatures in those receiving the vitamin C supplement decreased less after cold exposure than the control group [24].

Table 2. Recommended Dietary Allowance (RDA) for Vitamin C [24].

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	40 (AI)	40 (AI)
Infants	7-12 months	50 (AI)	50 (AI)
Children	1-3 years	15	15
Children	4-8 years	25	25
Children	9-13 years	45	45
Adolescents	14-18 years	75	65
Adults	19 years and older	90	75
Smokers	19 years and older	125	110
Pregnancy	18 years and younger	-	80
Pregnancy	19-years and older	-	85
Breastfeeding	18 years and younger	-	115
Breastfeeding	19 years and older	-	120

The deficiency of vitamin C leads to many diseases: scurvy, plug poisoning, liver disease, allergic reactions, arteriosclerosis, etc. Keeping in view its importance, the analysis of pharmaceuticals containing this vitamin assumes significance. The determination of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical and food application [25].

1.8. Review of Vitamin C assay methods

A large number of methods have been reported for the determination of acid ascorbic: titrimetry, voltammetry, potentiometry, spectrophotometry, flow injection analysis and chromatography [25].

1.8.1. Spectrophotometric methods

A method based on Ce(III)-arsenazo III complex formation for the determination of vitamin C is described. The acidic cerium(IV) solution gets reduced to cerium(III) by addition of ascorbic acid. The Ce(III) thus obtained forms a blue complex with arsenazo III in acidic medium. The absorption spectrum of the complex exhibits absorption bands at 420, 610 and 650 nm. Due to the highest absorbance of the last peak and the lowest contribution of the blank, absorbance measurements were carried out at 650 nm. The stoichiometry of the reaction between cerium (III) and arsenazo III is reported to be 1:1, with a molar absorptivity of $5.6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 650 nm. The absorbance of the complex was measured at 650 nm against a reagent blank. The proposed procedure was employed in the analyses of various pharmaceutical formulations, including vitamin C tablets and multivitamin products. The ascorbic acid content was computed from the standard calibration curve [26].

Another very simple spectrophotometric method has been developed for indirect determination of L-ascorbic acid using flow injection system based on the redox reaction between iron(III)-thiocyanate complex and L-ascorbic acid in acidic medium. A negative peak results from an injection of L-ascorbic acid into an iron(III)-thiocyanate complex carrier stream when absorbance is monitored at 462 nm. The height of the negative peak is proportional to the concentration of L-ascorbic acid in the sample [27].

A new, selective and accurate direct spectrophotometric method was also developed for the determination of L-ascorbic acid in pharmaceuticals with background correction based on the oxidation of L-ascorbic acid by nitrite in an acidic medium. The method is quite useful and allows the determination of L-ascorbic acid in the presence of common ingredients usually encountered in pharmaceutical formulations [28].

Microfluorometric is also an official method for determination of vitamin C based on oxidation of ascorbic acid to dehydroascorbic acid in the presence of Norit. The oxidized form is reacted with o-phenylenediamine to produce a fluorophor having activation maximum at ca 350 nm and fluorescence maximum at ca 430 nm. The fluorescence intensity is proportional to concentration. Development of fluorescent derivative of vitamin is prevented by forming H_3BO_3 -dehydroascorbic acid complex prior to addition of diamine solution. Any remaining fluorescence is due to extraneous materials. This serves as blank. Ascorbic plus dehydroascorbic acid is calculated by comparing the corrected fluorescence reading for sample with that of the standard similarly oxidized and treated [29].

1.8.2. Titrimetric methods

2, 6-Dichlorophenolindophenol (DCIP) has been used as the titrant for the determination of ascorbic acid. The method is based on the reduction of DCIP with ascorbic acid in acidic solution. It is an official method, for many pharmaceutical preparations, which do not contain Fe (II), Sn(II), Cu(I), SO_2 , SO_3^{2-} and $\text{S}_2\text{O}_3^{2-}$ ions which are usually associated with mineral preparations. The applicability of the method is restricted to only those multivitamin tablets, which do not contain minerals. Moreover, DCIP has been found to oxidize thiols [10]. Consequently, a mild oxidizing agent like DCIP (redox potential +0.217) or any other oxidizing agent could not be used for the determination of ascorbic acid in the presence of thiols. On a project on the determination of sulfur compounds, it was found that tetrachlorobenzoquinone reacts stoichiometrically with ascorbic acid but does not react at all with strong reducing agents like thiols under identical conditions [23].

1.8.3. Electrochemical methods

A flow injection system using a tubular electrode based on the redox properties of copper (II) ions occluded in polyethylene- co-vinyl acetate (EVA) membrane was developed for L-ascorbic acid determination. The polyethylene- co-vinyl acetate (EVA 40% m/m) matrix was doped with

copper (II) ions and dispersed on the surface of a graphite/epoxy tubular electrode. The electrode showed a super-Nernstian response for L-ascorbic acid concentration between 10^{-3} and 10^{-1} mol L^{-1} . The FIA system permitted the solution of some problems that the potentiometric electrode based on an EVA membrane doped with copper (II) ions presented and improved the response and regeneration time of the electrode. This methodology can be applied with good precision to the quality control of vitamin C in pharmaceutical samples [30].

In addition a chemically modified carbon paste electrode with 2, 7-bis (ferrocenyl ethyl) fluoren-9-one (2, 7-BFEFMCPE) was employed to study the electrocatalytic oxidation of ascorbic acid in aqueous solution using cyclic voltammetry, differential pulse voltammetry and chronoamperometry. It has been found that under an optimum condition (pH 7.00), the oxidation of ascorbic acid at the surface of such an electrode occurs at a potential about 300 mV less positive than that of an unmodified carbon paste electrode. The catalytic oxidation peak currents show a linear dependence on the ascorbic acid concentration and linear analytical curves were obtained in the ranges of 8.0×10^{-5} M - 2.0×10^{-3} M and 3.1×10^{-5} M – 3.3×10^{-3} M of ascorbic acid with correlation coefficients of 0.9980 and 0.9976 in cyclic voltammetry and differential pulse voltammetry, respectively [31].

Strohl and Curran reported for the first time the use of a reticulated glassy carbon electrode as an amperometric flow-through detector in the flow injection determination of vitamin C. Amperometric flow injection methods using immobilized enzyme reactors or photochemical reduction of methylene blue have also been recommended for the assay of ascorbic acid in 0.1 M phthalate buffer (pH 3.8). The methylene blue method allows the determination of ascorbic acid in the range 5.0-90.0 $\mu\text{g/ml}$ [32].

1.9. Ion selective electrodes

An ion-selective electrode is defined as an electroanalytical sensor with a membrane whose potential indicates the activity of the ion (the analyte) to be determined in a solution Eq.6.

$$E_{\text{mem}} = \text{Constant} - \frac{RT}{z_i F} \ln a_i \quad (6)$$

Where z_i and a_i represent the charges and the activity of the ion of interest respectively.

The membranes of ISEs consist either of liquid electrolyte solutions or of solid or glassy electrolytes that usually have negligible electron conductivity under the conditions of measurement [33]. The development and application of ion-selective electrodes (ISEs) continue to be exciting and expanding areas of analytical research. This is related to their ability to make direct or indirect measurements in complex samples [34].

ISEs have been increasingly used in the determination of concentration of ions in varieties of fields like environmental science, life science, clinical, diagnostic, pharmaceutical, and water chemistry and so on. A number of ion selective electrodes selective to different ions are now commercially available but they are too expensive for the teaching institute of developing countries who cannot afford to procure such electrodes for their educational purpose. This greatly hindered in the teaching of analysis with ion selective electrode. The method of fabrication of ISE is usually patented and guarded as trade secret. However, fabrication of workable inorganic precipitate based ISE in the laboratory is not insurmountable task but demand some effort to prepare well behaved ISE. No doubt the performance of electrodes with respect to Nernstian behavior, response time and detection limit all depends upon the method of fabrication and surface characteristics of the electrode [33, 34].

Several types of sensing electrodes are commercially available. They are classified by the nature of the membrane material used to construct the electrode. It is this difference in membrane construction that makes an electrode selective for a particular ion. Solid state electrodes utilize relatively insoluble inorganic salts in a membrane. It may exist in homogeneous or

heterogeneous forms. In both types, potentials are developed at the membrane surface due to the ion-exchange process [35].

The use of Ion Selective Electrodes in environmental analysis offer several advantages over other methods of analysis, (a) they do not affect the test solution; (b) they could be portable; (c) they are suitable both for direct determinations and as sensors for titrations; (d) the expense is considerably less than other methods, such as Atomic Adsorption Spectrophotometry or Ion Chromatography; and (e) they are not subject to interferences such as color of the sample. Moreover, there are few matrix modifications needed to conduct these analyses [36].

Potentiometric measurements are based on monitoring potential values under a zero current flow regime, in order to determine the analytical concentration desired components in an analyte. In these methods the potential difference between an indicator electrode (i.e. ion-selective electrode, redox electrode, metal-metal oxide electrode) and the reference electrode is measured as the analytical potential. ISEs have also been used as detectors for titrations. The concentration of the ion in the sample is back calculated from the volume of the titrant used in the titration [37].

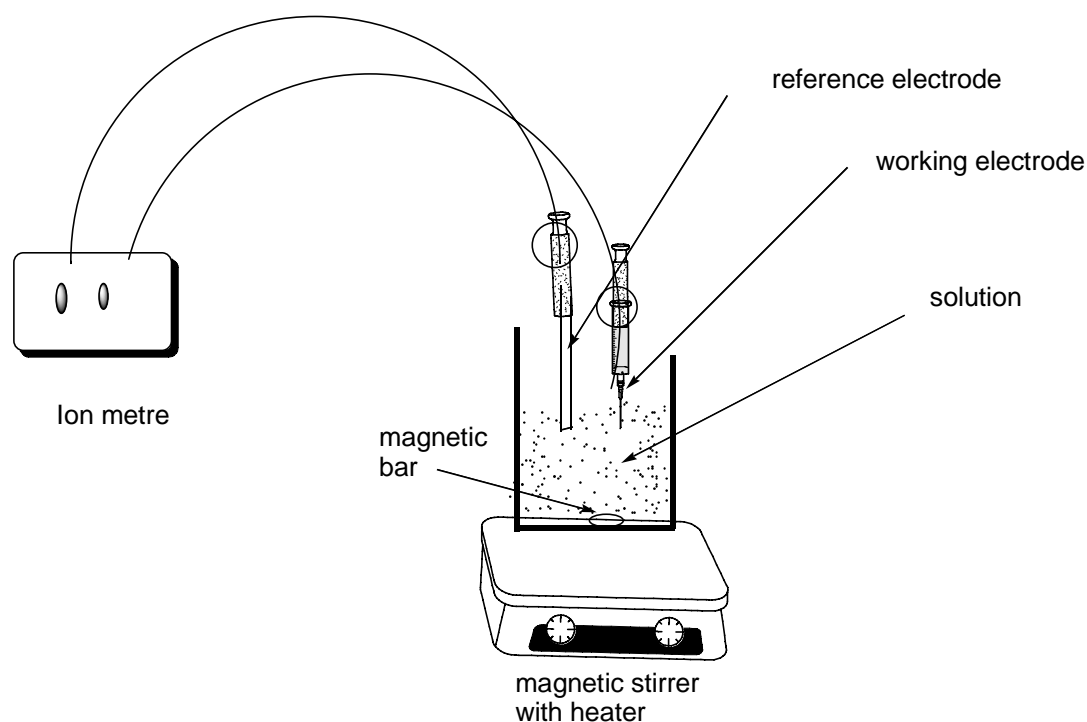


Figure 3. Schematic representation of potentiometric cell.

1.10. Iodide Ion Selective Electrode

Halide (X^-) ion- selective electrodes are generally prepared by co-precipitation of Ag-halide and Ag_2S . To an equal molar solution of potassium halide and sodium sulfide excess silver nitrate solution was added till complete precipitation and ion selective electrode is fabricated [38].



The iodide ion-selective electrode consists of a compressed pellet of silver sulfide and silver iodide. 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 nernstian 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 [39].

Pungor et al reported that the performances of the laboratory made ion selective electrodes are compared with commercial EDT- Qualiprobe Ag^+ electrode and Orion 94-16 Model Ag^+ ion selective electrode. The results, tabulated in Table 3, reveal that some ISEs can be designed for analytical applications in laboratories of even developing countries to counteract the high costs of commercial ISEs [38].

Table 3. Comparison of laboratory made (Ag_2S and $\text{Ag}_2\text{S-AgI}$) electrode with commercial electrodes [38].

Electrode	Determinant ion	Slope mv	Intercept	Linear range	Coefficient of determinant
Ag_2S	Ag^+	59.8	603	$1 \times 10^{-1} - 1 \times 10^{-5}$	0.9933
$\text{Ag}_2\text{S-AgI}$	I-	57.5	-339	$1 \times 10^{-1} - 1 \times 10^{-5}$	0.9865
EDT- Qualiprobe Ag^+	Ag^+	59.5	594	$1 \times 10^{-1} - 1 \times 10^{-5}$	0.9992
Orion 94-16 Model Ag^+	Ag^+	59	553	–	–

Several works were reported from this Laboratory on ion sensors based on ion association salts of triphenylmethane dyes (e.g. Crystal Violet, Brilliant Green) for many anions including saccharin [40], perchlorate and periodate [41], and complex anions like tetrachloroferrate (III) [42], tetrathiocyanato zinc(II) [43], and Hexafluorotantalate(V) [44].

1.11. Analytical application of Iodide Selective Electrodes

1.11.1. Indirect determination of iodine-containing drugs

The application of iodide electrodes for drugs containing combined iodine or for indirect determinations through reactions of iodine with reducing agents is well established. One example of an iodide electrode application for indirect determination of iodine containing drugs includes for Iodiquinol and Levothyroxin [45]. This involves the use of sodium metal for alkaline fusion. For an appropriate amount of finely powder tablet was added to hot tube, and the resulting mixture was completely burned. The iodide released from the decomposition of the drug was determined by potentiometric titration method using standardized silver nitrate, the proposed electrode as indicator electrode. The same procedure was applied for iodide determination in L-throxin in its tablets. There results had shown satisfactory agreements between the determined and declared amounts of the drugs.

1.11.2. Determination of vitamin C in pharmaceutical products

Potentiometric titrations have been employed for the determination of vitamin C (L-ascorbic acid) using N-bromosuccinimide, iodine, tetrachlorobenzoquinone and hexamminecobalt(III) triscarbonatocobaltate as titrants. The method is useful only for drugs without additives. Also, it lacks accuracy in the analysis of multivitamin formulations containing minerals or other oxidising compounds. Titrations involving hexacyanoferrate(III) are to be carried out in 4.5-5.5 M sulfuric acid medium under an inert atmosphere and in 10-12 M phosphoric acid. Micro-determination of L-ascorbic acid based on its oxidation by iodine in chloroform or methanol has also been carried out, which involves the determination of iodide ion formed with an iodide ion selective electrode; however sulfide ion is expected to interfere [46].

1.11.3. Potentiometric titration of iodide ion with silver nitrate

Iodide-selective Electrode based on Copper Phthalocyanine (CuPc) was found to work well for potentiometric titration of iodide ion with silver nitrate [47]. Membrane coating solutions were prepared by dissolving various amounts of the ionophore (CuPc), together with appropriate quantity of plasticizer and PVC to give a total mass of 200.0 mg in 5.00 ml THF. The electrode was successfully applied as indicator electrodes for the titration of 50.0 ml of 1.0×10^{-3} M KI solution with 0.005 M AgNO_3 . The average recovery for detection of iodide using this electrode was 98.7% with the mean standard deviation of 1.8%. The high degree of iodide selectivity exhibited by the CuPc-based membrane electrode makes it potentially useful for monitoring the concentration of iodide in natural water samples.

2. Objectives

2.1. General objectives

The main objective of this project was to construct and assess the analytical application of iodide ion selective electrode.

2.2. The specific objectives

1. To determine the amount of vitamin C in pharmaceutical products and in fruit juice (like orange).
2. To determine the amount of iodine in iodized salts.

3. Experimental

3.1. Reagents and chemicals used

Potassium iodate 99.5% purity (BDH, England), Potassium chloride (Riedel-de Haen, AG), Potassium nitrate (Research Chemicals Ltd), Potassium iodide (BDH, England), Sodium thiosulfate (Riedel-de Haen, AG), Sodium Chloride (Oxford Laboratory Reagent, Extra pure (99.5%), India), Sodium carbonate (BA, A.A.U), Sodium sulfide (BDH, England), Silver nitrate (Scharlau Chemie S.A, EU), Parafilm (American can company, America), and distilled water were used throughout the experiment.

3.2. Apparatus and Instruments

Potentiometric measurements were made at room temperature using Jenway 3345 Model Ion Meter with graphite coated electrode (GCE) against Ag/AgCl external reference electrode (Hanna Model HI5412). Stable potentials were recorded within 1-5 minutes after dipping the GCE and the reference electrode in the test solution stirred using a teflon- coated magnetic stirring bar.

3.3. Samples

Iodized salts purchased from super markets were manufactured by (a) Saudi Salt Refinery, Saudi Arabia (label claim 70-100 mg/Kg as iodine), (b) Klinge Foods LTD, Scotland, 47.5 mg/Kg as iodine) (C) American Garden product, USA (0.006% as potassium iodide). The Vitamin C tablets purchased from local pharmacies were produced from the Ethiopian Pharmaceuticals Manufacturing Industry and Chemical Industrial Development, (Egypt). The latter is sold as Effervesce vitamin C tablet. Egyptian Orange fruit, and whole Ethiopian orange (yellow and Green orange) were also purchased from supermarkets.

3.4. Electrode preparation

A 2 cm long graphite rod taken from dry cell was cleaned and inserted into a ball point pen holder 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 parafilm, to avoid the passage of test solution in to the copper wire, which was used to connect the electrode with the ion meter. 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 ($\text{Ag}_2\text{S-AgI}$) 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 inside the pen holder to insure electrical conductivity between the electrode and the copper wire to be connected to the pH/mV meter. The electrode was allowed to stay for 2 hours and finally conditioned for at least an hour before use [48].

3.5. Solution preparation

3.5.1. 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. 1×10^{-2} M, 1×10^{-3} M, 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M solutions were prepared by serially diluting the stock using 0.1 M KNO_3 to keep the ionic strength constant at 0.1.

3.5.2. Solution preparation for standardization of sodium thiosulfate

0.07 M $\text{Na}_2\text{S}_2\text{O}_3$ was prepared by dissolving 8.7 g of $\text{Na}_2\text{S}_2\text{O}_3$ in 500 ml of freshly boiled distilled water containing 0.05 g of Na_2CO_3 . 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 2 g of solid KI and 10 ml of 0.5 M H_2SO_4 was added.

3.5.3. Preparation of Iodized salt and solution preparation for titration

25 gram of pure (99.5%) NaCl was dissolved in 100 ml of 7.5×10^{-6} M KIO_3 . This solution was acidified by 1 ml of 0.3 M H_2SO_4 and 2 gram of KI was added in excess to stabilize iodine in aqueous solution by forming triiodide ion.

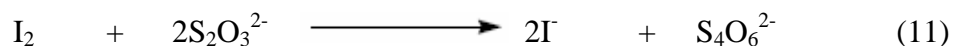
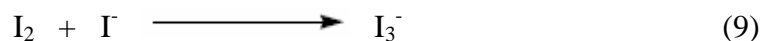
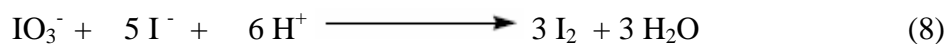
3.5.4. Solution preparation to determine titratability of iodized salt

1000 ppm of stock KIO_3 was prepared in 250 ml flask. From this 10 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm solutions were prepared in 200 ml flasks by serial dilutions of the stock solution. The same amount of excess KI (4 mg) and 0.3 ml of 0.3 M H_2SO_4 was added to each of the above solutions.

3.6. Methods for sample analysis

3.6.1. Methods for analysis of vitamin C

Ascorbic acid (vitamin C) is a mild reducing agent that reacts rapidly with triiodide. In this experiment triiodide was generated by the reaction of iodate with excess iodide in the presence of sulfuric acid (Eq. 8 & 9). The reaction was allowed to proceed with ascorbic acid, and then the excess I_3 was potentiometrically back titrated with standard thiosulfate solution (Eq. 10 & 11). The analyses of vitamin C in vitamin C tablets were conducted as follows; contents of ten tablets/capsules were weighed and crushed to powder. An amount equivalent to 200 mg of ascorbic acid was dissolved in 60 ml of 0.3 M H_2SO_4 . 2 g of KI and 50 ml of 0.01 M KIO_3 was added to produce triiodide ion which was titrated as mentioned above [49].



3.6.2. Methods for analysis of iodized salts

The iodine content of iodized salt samples was measured using iodometric titration. Usually iodate is added for iodization than iodide because iodate is more stable under adverse climatic condition than iodide. Analysis of these salts were made by dissolving 25 g of the sample with 100 ml distilled water and acidifying the solution with H_2SO_4 , free iodine was liberated from the iodate in the salt sample. Excess KI was added to help solubilize the free iodine (to form I_3^- , Eq. 9), which is quite insoluble in pure water under normal conditions. The I_3^- formed was potentiometrically titrated by standard thiosulfate solution. The amount of thiosulfate used was proportional to the amount of free iodine liberated from the salt (Eq. 11). For samples which are iodized by iodide rather than iodate the concentration of iodine was calculated from the calibration curve [50].

4. Results and discussion

4.1. Calibration of the electrode

The EMF of the cell for the $\text{Ag}_2\text{S}-\text{AgI}$ membrane electrode coupled with saturated calomel reference electrode plotted against $-\log [\text{I}^-]$ is shown in Fig.4. The EMF of the cell was found to follow the following equation.

$$E = -85.97 + 56.85 \log [\text{I}^-]$$

The electrode showed linearity in the EMF vs. $-\log [\text{I}^-]$ plot over the concentration range of 10^{-1} M to 10^{-6} M with a slope of 56.85 mV and R value 0.99984 per decade change in concentration of iodide ion at 25°C . The slope is slightly less than the expected value from Nernst equation but is quite acceptable for a well behaved solid state membrane electrode. Detection limit of the electrode was 6×10^{-7} M with standard deviation of 1.35 (n=10) at 5×10^{-4} M iodide. The sensors have response time of 1-5 minutes and can be used for at least 2 months without any considerable divergence in their potential response.

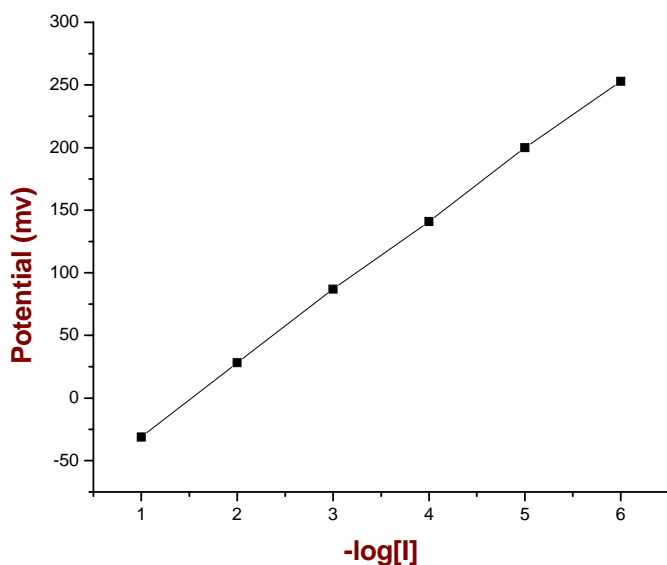


Figure 4. Plot of Cell EMF against $-\log [\text{I}^-]$

4.2. Standardization of sodium thiosulfate

Standardization of sodium thiosulfate, which was used as a titrant in all the experiments below, was done by using iodide selective electrode. The solution prepared as in 3.5.2 was immediately titrated with thiosulfate solution. The concentration of the thiosulfate was calculated from the potentiometric titration curve shown in Fig.5. The result of this experiment was compared with other methods, which use disappearance of the dark brown color of I_3^- to detect the end point. Using this method the volume of sodium thiosulfate solution equivalent to 50.00 ml of the 0.010 M KIO_3 solution from three determinations was 42.48 ml. Since the number of moles of KIO_3 consumed in this reaction was 5.05×10^{-4} , the number of moles of thiosulfate should be $6 \times 5.05 \times 10^{-4}$. In this way, the concentration of the thiosulfate solution was calculated to be 0.071 M [50].

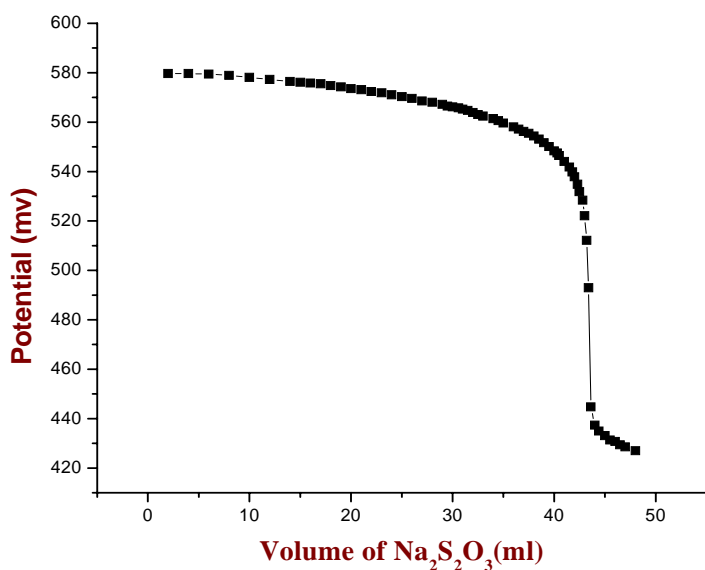


Figure 5. Application of the electrode for standardization of $Na_2S_2O_3$ by potentiometric titration of 50 ml of 0.01 M KIO_3 with 0.06749 M $Na_2S_2O_3$ (calculated from the curve).

4.3. Method validation for the determination of commercial iodized salts using Laboratory prepared iodized salts.

Method Validation for measuring iodine content of commercial iodized salts was made by titrating and calculating iodine in laboratory formulated iodized salt. For this experiment synthetic iodized salts (prepared as in section 3.5.3) were potentiometrically titrated with 3×10^{-3} M $\text{Na}_2\text{S}_2\text{O}_3$ using iodide selective electrode. Iodate recovery was found to be 98.6 % (64.6 mg/Kg out of 65.5 mg/Kg). Triplicate measurements were made to observe the repeatability of the experiment. As it is indicated in Fig.6 end points were recorded as 15 ml (A), 15.3 ml (B), 15 ml (C) under repeatability conditions. Thus, the results prove that this method can easily be used for quantification of iodate in commercial iodized salts.

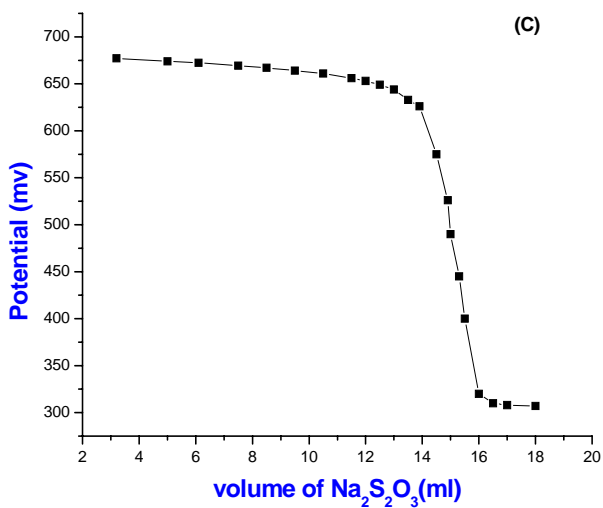
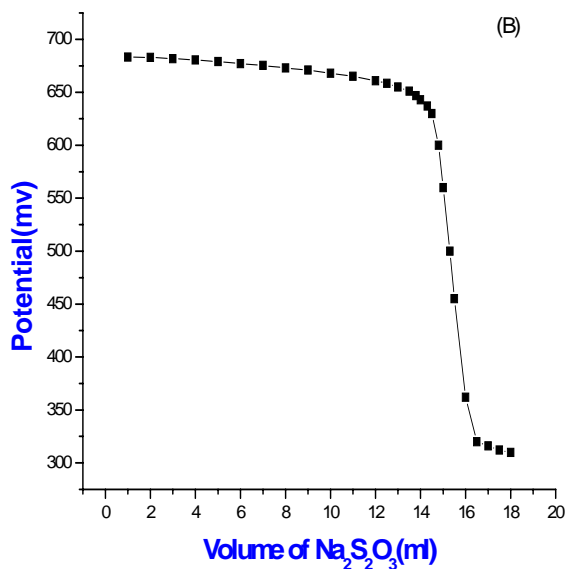
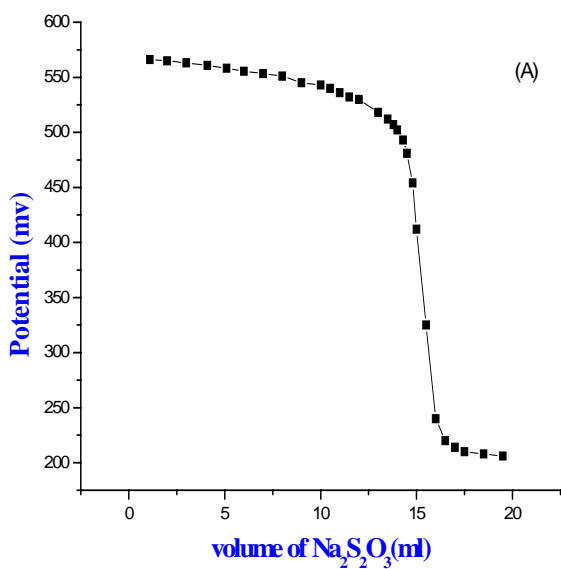


Figure 6. Potentiometric titration of laboratory prepared iodized salt (47.5 mg iodine /Kg NaCl or 65.4 mg KIO_3 /kg NaCl) with 3×10^{-3} M $\text{Na}_2\text{S}_2\text{O}_3$ end points were detected at A) 15 ml B) 15.3 C) 15 ml.

4.4. Titrability of different concentrations of potassium iodate with $\text{Na}_2\text{S}_2\text{O}_3$

Titration of different concentration of KIO_3 (in presence same excess of iodide and the same acidity) with $\text{Na}_2\text{S}_2\text{O}_3$ was studied. Fig.7 shows their titration curves with inflection points that can be used to locate the end points for the solutions ranging from 10 ppm to 200 ppm as iodate. This was due to the presence of iodide, which was initially added in excess. In other words, KIO_3 was the limiting reactant in the reaction and iodide selective electrode measure total amount of iodide (iodide from the reaction of I_2/I_3^- with $\text{Na}_2\text{S}_2\text{O}_3$ and I^- which was left unreacted with KIO_3). Moreover even though the same amount of KI was added to each solution (A-E), in the titration curve A initial concentration of iodide was small (more positive potential) than titration curve E (smaller potential). This was due to relatively excessive amount of KI has been reacted with KIO_3 (200 ppm in A than in the 10 ppm -150 ppm) in the titration curves E to B) and converted to I_2/I_3^- . Obviously, the largest volume of $\text{Na}_2\text{S}_2\text{O}_3$ is consumed by the titrand A.

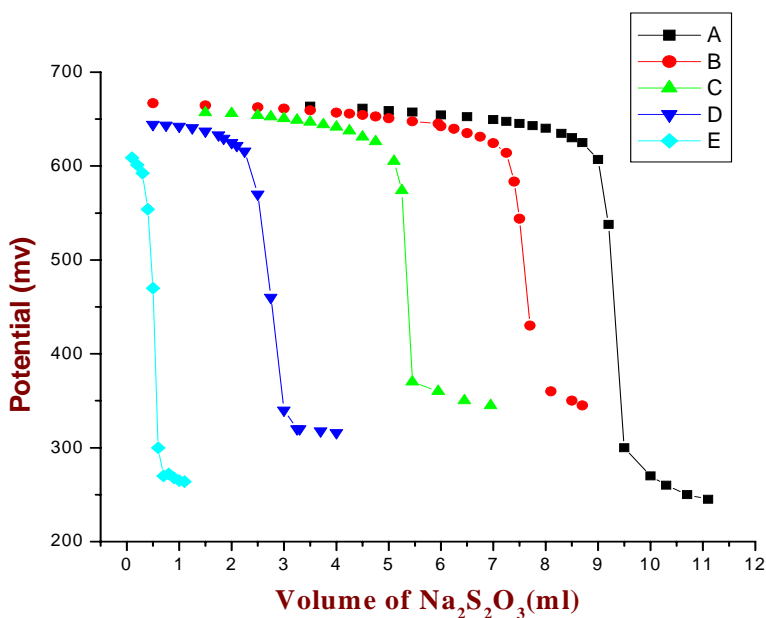


Figure 7. Titration of A) 200 ppm, B) 150 ppm, C) 100 ppm, D) 50 ppm, E) 10 ppm KIO_3 in the presence of 4 mg potassium iodide with 2×10^{-4} M $\text{Na}_2\text{S}_2\text{O}_3$

4.5. Analysis of Laboratory-formulated and commercial iodized salts

Table 4. Iodine/iodate content of commercial iodized salts produced in different countries.

Sample No.	Producers	SD at n=3	Added or Label value (mg/Kg) as			Calculated (mg/Kg) as		
			I ₂	KIO ₃	KI	I ₂	KIO ₃	KI
1.	Lab-formulated	0.739	47.5	65.45	-	46.90	64.63	-
2.	Saudi Arabia P. 9/2008	0.815	70-100	96.46-137.80	-	78.44	108.08	-
3.	Saudi Arabia P. 2/2007	0.914	70-100	96.46-137.80	-	72.72	100.20	-
4.	Scotland, P. 11/2007	1.20	47.5	65.45	-	42.45	58.5	-
5.	America, P. 09/2008	0.99	43.5	-	60	34.8	-	48

In this work, the iodine contents of different commercial iodized salts were estimated. The results of replicate analyses (n = 3) showed that determined values for samples 2 and 3 were within the range of the label claims by the manufacturers. However, the iodine contents of all other iodized salt samples are lower than the label value, which can be attributed to many factors. The actual availability of iodine from iodized salts at the consumer level can vary over a wide range because of uneven distribution of iodine in the iodized salts and individual bags, loss of iodine due to salt impurities, packing and environmental conditions during storage and distribution. Moreover, even though sample 2 & 3 were purchased from the same manufacturer

the amount of iodine calculated in these samples is different this may be related to their production date. Sample three, which is produced earlier than sample 2 has lost more iodine relative to sample 2. Iodine losses from point of production to consumption can then be well in excess of 50% [52].

4.6. Analysis of pharmaceutical products

It was found that the differential potentiometric titration curves are sharp, smooth, and symmetric; hence, one can easily locate the end-points, indicating the normal behavior of solid-state iodide selective electrodes. These electrodes were found to be suitable for this type of oxidation reduction titration and they were employed in this work.

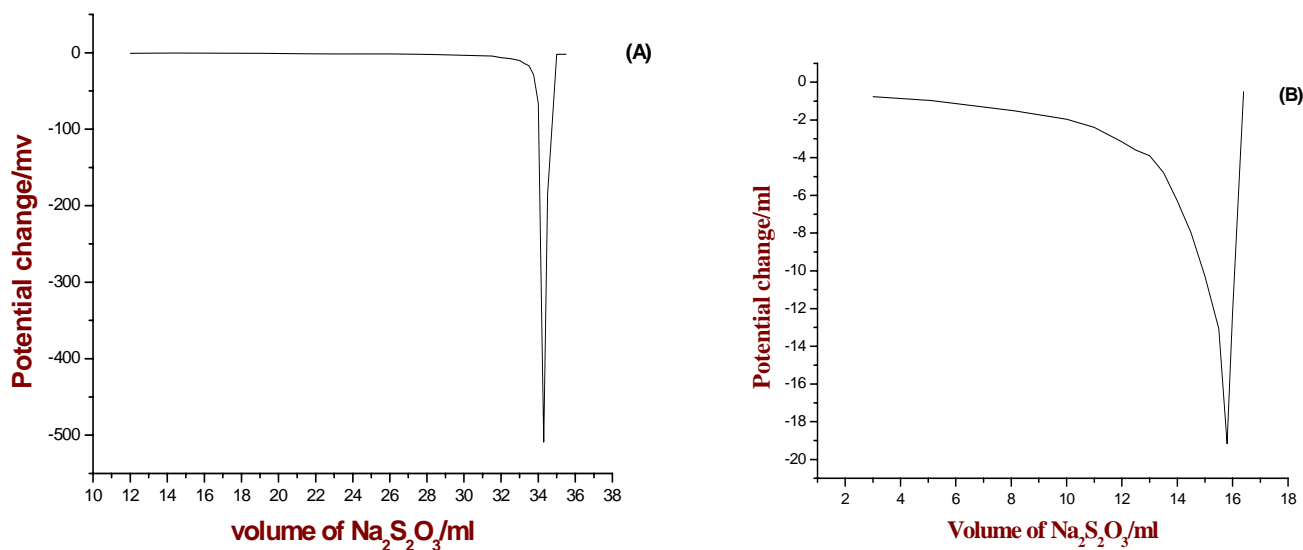


Figure 8. First derivative titration curves for the titration of pharmaceutical products A) Effervescent tablets, product of Chemical Industry Development, Egypt. B) Vitamin C tablets from Ethiopia Pharmaceutical Manufacturing Industry.

The amount of vitamin C recovery observed in triplicate measurements of both pharmaceutical products was less than the label value because additives for color, taste and other binding materials are usually added at the production stage. But, the amount of vitamin C was found to be higher in tablet B (431.5 ± 1.05 mg from 500 mg) this may be due to freshness of the product and hence oxidation of vitamin C by oxygen and other oxidants is lower. Whereas in case of tablet A it was two months away from the expiry date when the experiment was conducted and some of it was lost compared to its total mass (451.66 ± 0.89 mg from 1000 mg).

The results were compared with those obtained by a novel spectrophotometric method. The procedure involves the use of Cu (II) as vitamin C oxidant. This method is used for determination of vitamin C in fruits and pharmaceutical products. The percentage recovery of ascorbic acid for pharmaceutical samples (vitamin C tablet) was 245.6 mg/ 250 mg tablet [53].

4.7. Analysis of vitamin C in commercial orange fruit

Redox titration with potentiometric end point detection for L-ascorbic acid (vitamin C) determination was designed. The method is based on L-ascorbic acid oxidation to dehydroascorbic acid, in acidic medium, using iodine-iodide solution as oxidizing reagent. The iodine amount consumed in the redox reaction was detected potentiometrically and it was proportional to the amount of L-ascorbic acid from the sample [54].

Table 5. Vitamin C content of commercial orange

Orange sample	mg of vitamin C per 100 ml* of orange juice
Orange(Egyptian, yellow)	51.92 ± 1.10
Orange (Ethiopian, partially yellow)	41.4 ± 1.50
Orange (Ethiopian, green)	32.4 ± 1.30

* Mean \pm SD, n = 3 for each sample

The amounts of vitamin C obtained in commercial orange fruits were comparable with values of other titrimetric methods which use dichlorophenol indophenol (DCPIP) as a titrant. In this method a sample of 2.5 ml clear Orange juice (Greece) obtained by fruit pressing was diluted with distilled water to a final volume of 10 ml. Then, it was titrated with the DCPIP 5×10^{-4} mol L⁻¹ solutions until a pink tint appears that persists for about 30 seconds. The amount of vitamin C per 100 ml of juice was found to be 30.48 mg. Other results indicate a vitamin C content of 33 – 50 mg/100 ml for orange juice (Valencia) obtained by squeezing the fruits [21].

In addition, comparison of the amount of vitamin C in partially yellow and green orange (purchased from the same source) was made. As it is shown in the table, the amount of vitamin C was higher in partially yellow fruit than in the green one.

5. Conclusion

The application of laboratory made low cost solid state silver sulfide based iodide ion selective electrodes provides a sensitive and economic method for determination of iodide down to 1.0×10^{-6} M. The performance of these electrodes is comparable with the expensive commercial electrodes with respect to the linear range, and response time. The electrode can be used for the analysis of iodide, direct potentiometric titration of iodized salts and potentiometric back titration of vitamin C. ISE methods require cheaper instrumentation and a simple electric circuit with only two coupled electrodes. The results reflect that the inflection point locating the end points using the iodide selective electrode potentiometric method were defined down to 10 ppm potassium iodate. Starch-based indication of iodometric titrations can be replaced by the iodide ISE method after further work for comparing the two methods has been conducted. Potentiometric titration experiments for undergraduate students can be also designed to determine L-ascorbic acid as well as iodide- or iodate-iodized salts.

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