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EXTRACTION AND ESTIMATION OF NICOTINE IN TOBACCO USING POLARIMETRY

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Abstract

The polarimetry titration technique can make metal ions an optically active compound. Such titration involves recording changes in the optical activity value of the active titer with each addition of titrant, usually a metal ion. In such titrations, an optically active compound acts as a self-indicator. Metal ion will form a stereoselective complex when added to a ligand system (e.g., tobacco extract) under the optimized conditions. This idea was successfully applied to titrate nicotine, an important optically active component in tobacco extract (a multi-component system). This idea has been experimentally verified and validated. Zinc (II) ions have been used to estimate nicotine in five different varieties of leaves of Nicotiana tabacum of Punjab, Pakistan. This study provides an economical, rapid, and relatively simple analysis method for nicotine.

Keywords
Nicotine; Polarimetric Titration; Tobacco; Zinc.

1. Introduction

Tobacco is used in various medicines including intoxicant drugs, etc. It is extensively used in a germicide spray for plants worldwide (Bakht *et al,* 2012). It also finds its use in smoke-pipe and cigarettes universally. It has been declared as one of the major causes of lungs cancer. (Proctor, 2001). Estimation of any compound requires specific methods with usual long procedures. For example, harmine and harmaline from peganum harmala, caffeine, and gallic acid from tea leaves have been estimated using complexation and then

isolation (Munir *et al*, 1994). Munir, Khokhar and Ahmad (1986) first introduced the complexation method's estimation of optically active compounds. Later, this technique was used by Chohan and Munir to determine the metal-ligand ratio of amino acids with Cu^{2+} complexes. Schlesinger completed the first quantitative determination of nicotine in tobacco in 1846. It rests on the liberation of the nicotine by ammonia, extraction with ether, deduction of the ammonia by heating, and titration of the residue with regular sulphuric acid (Chapin, 1911). The present study has employed a similar approach to estimate the nicotine in tobacco leaves. The chemistry of pyridine alkaloids has brought about many other approaches for determining tobacco alkaloids quantitatively. Some of these old procedures are very laborious and time-consuming. These approaches can be used in the absence of current methods. The main alkaloid of attention in tobacco is nicotine. The procedures used to determine nicotine in tobacco are steam distillation, chromatographic, and auto-analyzer (Atkinson *et al*, 1984). Circular dichroism (CD) spectropolarimeter was used as the indicator for the determination of (S)- (-)-nicotine in chopped tobacco leaves after a single direct withdrawal of the analyte into methanolic KOH. Other compounds extracted from the leaf absorbance. In the UV range, but none is CD active, excluding all potential interferences. Some results are described for the nicotine contents in some smokeless tobaccos and cigarettes (Omara *et al*, 2014). To determine the total tobacco alkaloids and nicotine, a spectrophotometric method was described based on the bromination of nicotine to form dibromonicotine, which reacts with potassium iodide in the presence of starch to form a water-soluble blue complex. The complex gives maximum absorbance at 580 nm. The method was applied to determine total alkaloids and nicotine in tobacco leaves, cigarette smoke, and biological samples. The process is very delicate and was compared with different stated approaches (Rai *et al*, 1994). Polarimetery studies optical rotation to find and characterize biopolymers, natural polymers, and artificial polymers (Faes *et al*, 2021). Impreciseness in the technique was checked out by Spies (1937) who mentioned their method of precipitation with silicotungstic acid as more acceptable. Young (1927) volumetrically determined nicotine by the method of potassio-mercuric iodide. Before this, the desired components from plants were commonly estimated after isolation (Gray *et al*, 2012) which is quite a laborious process. It involves washing, drying, grinding, soaking, fractional distillation, column chromatography and thin-layer chromatography. Isolation may take 14-28 days. The chemistry of pyridine alkaloids has brought about many other approaches for determining tobacco alkaloids quantitatively. Some of these old procedures are very laborious and time-consuming. The main alkaloid of attention in tobacco is nicotine. The procedures used to determine nicotine in tobacco are steam distillation, chromatographic and auto-analyzer (Piade & Hoffmann, 1980). Circular Diachroism

(CD) Spectropolarimeter was used as the indicator for the determination of (S)- (-)-nicotine in chopped tobacco leaves after a single direct withdrawal of the analyte into methanolic KOH. Other compounds extracted 1rom the leaf absorb in the UV range, but none is CD active, excluding all probable interferences. Some results are described for the nicotine contents in some smokeless tobaccos and cigarettes (Clough *et al,* 2021). This research work estimates optically active nicotine from tobacco extract using a metal ion. i.e, Zn. Experimental observations have proved that metal ion can be used to estimate the desired component of plant extract with polarimetric technique, provided it is optically active and have ligand properties.

2. Materials and Method

Kirschner & Bhatnagar (1963) and then later Pearson & Kirschner (1969) were the pioneers in introducing spectropolarimetric titrimetric methods. They successfully carried out titrations such as metal-ligand titrations. In this work, we have successfully carried out spectropolarimetric titrations using five different tobacco extracts using Zn^{2+} ion as a complexing agent with nicotine as a target analyte and can act as a self-indicator.

2.1. Materials

The chemicals used were Zinc Chloride, $ZnCl_2$, and S (-) Nicotine, $C_{10}H_{14}N_2$ by Alfa Aesar Company. Different Tobacco samples from Mailsi, Nana Dogar, Sahiwal, Changa Manga, and Toba Tek Singh. The Solvents used were Dilute HCl (0.0098 M), Distilled water, and Isopropyl alcohol to remove the chlorophyll from tobacco extract. The Instruments used were Weighing Balance manufactured by Schwarzenbek and Automatic Digital Polarimeter by Rudolph Instrument.

2.2. Method

Before starting the actual titrating tobacco extract with zinc ions, it was decided first to titrate pure nicotine under similar conditions. Equimolar solution of Nicotine and HCl (0.0098M) was titrated with 0.0098 M $ZnCl_2$ using a digital polarimeter for recording changes in rotation α values. This experiment was done to check whether the proposed method works for the selective estimation of nicotine. Pure Alfa Aesar standard S (-) nicotine was purchased, and its stock solution was made by mixing an equimolar quantity of nicotine with HCl. Then 10mL of nicotine stock solution was taken in ten 50 mL flasks. Then 0.0098 M $ZnCl_2$ solution was added to the flasks with an incremental increase of 0.5mL. Finally, the volume of each flask was raised to the mark and let stay for about 30 minutes. Then these flasks were taken to the automatic digital polarimeter. This gives optical rotation α. Specific rotation is calculated using the formula. The same method was used for all titrations. Optical rotation, α values and specific rotation was also calculated. The results are given in Table 1.

Table 1. Observed Rotations of Preliminary Titration.

Equimolar solution of Nicotine & HCl	0.0098 M $ZnCl_2$ (mL)	Total Volume (mL)	Observed Rotation α°	Specific Rotation [α]
10	0	50	-1.96°	-98
10	0.5	50	-1.94°	-97
10	1	50	-1.91°	-95.5
10	1.5	50	-1.8°	-90
10	2	50	-1.72°	-86
10	2.5	50	-1.63°	-81.5
10	3	50	-1.58°	-79
10	3.5	50	-1.52°	-76
10	4	50	-1.48°	-74
10	4.5	50	-1.46°	-73
10	5	50	-1.41°	-70.5
10	5.5	50	-1.41°	-70.5
10	6	50	-1.41°	-70.5
10	6.5	50	-1.41°	-70.5
10	7	50	-1.41°	-70.5

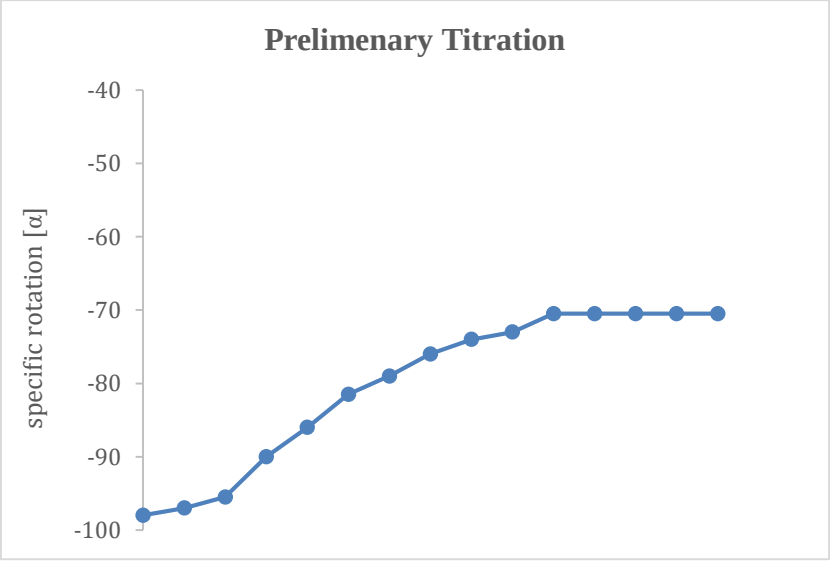

Fig. 1: Graph of Preliminary Titration

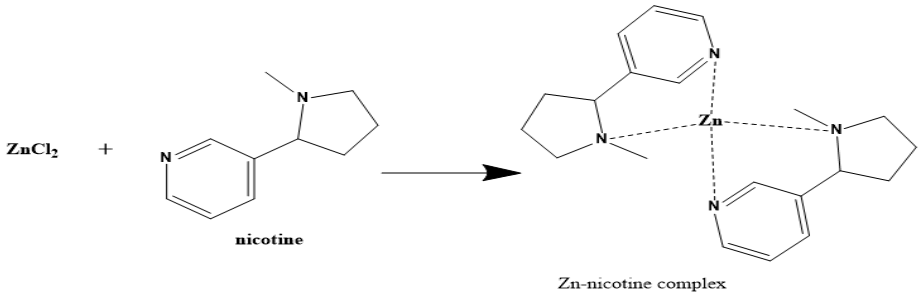

Fig. 2: Zn-nicotine complex formation in the tobacco extract.

Raw tobacco was purchased from the market and ground in a grinder. 10g of powdered tobacco was taken and treated with isopropyl alcohol to remove its chlorophyll. It was filtered, and the filtrate was discarded. The remaining powdered tobacco was again treated with isopropyl alcohol. This procedure was repeated three times to remove the maximum amount of chlorophyll of tobacco. In the next step, dilute HCl was added to the chlorophyll-free tobacco powder and stirred continuously for approximately 2 hours. The solution thus obtained was called tobacco extract. This tobacco extract solution was poured into the 500mL beaker and covered properly with aluminum foil. 10 mL of tobacco extract solution was then added in 10 different 50mL flasks. 0.1 molar solution of zinc chloride solution was prepared and added in each of the 10 flasks with 0.5mL increment. In the end, volume was raised with water up to the 50mL mark. Then these flasks were taken to the automatic digital polarimeter, and the change in optical rotation value of all the solutions was recorded from the digital polarimeter.

3. Results

3.1. Estimation of Nicotine in Tobacco of Changa Manga

Table 2: Observed Rotations of Changa Manga Tobacco.

Tobacco extract (mL)	0.1M $ZnCl_2$ (mL)	Total Volume (mL)	Observed Rotation α^o	Specific Rotation [α]
10	0	50	-0.430°	-86.0
10	0.5	50	-0.410°	-82.0
10	1	50	-0.385°	-77.0
10	1.5	50	-0.357°	-71.5
10	2	50	-0.342°	-68.5
10	2.5	50	-0.310°	-62.0
10	3	50	-0.285°	-57.0
10	3.5	50	-0.285°	-57.0
10	4	50	-0.285°	-57.0
10	4.5	50	-0.285°	-57.0

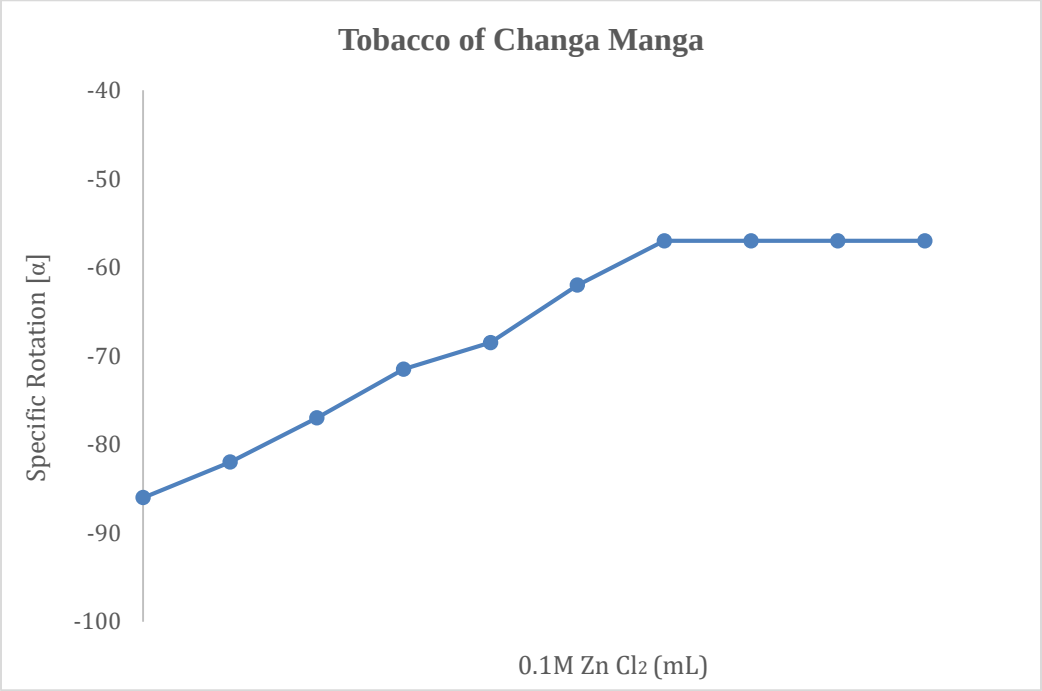

Fig. 3: Graph of Titration with Changa Manga Tobacco

As 10 grams of Changa Manga Tobacco contains 0.0973 grams of nicotine (Calculated), therefore, percentage of nicotine in Changa Manga Tobacco is 0.97%.

3.2. Estimation of Nicotine in Tobacco of Mailsi

Table 3: Observed Rotations of Mailsi Tobacco

Tobacco extract (mL)	**0.1M $ZnCl_2$ (mL)**	**Total Volume (mL)**	**Observed Rotation α^o**	**Specific Rotation [α]**
10	0	50	-0.425°	-85.0
10	0.5	50	-0.385°	-77.0
10	1	50	-0.372°	-74.5
10	1.5	50	-0.357°	-71.5
10	2	50	-0.330°	-66.0
10	2.5	50	-0.313°	-62.6
10	3	50	-0.280°	-56.0
10	3.5	50	-0.260°	-52.0
10	4	50	-0.260°	-52.0
10	4.5	50	-0.260°	-52.0
10	5	50	-0.260°	-52.0

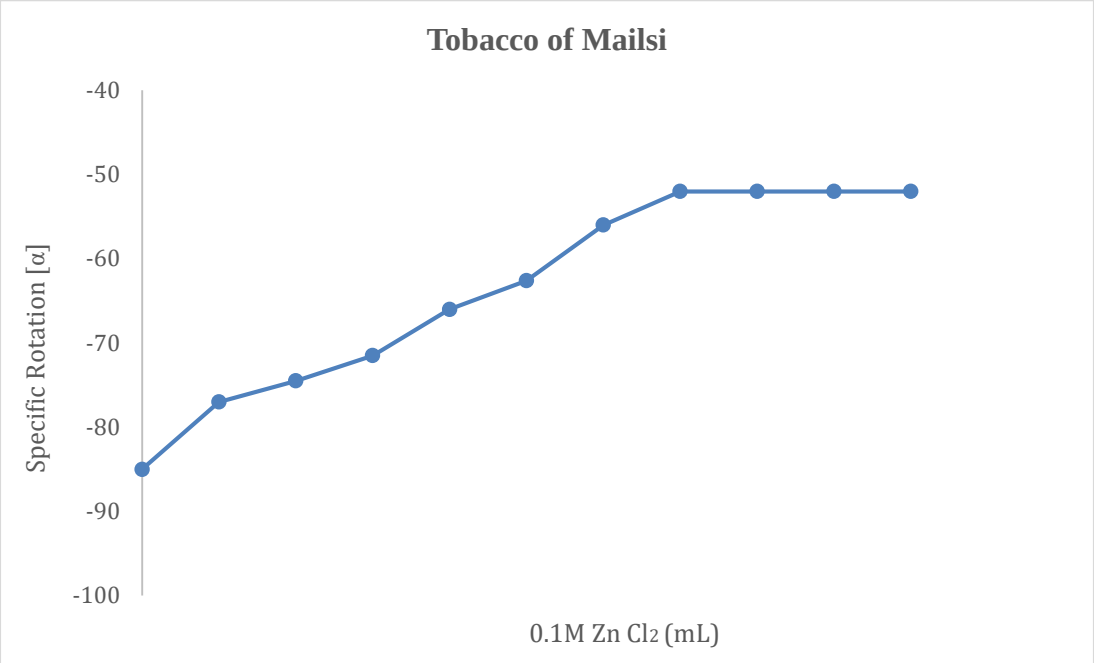

Fig., 4: Graph of Titration with Mailsi Tobacco

As 10 grams of Mailsi Tobacco contains 0.1135 grams of Nicotine, therefore, percentage of nicotine in Mailsi Tobacco is 1.13%.

3.3. Estimation of Nicotine in tobacco of Nana Dogar

Table 4. Observed Rotations of Nana Dogar Tobacco

Tobacco extract (mL)	**0.1M $ZnCl_2$ (mL)**	**Total Volume (mL)**	**Observed Rotation α°**	**Specific Rotation [α]**
10	0	50	-0.415°	-83.0
10	0.5	50	-0.390°	-78.0
10	1	50	-0.367°	-73.5
10	1.5	50	-0.352°	-70.5
10	2	50	-0.325°	-65.0
10	2.5	50	-0.307°	-61.6
10	3	50	-0.280°	-56.0
10	3.5	50	-0.255°	-51.0
10	4	50	-0.237°	-47.5
10	4.5	50	-0.237°	-47.5
10	5	50	-0.237°	-47.5
10	5.5	50	-0.237°	-47.5

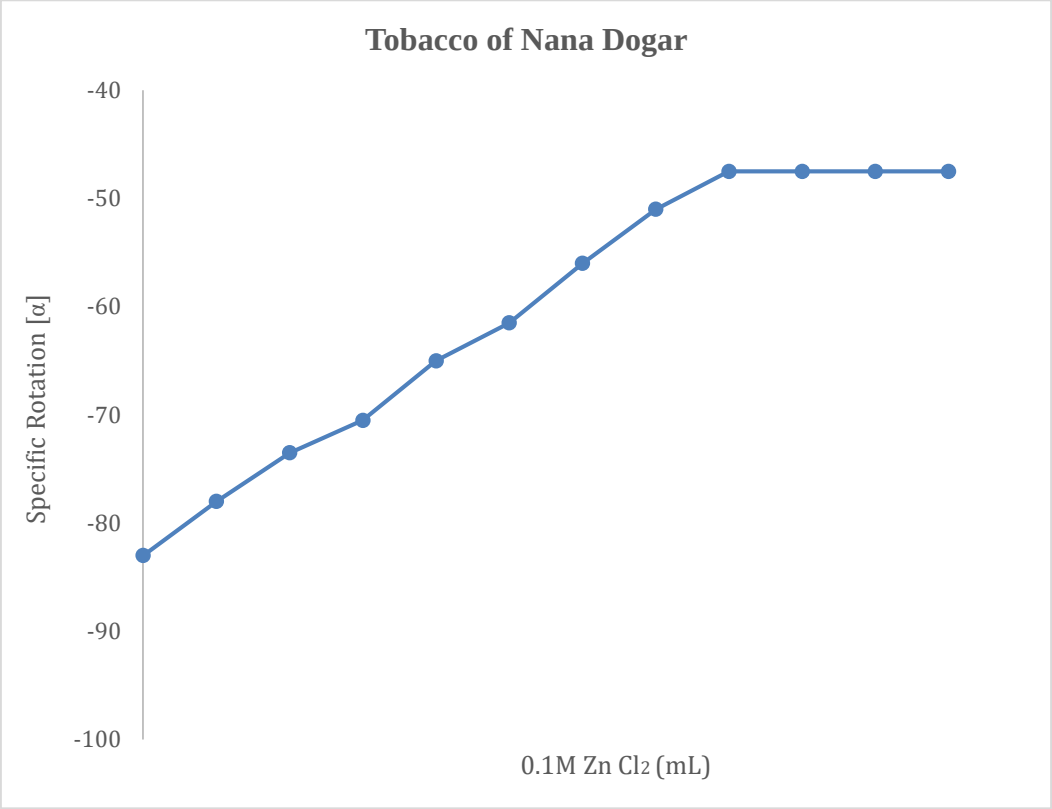

Fig. 5: Graph of Titration with Nana Dogar Tobacco

As 10 grams of Nana Dogar Tobacco contains 0.1297 grams of nicotine, therefore, percentage of nicotine in Nana Dogar Tobacco is 1.29%.

3.4. Estimation of Nicotine in Tobacco of Sahiwal

Table 5. Observed Rotations of Sahiwal Tobacco

Tobacco extract (mL)	**0.1M $ZnCl_2$ (mL)**	**Total Volume (mL)**	**Observed Rotation α°**	**Specific Rotation [α]**
10	0	50	-0.405°	-81.0
10	0.5	50	-0.387°	-77.5
10	1	50	-0.370°	-74.0
10	1.5	50	-0.357°	-71.5
10	2	50	-0.322°	-64.5
10	2.5	50	-0.305°	-61.0
10	3	50	-0.270°	-54.0
10	3.5	50	-0.252°	-50.5
10	4	50	-0.252°	-50.5
10	4.5	50	-0.252°	-50.5
10	5	50	-0.252°	-50.5

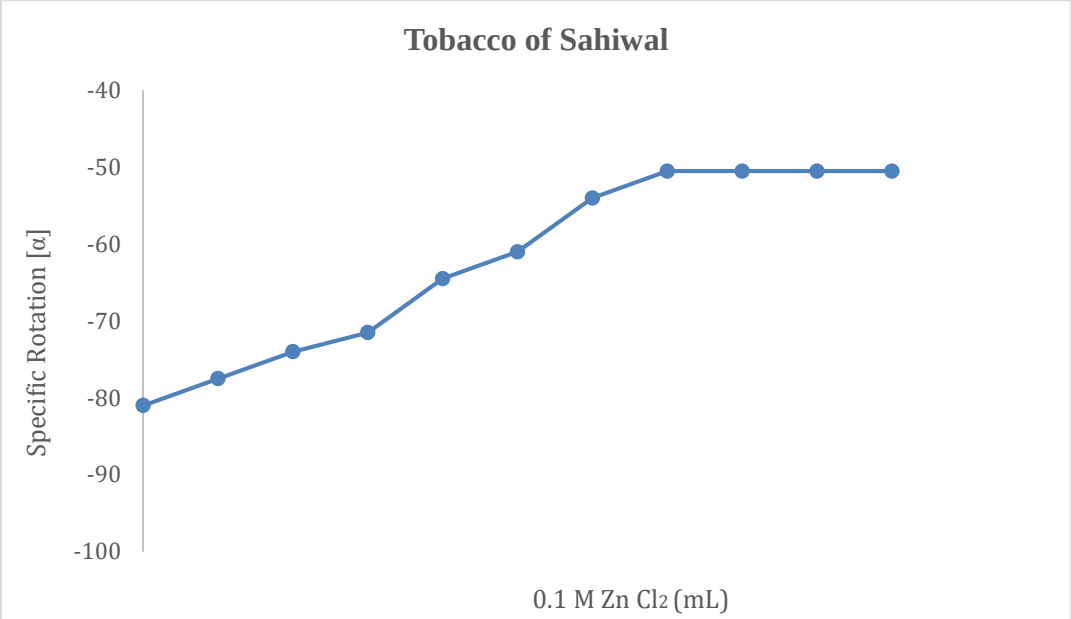

Fig. 6: Graph of Titration with Sahiwal Tobacco

As 10 grams of Sahiwal Tobacco contains 0.1135g of Nicotine, therefore, percentage of nicotine in Sahiwal Tobacco is 1.13%.

3.5. Estimation of Nicotine in Tobacco of Toba Tek Singh

Table 6: Observed Rotations of Toba Tek Singh Tobacco

Tobacco extract (mL)	**0.1M $ZnCl_2$ (mL)**	**Total Volume (mL)**	**Observed Rotation α^o**	**Specific Rotation [α]**
10	0	50	-0.450°	-90.0
10	0.5	50	-0.440°	-88.0
10	1	50	-0.415°	-83.0
10	1.5	50	-0.407°	-81.5
10	2	50	-0.400°	-80.0
10	2.5	50	-0.395°	-79.0
10	3	50	-0.380°	-76.0
10	3.5	50	-0.370°	-74.0
10	4	50	-0.355°	-71.0
10	4.5	50	-0.335°	-67.0
10	5	50	-0.335°	-67.0
10	5.5	50	-0.335°	-67.0
10	6	50	-0.335°	-67.0

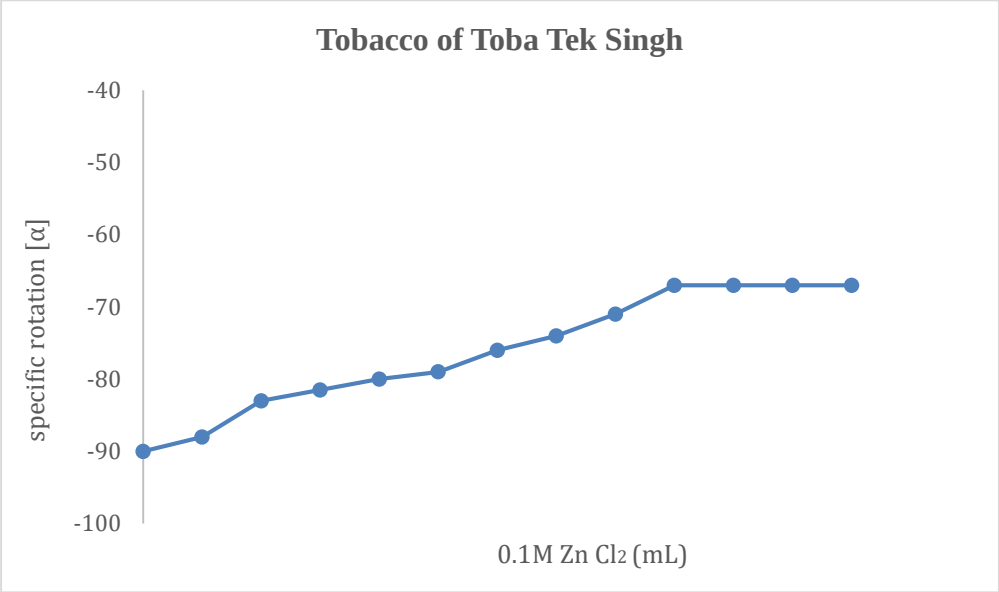

Fig. 7: Graph of Titration with Toba Tek Singh Tobacco

As 10 grams of Toba Tek Singh Tobacco contains 0.1460 grams of Nicotine, therefore, percentage of nicotine in Toba Tek Singh Tobacco is 1.46%.

4. Discussion

Estimation of nicotine in different varieties of tobacco was carried out by the Polarimetric Titrimetric method. Results are given table below. Experiments show that the amount of nicotine Toba Tek Singh tobacco sample is more than in the other four cities.

Table 7: Observed Rotations of Mailsi Tobacco

Tobacco	Nicotine Percentage
Changa Manga	0.97%
Mailsi	1.13%
Nana Dogar	1.29%
Sahiwal	1.13%
Toba Tek Singh	1.46%

Different varieties of tobacco were tried. Results show that zinc preferentially reacts with nicotine compared to many other ligands and non-ligand components in the tobacco extract. With careful selection of metal ions (here zinc), selective coordination of a particular component in a mixture of the multi-component system is possible. This idea can be used for the extraction and estimation of a compound of interest in a mixture of plant extract. This idea has been experimentally successfully verified in the present research with direct estimation of nicotine without its isolation from tobacco extract. It may be pointed out; Polarimetric titrimetry technique has been used for the first time for quantitative estimation of nicotine in a

variety of tobacco samples. This method should have been tried on standard tobacco samples with known nicotine contents to confirm the obtained results.

5. Conclusion

When added to a plant extract, which contains many different compounds, e.g. alkaloids, terpenes, flavonoids, proteins, tannins, etc. metal ions may selectively coordinate with a particular component, then tobacco in Nicotine component can easily be estimated. The selective complexation, of course, depends upon the nature of the metal and the ligand; for example, Zn^{2+} ion, when added to tobacco extract, selectively coordinated with the nicotine. The nature of metal and ligand is vital for selective coordination, but experimental conditions also play an important role in this respect. Thus under optimum conditions, it is possible to estimate the amount of a single component from plant extract using metal ions.

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