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Nicotine and Colored Complexes

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Abstract An activity for nicotine extraction and detection in tobacco. The procedure is based on the extraction of nicotine from tobacco, followed by the reaction between nicotine with Co(II) and Cu(II) ions. A qualitative experiment is proposed in order to demonstrate and visualize the presence of nicotine in tobacco and to demonstrate metal-nicotine complexes formation and their mass spectrometry (ESI) analysis.

Keywords: *nicotine, qualitative analysis, mass spectrometry (ESI), metal complex, naked-eye experiment, tobacco, first-year undergraduate/general, general public, high school/introductory chemistry*

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1. Introduction

Nicotine ($C_{10}H_{14}N_2$, **NIC**) is one of the alkaloids which is found in certain plants, it is well recognized for its toxin activity, and its chemistry is an important topic, because of its health implications. Nicotine is found in tobacco plants (*Nicotianatabacum*), where it is synthesized in the roots and accumulated in the leaves, making up 0.6 to 5.0% of the dry weight of tobacco. [1,2,3,4,5] It is an oily liquid and it is miscible with water in its base form. Nitrogenous atoms of nicotine form salts with acids, which are soluble in water. When tobacco is smoked, nicotine is absorbed through the wall lining of the small air sacs in the lungs. When sniffed or chewed, it is absorbed through the mucous membranes of the nose or mouth.

Nicotine or 3-(2-(N-methylpyrrolidinyl))pyridine, has a wide spectrum of detrimental biological activities on the cardiovascular and gastrointestinal systems, as well as dependence and neuromuscular effects and beneficial activity, which has been shown on neurodegenerative diseases, like Alzheimer’s and Parkinson’s, attention deficit, hyperactivity disorder, Tourette’s syndrome and schizophrenia [6]. This beneficial activity seems to occur as a result of nicotine’s interaction with nicotinic acetylcholine receptors in peripheral and central nervous system [7]. Specific chemical groups presented in the nicotinic acetylcholine receptors, include a quaternizedamino nitrogen, situated at a certain distance from a hydrogen-bond acceptor, the pyridine nitrogen in nicotine. This heterocyclic nitrogen is able to interact with transition metal ions and several studies on the preparation and characterization of transition metal complexes with nicotine have been reported [8,9]. On the other hand, it is well known that some metal complexes could retain or modulate the biological activity of the organic ligands [10].

The structure of nicotine has a unique functionality. Nicotine possesses the methylated pyrrolidine nitrogen ($pKb1 = 6.16$) and the less basic pyridine nitrogen ($pKb2 = 10.96$), connected by a chiral center. Both of the rings contain nucleophilic nitrogen atoms, which act competitively when reacting with electrophiles. An important aspect of nicotine reactivity is its metal complexation [11,12,13]. Different studies have highlighted the fact that biologically active compounds become more carcinostatic or bacteriostatic upon chelation or coordination with metal ions [14,15].

In this experience we try to answer questions like: “What contains the tobacco?”, “Which is the structure of the nicotine and how to extract it?”, “How nicotine chemically reacts with the metal ions?”.

Herein, we investigate the extraction of the alkaloid nicotine (NIC) from tobacco and the formation of several Co(II) and Cu(II) complexes. One of the goals of this demonstration is to show complex formation reactions with nicotine as a ligand and Co(II) and Cu(II) as the ions to be coordinated with it.

In general metal coordination complexes of biological molecules have much potential for designing novel therapeutic and diagnostic agents, which target specific properties and show reduced side effects, avoidance of resistance and improved selectivity, as they can be used for treating a wide range of important human diseases [16,17].

Additional challenges of this experience are the possibility to better understand the mechanism of action of the small molecules (as nicotine),in order to make a further evaluation and modulation of the chemical composition and reactivity. Of particular importance in the field of syntheticand biological chemistry are copper(II) and cobalt(II) complexes, because of the role this element plays in biological systems [18,19]. The interaction of transition metal ions with drugs is a subject of considerable interest, as nicotine has been previously reported to form molecular complexes with Ag(I), Zn(II),Cu(II) and Co(II) ions [20,21].

2. Experiment

The times pan of this experiment is two, 4 h sessions, with a prelab quiz administered at the beginning of each session. The experiment is carried out individually.

In the first session, students extract nicotine from tobacco. Add 200ml of NaOH 1M with stirring to 10 g of tobacco leaves in beaker (for 30 min). Filter the mixture in Buchner funnel using glass wool and press the cigarettes (than wash with 20 ml of water three times). Collect the filtrate together, if there is any impurities re-filter). Extract by 30 ml diethyl ether and repeat the extraction 4 times. Gather the 4 filtrates in a conical flask and dry by using 1 teaspoon anhydrous potassium carbonate or sodium sulfate. Filter and evaporate ether on water bath (Avoid extra heat because nicotine is hydrolyzed by extreme heating) and add 1 ml methanol, in order to dissolve the resulted pale yellow oil (see Figure 1). Alternatively, for Soxhlet extraction, extract 10 g of tobacco leaves with methanol for 2 h in a Soxhlet system at refluxing temperatures. Allow the liquid to cool, transfer it into a 100-ml volumetric flask and concentrate it, in order to obtain 2-5ml of concentrate yellow oil [22].

Figure 1. From the left: tobacco leaves, basic solution for extraction, concentrate solution containing nicotine

In the second session, each student is assigned either Cu^{2+} or Co^{2+} ion, from which to prepare a metal−**NIC** complex. In two test tubes, Copper or Cobalt salt are dissolved in methanol on a steam bath, and some drops of the extracted nicotine are added. The color changing and any appearance are recorded. Students record the ESI spectrum and ESI-MS/MS spectra of the principal peak for the nicotine, metal salt and metal complexes solutions. Students simulate mass spectra with free mass spectra simulator, in order to compare it to experimental data. Students collate results, so that they can comment on the differences between the cobalt and copper complexes. A detailed description of the experimental procedure is reported in the Supporting Information.

3. Hazards

Students must wear safety glasses and laboratory coats at all times. The extraction steps should be performed in a fumehood. The copper(II) and iron(III) chloride salts are harmful if swallowed and by inhalation. Ninhydrin is harmful if swallowed and causes skin, respiratory and eye irritation.

Diethyl ether, ethanol and methanol are highly flammable and toxic, the latter being particularly toxic when absorbed through the skin. Students are provided with access to the material safety data sheets for all chemicals, and are asked questions on safety aspects of the experiment as part of their prelab work.

Caution is needed in preparing the solutions, as pure nicotine is an extremely deadly poison. Sodium hydroxide pellets are caustic. Avoid contact with skin, especially near the eyes. To make up a solution, add the weighed pellets to cool water with stirring. When NaOH dissolves in water, heat is evolved. Remember to wear safety glasses when preparing or presenting the experiment.

4. Results and Discussion

4.1. Qualitative Analysis

TLC was performed on 10×20 cm TLC sheets, coated with 0.25 mm layers of silica gel 60 F_{254} (E. Merck). After the application of the extract (about 10μl), the sheets were developed in paper-lined all-glass chambers with 10 ml of dichloromethane-methanol (90-10), previously left to equilibrate for at least 15 min. The visualization of the nicotine was achieved by spraying the sheets with ninhydrine solution (0.2 % in ethanol), letting them evaporate and then gently warming them up on the plate. Usually a light pink coloration may develop on a background. Blue-pink color is due to the formation of Ruhemann's purple. Spots are visualized also with a UV lamp. The distance to the center of the spot (dspot) and the solvent front distance (dsolv) from the spot line are measured with a ruler, and the Rf (retention factor) for the spot is calculated from Rf =dspot/dsolv. The TLC method uses commonly available supplies and laboratory glassware, facilitating its use in the general chemistry laboratory, such as 100 ml beakers, filter paper, and aluminumfoil to cover the beaker (the “TLC chamber”). The TLC developing solvent efficiently separates nicotine (Rf = 0.20−0.25).

Figure 2. TLC treatment with ninhydrin should result in a dramatic purple color if the nicotine is present

4.2. Complexes with Cobalt(II) and Copper(II) Solutions

The Copper solution has to be prepared by dissolving 50 mg of $Cu(NO_3)_2*3H_2O$ in 10 ml of methanol. The Cobalt solution has to be prepared by dissolving 50 mg of $CoCl_2*6H_2O$ in 10 ml of methanol. All the solutions must be prepared using distilled water. Observe any color changing.

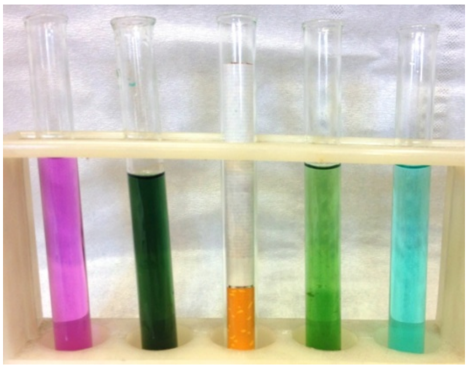

Figure 3. From the left: $CoCl_2$ (pink); $CoCl_2$ and nicotine (green complex); cigarette for extraction; $Cu(NO_3)_2$ and nicotine (green complex); $Cu(NO_3)_2$ solution (light blue)

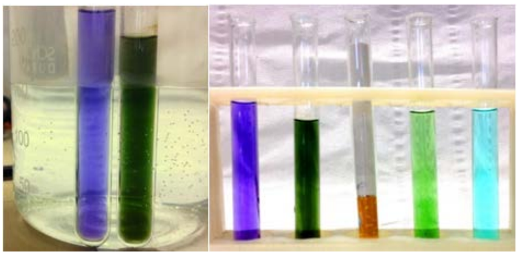

Figure 4. Hot solution presented in Figure 3, observe the differences in the Co solutions

In two tubes transfer 5 ml of the metal solution and add 3-4 drops of concentrate nicotine solution, obtained from the extraction of tobacco (Figure 3). Heat in a water bath for 15 minutes and observe any color changes (Figure 4).

4.3. ESI-MS/MS Spectra

ESI (Electro Spray Ionization) experiments were conducted with a Thermo Finnigan Advantage Max Ion trap spectrometer in positive ion acquiring mode; sheath gas flow rate was set at 25 (arbitrary unit), auxiliary gas flow rate at 5 (arbitrary unit), spray voltage at 3.25 (KV), capillary temperature at 270 C, capillary voltage at 7 (V), and tube lens offset at 60.00 (V). Nitrogen was used as sheath and auxiliary gases.

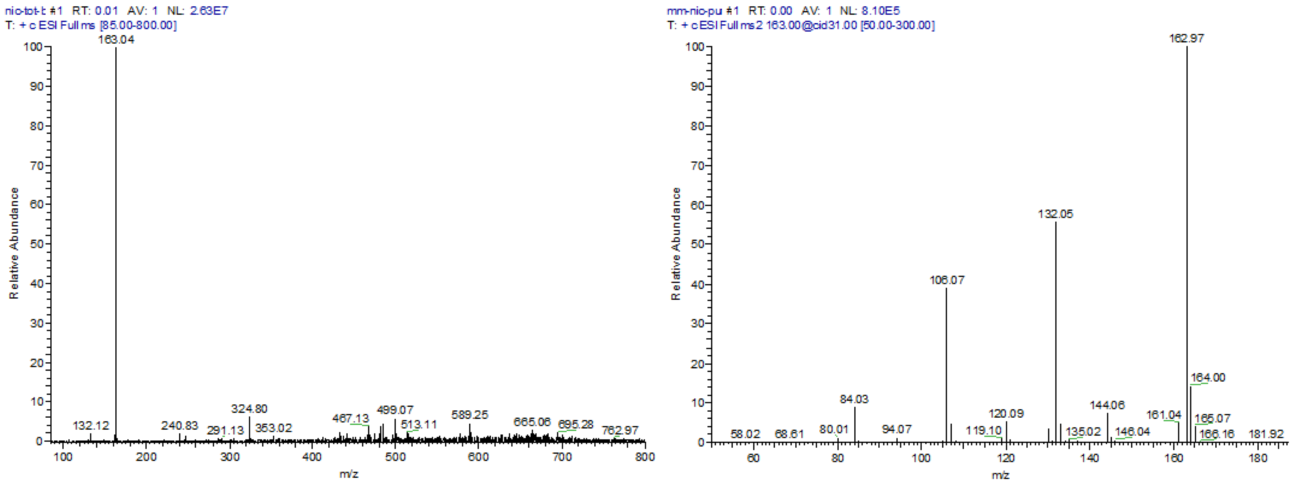

Figure 5. a) ESI spectrum of nicotine from tobacco extraction; b) ESI-ms/ms spectra of peak at 163.04 and fragmentation patterns of nicotine

The ESI spectrum reported in Figure 5a) shows a major peak at m/z=163.04, relative at the pure nicotine extract from tobacco leaves. The mass spectrum reported in Figure 5 b) shows the complicate fragmentation obtained in ESI-MS/MS spectrum; in SI we report the previously proposed structure of major fragments [23]. Similar molecular structures yielded similarities in the ESI-MS/MS fragmentation patterns of nicotine and its relative metal complexes.

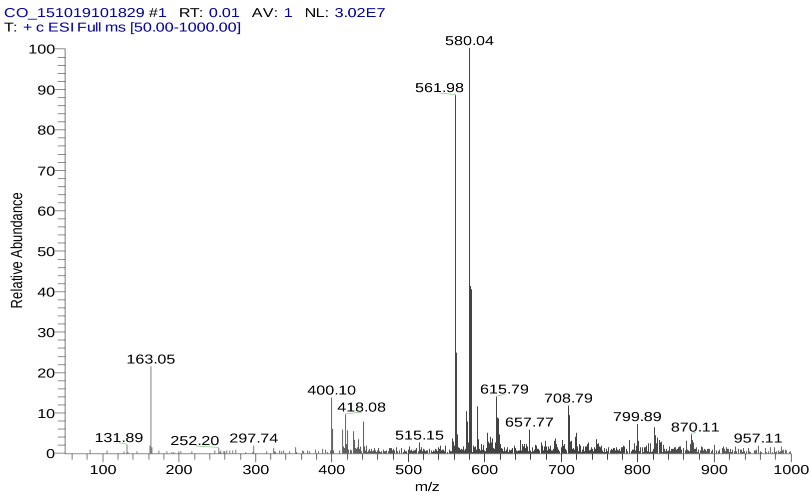

Figure 6. ESI spectrum of Co-nicotine solution from the green solution obtained in the qualitative experiment

The mass spectrum reported in Figure 6 is relative to the Cobalt solution with nicotine. Figure 6 shows two major peak at m/z=561.98 and 580.04, attributable to the corresponding complexes m/z= 561.98= $[Co(NIC)_3OH]^+$ and580.04= $[Co(NIC)_3Cl]^+$. The isotopic patterns confirm peaks assignation (see SI).

In the complex m/z= 561.98= $[Co(NIC)_3OH]^+$, the presence of OH^- group is attributable to the protonation of the free nicotine (OH^- comes from the water of crystallization, $CoCl_2$*$6H_2O$ reagent).

On these peaks tandem mass spectrometry analysis was performed in order to obtain more structural pieces of information (Figure 7). The clear fragmentation obtained in MS/MS spectrum revealed that both the complexes can lose a nicotine ligand (579.94-418.00=161.94; 561.92-400.04=161.88, $NIC+H^+$m/z=163.05). The stoichiometry of the two complexes are confirmed by isotopic pattern (see SI).

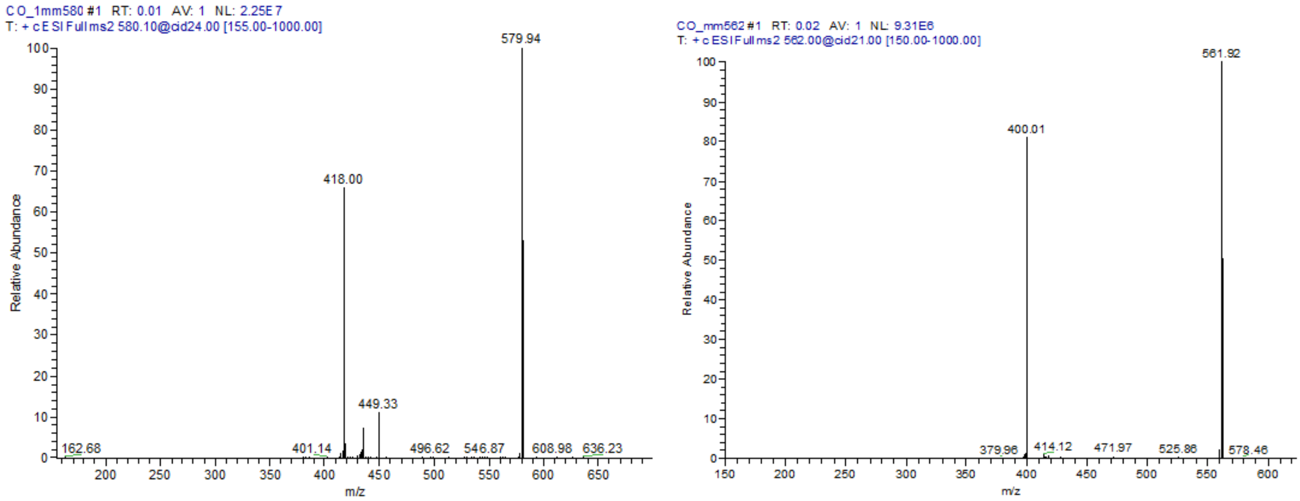

Figure 7. ESI-MS/MS spectra of Co-NIC complexes. On the left: ESI-MS/MS spectra of the peak at m/z= 580 and fragmentation patterns (loss of a nicotine ligand); on the right: ESI-MS/MS spectra of the peak at m/z= 561 and fragmentation patterns (loss of a nicotine ligand)

The mass spectrum reported in Figure 8 shows a single major peak at m/z=448.93=$[Cu(NIC)_2NO_3]^+$. The isotopic patterns confirm the peak assignation (See SI). Other minor peaks are assigned:

m/z= 351.19= $[Cu(NIC)(CH_3OH)_2(NO_3)]^{+1}$
m/z= 369.23= $[Cu(NIC)(CH_3OH)_2(H_2O)(NO_3)]^{+1}$
m/z= 383.25= $[Cu(NIC)(CH_3OH)_3(NO_3)]^{+1}$.

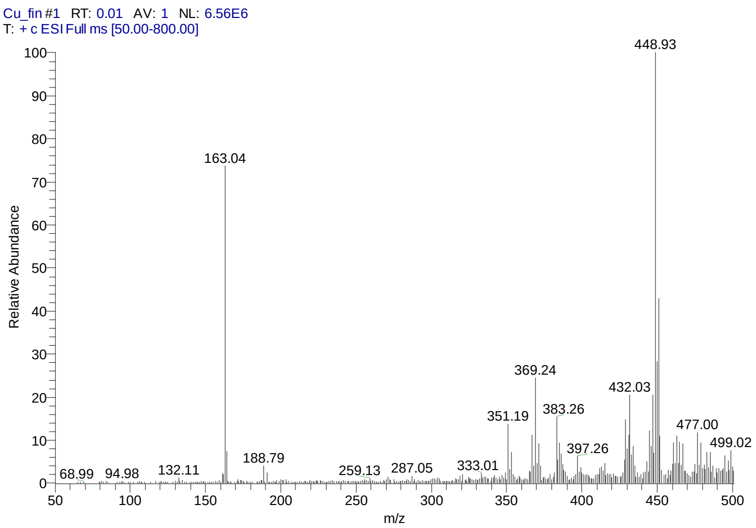

Figure 8. ESI spectrum of Cu-nicotine solutionfrom the green solution obtained in the qualitative experiment

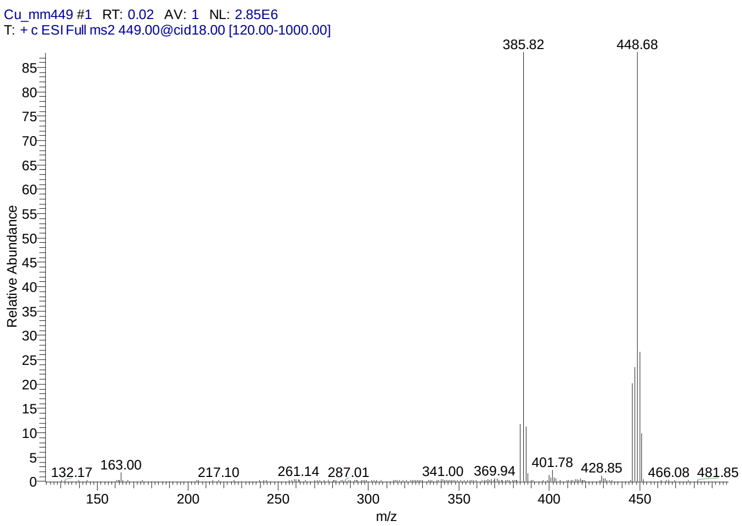

Figure 9. ESI-MS/MS spectra of the peak at m/z= 448.69 and fragmentation patterns (loss of a nitrate)

Also on the major peak, tandem mass spectrometry analysis was performed, in order to obtain more structural information (Figure 9). The clear fragmentation obtained in MS/MS spectrum revealed that the complex can lose a nitrate (448.93-385.82=62.86;).

5. Discussion

Many important features of the chemistry of cobalt and copper complexes cannot be explained in terms of the Valence Bond Theory. Crystal Field Theory can be used to justify the diverse colors of the Co(II) and Cu(II) methanol solution and of the different nicotine complexes or, conversely, the colors can be used to give a visual demonstration of the theory [24]. Although these colors are possibly familiar to all general chemistry instructors, and to some students, their juxtaposition in this demonstration can be used to dramatize several ideas: the changes in color is due to displacement of different equilibria.

COBALT

Transition metal ions in solutions generally exist as complex ions in which solvent molecules or other ligands, acting as Lewis bases, coordinate or bond with the metal cation.

Cobalt can form compounds in two diverse oxidation states, +2 and +3. The +2 state is more common. The ion Co^{2+} (aq) is pale pink. Other compounds of Co(II), which include both anhydrous Co^{2+} and complex ions, are commonly deep blue.If a solution contains both cobalt(II) and chloride ions, the blue ion $[CoCl_4]^{2-}$ forms, in equilibrium with the pink Co^{2+} (aq) ion.

$$(CoCl_4)^{2-}(aq) \leftrightarrow Co^{2+}(aq) + 4Cl^{1-}(aq).$$

Blue **Pink**

At relatively low concentrations of chloride, the equilibrium lies far to the right, and the solution is pink. If there is a large concentration of excess chloride, the equilibrium tends to the left, and the solution tends to be blue. The equilibrium is sensible to temperature, as well as to concentration of solutes. At lower temperatures, the equilibrium tends to the right and the solution looks like pink; at higher temperatures, it lies to the left and the solution appears more blue. The color changes are reversible. In this experience it is possible to visualize both the colored solutions. After the nicotine addition, a mixture of $[Co(NIC)_3OH]^+$ and $[Co(NIC)_3Cl]^+$ forms and the solution becomes deep green. The color change is irreversible. Both the compounds $[Co(NIC)_3Cl]^+$ and $[Co(NIC)_3OH]^+$ are detected in mass spectrometry analysis. The isotopic patterns confirm the peaks assignation (See SI).

COPPER

Orgelfirst pointed out the application of crystal field theory to copper complexes. According to this theory, the complexes of copper are basically square planar [25,26] (complex with m/z=448.93=$[Cu(NIC)_2NO_3]^+$, m/z=383.25 =$[Cu(NIC)(CH_3OH)_3(NO_3)]^{+1}$, m/z=351.19= $[Cu(NIC)(CH_3OH)_2(NO_3)]^{+1}$, and see SI for m/z= 613.34= $[Cu(NIC)_3(NO_3)]^+$). Anyway Cu(II) can coordinate more than four ligands; if any additional ligands (NIC, NO_3^-, H_2O, CH_3OH) are available, they would take up the fifth and sixth coordination positions (peaksm/z=369.23= $[Cu(NIC)(CH_3OH)_2(H_2O)(NO_3)]^{+1}$, and m/z=693.44= $[Cu(NIC)_3(NO_3)(CH_3OH)_2(H_2O)]^+$, see SI). [27,28,29] However, because of the fact that the Cu orbitals always contain a pair of electrons, in most copper complexes the fifth and sixth ligands will not be able to approach the copper as closely as the ligands in the plane, and thus the complex will have preferably a tetragonal structure. We can observe that the $[Cu(NIC)_2NO_3]^+$is the major peak in the Figure 8.

The experiment has the following key learning outcomes:

• Improve synthetic skills (extraction of an alkaloid reagents, solvent extraction, vacuum filtration, preparation of metal complexes), assessed by yield and appearance of ligand and complexes;

• Exposure to a range of analytical techniques (Mass spectrometry, volumetric glassware), assessed by reported spectra;

• Improve data analysis (comparison between experimental and simulate mass spectra), assessed by MS/MS fragmentation, isotopic distribution and determination of stoichiometry;

• Improve understanding (for example, of the relationship between absorption spectrum and color and of complexes with varying ligand numbers), assessed through preparation of the experimental report.

This experiment was not related to any lecture content, but students were able to observe that the identity of the metal ion affected the composition of the complex itself. Furthermore, as the use of structure-drawing software was a generic learning outcome for this laboratory course, students drew reasonable structures for the resulting metal complexes (see Supporting Information). Furthermore, students were required to compare measured ESI-MS/MS and fragmentation patterns to literature values, which gave them the experience of searching the literature for such information. While this experiment studies only nicotine to Co^{2+} and Cu^{2+} binding, nicotine complexes of other transition metals have been previously reported and characterized. Here, we chose to focus on Co^{2+} and Cu^{2+}, owing to their strong color changes, but this experiment could readily be expanded to include other transition metal ions.

6. Conclusions

Nicotine has a long story, as it has often been produced and used by humans for pharmacological purposes, because of its numerous biological properties. The LD50 of nicotine is 50mg/kg for rats and 3mg/kg for mice: this makes it an extremely deadly poison. 40-60mg/kg of nicotine can be lethal dosage for adult human beings. Students are often surprised in this experience by the results of the extraction, which indicate a significant presence of nicotine in cigarettes and in tobacco in general. The experience is easy to handle and prepare, is adapted to the time spent by students in laboratory and the absence of toxic reagents and derivatives render this synthetic approach particularly useful for students in complete safety, without risks, and with low costs and ease to reproduce.

Different and stable species of Co(II) and Cu(II) with nicotine ligand in methanol solutions were prepared and observed by intense color changes. The products were analyzed by mass spectrometry in electrospray ionization (ESI) and identified through the exam of the isotopic patterns and with the fragmentation patterns obtained with MS/MS analysis.

In conclusion, in this experience it is possible to observe an example of extraction of organic compound from tobacco leaves, different complexation equilibria of nicotine Cu(II) and Co(II) complexes, which have much potential for designing novel therapeutic and diagnostic agents, in order to better understand the mechanism of action of small molecules, such as nicotine. This demonstration fits particularly well if students are doing qualitative analysis for cobalt (II) and copper(II) ion, in which these colors are very indicative, and, in some circumstances, are proof of their presence in solution.

At a somewhat more advanced level, other things which might be included in the discussion are the structures of the complexes, the splitting of the *d*-orbitals and the relative field strength of the ligands, as per crystal field theory, and their absorption of light, as students can properly understand the results.

7. Summary

This laboratory experiment was appropriate for an intermediate, general or inorganic chemistry course. The experiment used simple, inexpensive starting materials, and demonstrated an umber of important principles related to modern chemistry research.

Associated Content

Supporting Information

Experimental details, notes for instructor, hazards, question and answer for lab experiences.

Notes

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