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NEW ASPECTS IN THE PHARMACEUTICAL ANALYSIS OF QUININE AND SOME OF HIS PRODUCTS ON OXIDATION

EXTENDED ABSTRACT OF A PhD THESIS

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I. INTRODUCTION

The dissertation explores various aspects of the qualitative analysis of the alkaloids quinine and quinotoxine - representatives of the general alkaloid set of *cortex Chinae*. Quinine is a pharmacopoeial representative and is currently used in the therapy of malaria, and in particular in cases of its cerebral form. Quinotoxine has been used for approximately a decade in medical practice as a cerebral vasodilator. Numerous derivatives with radically different behavior and activity have been obtained from both alkaloids. Therefore, every single aspect of the analysis of these alkaloids is significant and contributes to the general knowledge in the field of this class of bioorganic compounds. Additionally, the qualitative analysis is an element of the general identification analysis of each pharmacopoeial and pharmacologically significant representative.

In this regard, the methods of qualitative alkaloid analysis are the main topic of the present work. Although adapted in essence, the analytical methods presented here carry their original features and innovation. In some of them, different interdisciplinary motives are even combined - those of physics, photochemistry, fine organic synthesis.

Not every one of the presented methods is reflected in the scientific literature, but its place there is completely permissible after conducting additional research; research that goes beyond the generally accepted framework for a dissertation.

II. AIMS AND TASKS

Aim:

To present new qualitative methods for the analysis of the alkaloids quinine and quinotoxine and evaluate their fundamental analytical characteristics.

Tasks for achieving the stated aim:

1. To evaluate the reactivity of the quinine alcohol group towards the oxidizer molten elemental sulfur.

2. To assess the possibility of the photo-initiated conducting of the so-called herapathite test.

3. To determine the ability of quinotoxine to form phenothiazine dyes.

4. To determine the chemiluminescent reactivity (or inertness) of quinine towards octaammonium dicerium octasulfate tetrahydrate.

5. To compose a quinine-imprinted polymer layer on the surface of a quartz resonator and determine its sorption behavior towards (+)- and (-)-carvone.

III. EXPERIMENTAL PART

1. MATERIALS AND METHODS.

All chemicals were of analytical grade and were used as received.

ATR-FTIR spectra were recorded using an FTIR spectrometer (model Tensor II; Bruker), equipped with an ATR module. Spectra were collected within the middle infrared (IR) range.

The ultraviolet-visible spectroscopy (UV-Vis) absobtion spectrum was measured in a 1.0 cm quartz cuvette using a UV-Vis spectrophotometer, T60, from PG Instruments (Leicestershire, UK).

2. SYNTHETIC / ANALYTICAL METHODS.

All details regarding the analytical and synthetic procedures/methods used can be found in the dissertation and related publications.

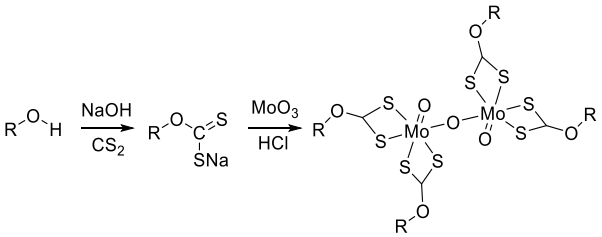
IV. RESULTS AND DISCUSSION

1. S_8 IN QUALITATIVE QUININE ANALYSIS.

The present study describes a simple analytical qualitative technique for determining the presence of secondary alcohol groups in the composition of the drug enantiomeric pair *(-)-quinine* and *(+)-quinidine*. The analysis is based on the oxidation potential of molten sulfur (S_8) and on the reactivity of the resulting H_2S to $Pb(OAc)_2$.

The qualitative analysis of raw drug substances containing secondary alcohol groups is a matter of paramount analytical importance [1,2]. As a rule, the pharmacopoeial analysis of complexly structured alcohols includes the following two main strategies [3]:

- implementation of complex-forming reactions; *i.e.* synthesis of distinctively colored complexes of mixed-ligand complexes:



and

- treatment with the *Lucas' reagent* [1].

[1] An equimolar mixture (a solution) of anhydrous $ZnCl_2$ and concentrated HCl.

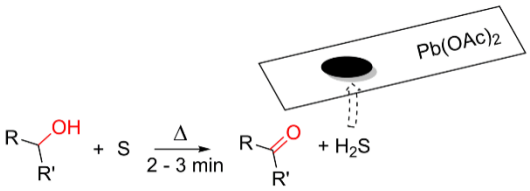
Although strictly specific, the first strategy requires the use of expensive, non-conventional and potentially toxic reagents - inorganic salts and oxides; as well as the usage of larger quantities of hazardous solvents - benzene, CS_2, *etc*.

Lucas' reagent, on the other hand, has been usually employed as a "measuring stick" for checking the quality of bottled (the so-called pure commercial) alcohols or as a reagent for the systematic analysis (or even identification) of lowmolecular-weight alcohols - saturated monofunctional alcohols having fewer than six or eight carbon atoms [4].

The oxidation-reduction analysis of secondary alcohols began early in 1957, when F. Feigl, V. Gentil and C. Stark-Mayer [5] accomplished qualitative analysis of several artificial and natural compounds in the presence of the inorganic reagent - elemental sulfur S_8. Shortly thereafter, however, this redox test lost its practical value, being substituted by the ones pointed out above and by other analytical methods [3].

From the chemical point of view, S_8 is principally used as a mild oxidizing agent, as a reagent for the *in situ* formation of carbonyl sulfide; S_8 is the reagent of choice when performing dehydrogenation of aromatizable substrates (hydrocarbons).

In the majority of cases, when non-volatile organic samples containing secondary alcohol groups are fused for a short time with sulfur, hydrogen sulfide is split off no matter what other functional groups or elements are present in the analytes' composition [4]. The evolved H_2S (gas), in turn, can be readily detected, even in traces, with a piece of filter paper moistened with $Pb(OAc)_2$ (Scheme 1).



Scheme 1. Schematic representation of the oxidation reaction of secondary alcohols in the presence of S_8.

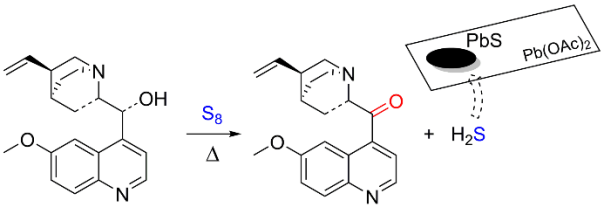
According to Feigl and co-authors [4], the current redox reaction seems to be especially realizable with analytes that melt at 120÷180°C.

In this context, an analogous pyrolytic splitting out of H_2S gas may occur when long-chain fatty acids (*e.g.*, palmitic, stearic, and oleic acid) and waxes (*non-aromatizable representatives*) are heated to about 250°C along with S_8 [4] by analogy with the ex-mentioned aromatizable hydrocarbons.

In these cases, however, the reaction proceeds so sluggishly that no traceable result can be sensitively (and readily) detected within the first three minutes; even when a lot of S_8 is used.

A series of positive analytical tests including the pharmacopoeial representatives *Chinidini sulfas* and *Chinini sulfas* was successfully carried out in order to boost the potential of Feigl's test when utilized for pharmaceutical analyses. A strategy for converting both drugs into analytically pure alkaloid bases was also employed. Moreover, the need of introducing S_8 in the form of a CS_2 solution was eliminated by the methodological modification imposed herein. The present work also presents an original microanalytical method for estimating the LOD values of the two alkaloids.

To supplement the potential of Feigl's test in the field of the pharmaceutical analysis, we initiated a series of tests for investigating with S_8 the reactivity of the medicines in question (Scheme 2). It should be pointed out that, in the cases where salts of organic bases are analyzed, it is necessary beforehand to release the organic constituent (low-melting organic base) from the inorganic one. That is why, in the current work, the procedures for the synthesis of the relevant alkaloid bases are presented in more details.



Scheme 2. Proposed route of the applied redox reaction between the used Cinchona' alkaloids and elemental S_8.

As expected, a positive analytical response was observed when an insignificant amount of the examined compounds was fused with elemental sulfur. The appearance of black-colored spots of PbS on the impregnated caps was recorded within one minute. The maximum time required to reach the level of assured analytical perception (maximum intensity), however, was estimated to be 3.0 minutes.

From an analytical point of view, however, the so-formed PbS spots may also be used as a means (analytical marker) for estimation of the so-called "limit of detection". Properly "configured", the present method can also be employed as an analytical tool for detecting traces of the investigated analytes.

Though, to register extremely small amounts of the analytes in question it is necessary to select reaction vessels with the smallest possible capacity. For this purpose, glass capillary microtubes with a total capacity of 45 microliters were selected as completely suitable. Regarding the precise and accurate transfer of submicrograms of both analytes in each microtube, the *dried droplet sample deposition technique* was employed in preparing all the samples [5,6]. Actually, this technique allowed us to examine the two analytes in the form of micro-residues evaporated from acetone (onto the surface of S_8 particles).

Aliquots of each working solution are withdrawn using a 10 μL GC micro-syringe[2] to be delivered exactly where needed, *i.e.* at the bottom of each capillary microtube; and then transferred directly into the volume of S_8 already introduced. As expected, the analysis revealed that the examined analytes thus deposited into the vessel content, do come into direct contact with the introduced oxidizing agent - S_8. Otherwise, a part of the analyte will be deposited outside the reaction zone, *i.e.* far from the deposited sulfur. As for the analytical sensitivity already achieved, its value will be reduced drastically in this case.

Special precautions must also be observed when evaporating the solvent (acetone) used. Much attention should be paid to avoid localized overheating the samples. Otherwise, significant amounts of the introduced samples can be pushed out of the capillary volume. That is why the resulting suspensions were allowed to evaporate slowly in a drying oven - at 40 °C in a matter of hours. The little that ultimately remains in every capillary must visually resemble the

[2] The total length of the needle used has, however, a direct bearing on the reproducibility of the test; it must reach the bottom of each capillary microtube.

sulfur implemented – the reagent used in excess. All capillaries were then plugged with small pieces of tightly wound filter paper. The latter should be wetted with a specific amount of freshly prepared solution of lead acetate; taking care, here, the droplets deposited not to exceed the sorption capacity of the paper stoppers used. After being thus charged and plugged, the capillaries were placed in a silicone bath preheated to 200°C. The total annealing time of the samples was 3.0 minutes. During this time, the main analytical (oxidation) reaction took place. Along with this, the resulting melt also changes both its color and its texture. The resulting dark brown color of the melts was preserved even after their cooling/solidifying. Cooled to room temperature, the samples were allowed to stand for another 3.0 minutes, but in a horizontal position. That allowed the residual H_2S to react exhaustively with the impregnated lead acetate. Thus conducted, the method is able to establish minimal amounts of the analytes studied.

Using the newly developed analytical protocol, we were able to establish accurately the limits of detection (LODs) of the two alkaloids - *quinine* and *quinidine*. The analysis showed that the magnitudes of these values for both analytes were equal to ~ 0.006 mg (or ~ 6.0 μg).

In order to justify the above-proposed route of the applied redox reaction and exclude a possible presence of false-positive reactions, additional tests were separately performed both with S_8 and with quinidine.

As expected, none of the blank samples used gave a false-positive outcome. A negative response was also registered even at fusing of larger amounts of the high-melting drug salts used - *Chinidini sulfas* and *Chinini sulfas.*

The presented analytical strategy selectively registers the presence of secondary hydroxyl groups in the analytes studied. Being accurate and reproducible, the presented approach appears to be completely applicable and suitable for the routine second identification analysis of both alkaloids.

2. THE QUINOTOXINE AS A PRECURSOR FOR THE SYNTHESIS OF "METHYLENE BLUE" DYE.

When investigating the literature on the qualitative analyses of the piperidine quinotoxine residue, nothing has been identified to date. Therefore, the present work is focused precisely on the qualitative analysis of this structural residue - a residue that distinguishes this alkaloid from its synthetic precursor, *quinine*.

The synthesis of quinotoxine was carried out according to the methodology of Biddle [7]. A minor modification was made regarding the selective isolation of the toxin from its concomitant impurities, representing an adapted concept from the manufacturing patent of *Viquidil hydrochloride* [8]. Furthermore, the overall duration of quinotoxine synthesis was also increased. To this end, the reaction mixture was monitored periodically with an external UV source[3].

The authenticity of the thus obtained product was established by ATR-FTIR and UV-Vis analysis (Figure 2).

[3] Exhaustion of the introduced quinine was evident by the loss of the fluorescent properties of the reaction medium.

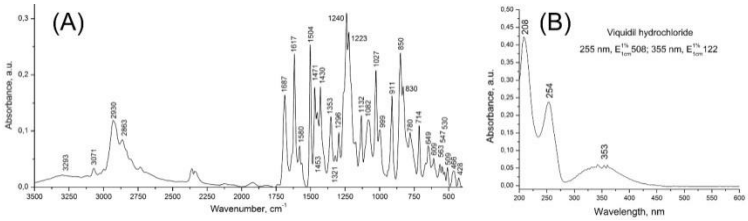
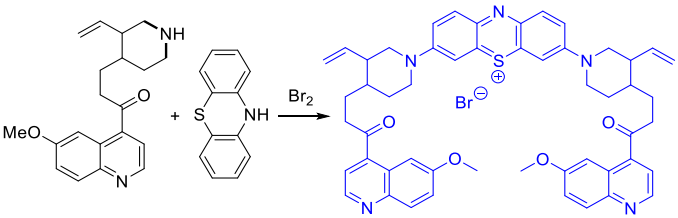


Figure 2 . ATR-FTIR (A) and UV-Vis (B) spectra of the alkaloid quinotoxine.

The purity of the product was determined by thin-layer chromatographic analysis, where the presence of no additional/side reaction product was witnessed.

As for the main analytical method, the strategy developed by Kehrmann [10] for the analysis of secondary amines was applied. Although the method was subsequently developed by Bröll and Fischer [11] for the analysis of cyclic amines, it has not yet found application in the analysis of natural compounds. Therefore, the aim of the present work was to establish the applicability of this test in the analysis of the alkaloid quinotoxine - a compound containing a secondary amino group in its piperidine moiety (Scheme 3).



Scheme 3. Proposed route of the applied *spot-test* reaction.

The expected analytical response is registered immediately upon the introduction of the tested alkaloid to the bromine–phenothiazine solution. By presumption, the appearance of the so-called methylene

blue color is considered as an irrefutable positive analytical sign for this test. The expressivity of the test can also be taken as an additional measure of the inertness of the remaining quinotoxine functional residues with respect to the specified reaction (piperidine) center.

To rule out the possibility of a false-positive result, the alkaloid quinine is also analyzed in parity. No indication of any analytical reflex to the quinuclidine-containing alkaloid is observed.

Actions were also taken to establish the limit of detection (LOD) of the specified test. The analysis showed that the magnitude of this value was equal to ~0.0075 mg (or ~7.5 μg).

The presented test can be perceived as a first attempt in the analysis of the piperidine residue of the alkaloid quinotoxine. Given that other available methods target the quinoline residue, which is common to this alkaloid class, the resented test has the potential to be the approach of choice to achieve the intended goal.

3. PHOTO-INITIATED SYNTHESIS OF HERAPATHITE.

The presence of the so-called quinine-herapathite test in textbooks on pharmaceutical chemistry and analysis is an indisputable fact. When utilizing this method in the analysis of quinine in tablets, it becomes clear that it can be easily modified. In other words, it is not necessary to isolate the alkaloid from the tablet content to obtain the characteristic crystalline (herapathite) precipitate. Furthermore, the analysis has been conducted even on a microscope slide, on which a very small portion of the tested tablet powder is placed. The appearance of rosettes of olive-green, cinnamon-brown or bluish crystals is instantly recorded by the authors [12].

Both the researchers mentioned and W.B. Herapath himself [13], as well as the authors who established the crystal structure of herapathite [14], use quinine, concentrated sulfuric acid and I_2 as reagents for growing the target crystallites. These reagents are pre-dissolved in an ethanol : acetic acid medium (1:1).

Knowing that KI is photosensitive, and that its prolonged exposure to air, including light, leads to the release of elemental iodine, we decided to modify the named test, using KI instead of iodine.

The quantities used and their stoichiometric ratios in the preparation of each analytical sample are presented in detail in the dissertation.

In every experiment conducted of each series, certain changes are imposed to detect or eliminate the occurrence of false-positive results.

In Series 1, the originally raised aim is set - to prepare an analytical reaction composition, similar to that of other authors, in which the elemental iodine is replaced with its precursor, potassium iodide (Sample A). In the subsequent compositions, the presence of some of the reagents used (Samples B, C and D) is deliberately removed, in order to detect the occurrence of false-positive results.

The expected changes in Sample A occur smoothly over time. The initially obtained, *in situ*, minimal amount of elemental iodine turns out to be insufficient for the full development of the specified analytical reaction. Upon subsequent exposure of these samples to sunlight, the amount of elemental iodine visibly increased, and a slight opalescence is observed in the solutions. After about a minute, the appearance of herapathite is reported. Upon prolonged exposure to solar radiation, the quantitative presence of herapathite in the samples increases, simultaneously with the enlargement of each herapathite crystallite. In the absence of one of the used reaction

precursors, acetic acid (Sample B), in the composition of the analytical solutions, the appearance of the target compound is not considered. Moreover, as a final result, in these samples, the appearance of a red insoluble product is observed. The latter most likely represents a molecular complex of the introduced alkaloid with iodine.

The resulting analytical responses for all samples from Series 1 are presented in Figure 3.

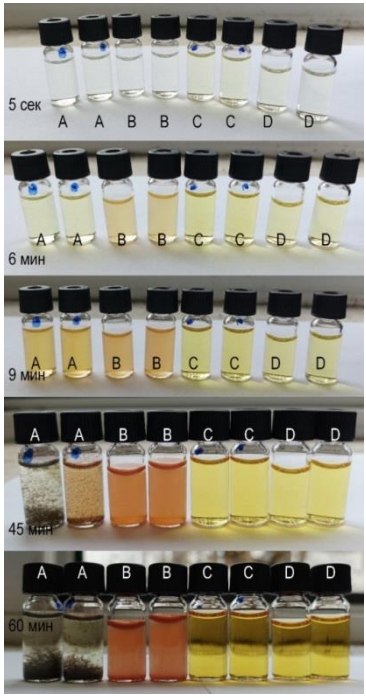


Figure 3. Photographs illustrating compositional changes over time in Samples A, B, C and D, Series 1, under sunlight exposure.

In the absence of quinine (Samples C and D) in the composition of the examined samples, only the appearance of the expected halogen element is witnessed.

A similar test was conducted, but using smaller quantities of H_2SO_4, KI and CH_3COOH (Series 2).

In general, the reported changes in each sample are identical to those recorded in Series 1. A significant difference is identified only in terms of the quantitative herapathite yield. The observation is, however, fully justified, given the smaller quantity used of the three mentioned herapathite precursors.

To verify the real contribution of the introduced sulfuric acid to the performance of this test, we conducted additional tests (Series 3). The quantity of sulfate ions introduced by the alkaloid itself turns out to be insufficient for the development of the analytical reaction in question. Moreover, in the absence of this acid, not even the appearance of elemental iodine - the other essential precursor in the thus modified test - is realized.

In a separate study, the behavior of the test mixture in an ethanol environment was investigated (Series 4). In an alcoholic environment, the introduced participants do not form a homogeneous solution. However, the appearance of the specific I_2 staining is recorded within minimal exposure to sunlight. When a total of 200 microliters of double distilled water is introduced into the mixture, the latter assumes a homogeneous appearance. But, even with approximately 110 minutes of residence time, the appearance of herapathite is not observed in this solution. Probably, the presence of a significant amount of ethanol in the reaction mixture hinders the formation of herapathite. Therefore, the authors, using the classic

methodology for the synthesis of herapathite, introduce the precursors in an equivolume mixture of water and ethanol.

With the presented modification of the test, the following analytical results are achieved: for the first time, the photoinitiated synthesis of herapathite is realized; the expected analytical result is achieved smoothly within a relatively short period of time (one hour); the need for participation of each analytical reagent in the synthesis of the target compound can be easily traced, and furthermore, the methodology of the experiment allows accounting for the stoichiometric requirements in terms of the necessary halogen molecule.

The test is easily implemented. It uses commonly available and relatively harmless reagents. This test, in its modification, can also be used for academic/educational purposes. It combines elements of photochemistry, chemistry (including the stability of compounds) of iodine, pharmaceutical analysis, and also the chemistry of clathrate compounds.

4. CHEMILUMINESCENT QUININE ANALYSIS.

The basis of Ce(IV)-chemiluminescent systems is the process of reduction of Ce(IV) to Ce(III) ions, accompanied by the emission of electromagnetic radiation. The reaction is carried out in an acidic environment (usually sulfuric acid) and finds applications in pharmaceutical analysis. Furthermore, the presence of certain pharmaceutical substances in the composition of dosage forms can be detected with the help of this analytical system. Success in this field is mainly associated with the development of the so-called flow-injection-chemiluminescence methods (flow-injection-chemiluminescence methods). The sensitivity of the method increases, in fact, with an increasing concentration of Ce(IV) ions. In

almost every Ce(IV)-based chemiluminescent system, there is also a sensitizer molecule (syn. fluorometric reagent - quinine, rhodamine B, rhodamine 6G, lanthanide (terbium and europium ions)) - a molecule that absorbs the energy released by the redox reaction and emits, in turn, electromagnetic radiation of greater intensity. Hence, most methods involving Ce(IV) ions are indirect.

Ce(IV)-chemiluminescent systems are widely used in the analysis of S-containing substances (tiopronin, captopril, menadione sodium bisulfite) and S-substituted benzamides, parabens, phenolic compounds, *etc*.

$(NH_4)_8[Ce_2(SO_4)_8]$ $4H_2O$ and $(NH_4)_2[Ce(NO_3)_6]$ complexes are usually used as Ce(IV) sources. $(NH_4)_8[Ce_2(SO_4)_8]\cdot 4H_2O$ (CAS) is a solid crystalline substance with a melting point of 140°C (with decomposition). It has a characteristic color - orange. It is soluble in water, dilute H_2SO_4 and dilute H_2SO_4 -MeCN solutions. The cerium ion is a stable, low-toxic reagent, a powerful one-electron oxidizer [15].

Considering the reactivity of CAS and the structural features of the commonly used sensitizer – quinine, we decided to conduct additional research on the propensity of the title alkaloid to interact with the oxidant used.

The result of the analysis is presented in Figure 4. From the figure it is evident that no luminescent yield is initially registered when the alkaloid is added to the available CAS solution. The yield manifests itself after some time - a few minutes. It is obvious that the received signal does not have a certain course, but passes, sporadically, through certain extreme values. Given the use of highly dilute solutions of the two analytes and also the choice of approach for the introduction of the quinine solution, the possibility of limited mass

transfer of the analytes in the solution is unlikely to occur and be a significant reason for the observed experimental behavior.

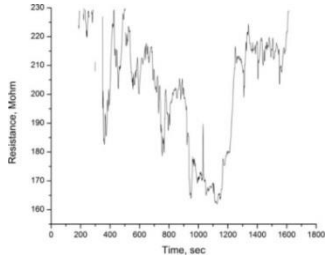


Figure 4. Graph illustrating the luminescence behavior of the tested quinine-cerium sulfate reaction pair.

However, in a repeat of this experiment, radically different spectral results were reported (Figure 5). The appearance of a luminescent response is also registered here after some time, but the total luminescent yield of the reaction is clearly much lower than that reported in the previous one; the absence of clearly defined emission bands in the presented spectrum is meant. The latter are rather discrete rather than continuous in nature. The higher intensity of these types of signals should be related to the physical characteristics of the used photoresistor and its threshold sensitivity to weaker signals.

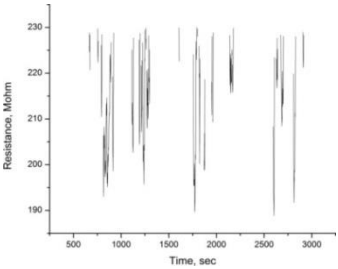


Figure 5. Graph illustrating the luminescence behavior of the tested quinine-cerium sulfate reaction pair.

In the third repetition of this experiment, the occurrence of photochemical emission from the investigated solution is not registered. The same regularity is reported in the subsequent experimental repetitions.

The lack of repeatability in this experiment encouraged us to continue and establish the obvious cause of the observed deviations in it. In the subsequent performance of the same experiment, but approximately 24 hours apart, we reported the appearance of a similar analytical result. Initially, the appearance of a continuous emission spectrum is reported, and then, in its second repetition, the appearance of discrete signals. The absence of electromagnetic emission in the subsequent experimental repetitions is again "witnessed", even when using freshly prepared solutions of both analytes.

In order to determine the intermediate reproducibility of this method, a change is imposed in only one conditional methodological element– the injection needle used. For these purposes, the metal needle used until now is replaced with teflon one.

Surprisingly, however, in none of the experiments conducted with the participation of the teflon needle, the appearance of a chemiluminescent signal was reported. What is observed is confirmed in multiple repetitions of the experiment.

Assuming that in the course of working with the used metal needle, metal ions are "introduced" into the test solution, an additional analysis is undertaken - an analysis that will establish the type of metal ions introduced in the injection solution. The inductively coupled plasma mass spectrometry (ICP-MS) method is selected as suitable. The analysis categorically confirmed the main presence of

four metals in the composition of the sulfuric acid eluate[4]– Fe, Al, Ti and Mn. In an analogous way, in the working solution, a certain quantity of the metal ions in question should enter with the introduced quinine solution. With each subsequent experiment, however, the total quantity of metal ions decreases, and with that the reported final fluorescence yield. The metal ions present, on the other hand, should assume a kind of participation in the chemiluminescence reaction. Their catalytic role is undoubted. Therefore, any loss in chemiluminescent yield reported in each subsequent experiment we associate with the extraction of metal ions from the inner/passable surface of the metal needle used.

Undoubtedly, the processes related to the participation of these metal ions resemble those characteristic of the so-called oscillating reactions (reactions in which, in the mixture of reacting chemical compounds, the concentration of one or more components changes periodically). In this case, a cascade of oxidation-reduction reactions is most likely realized between all the metal ions present, in a larger quantity in the reaction mixture.

Hence, when using a teflon needle, similar analytical behavior is not reported in any experiment.

Doubts about the reactivity of CAS towards the sensitizer used remained unresolved. In this connection, an engineering solution is imposed, which drastically increased the sensitivity of the photoresistor used. In order to overcome the limitations in the working range of the analytical instrument used, all readings of the photogenerated current are conducted in relation to the changes in

[4] Eluate obtained as a result of several consecutively applied washes of the test metal needle with the same volume of 0.1 M H_2SO_4.

the voltage of the constructed system. The obtained results are presented in Figure 6.

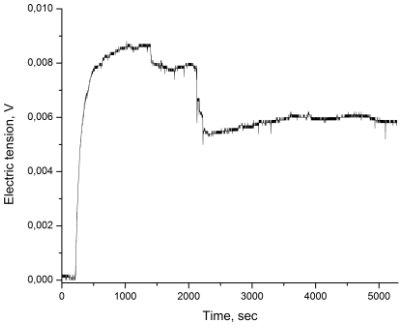


Figure 6. Spectral picture expressing the changes in the voltage of the constructed photoelectronic system as a function of the generated photocurrent over time.

It is essential to note that with the help of the measurement system constructed in this way, it is possible to register the so-called dark current (in the range 0.0000 ÷ 0.0002 V), with a resolution that allows even the thermal noise current of the used resistor to be recorded.

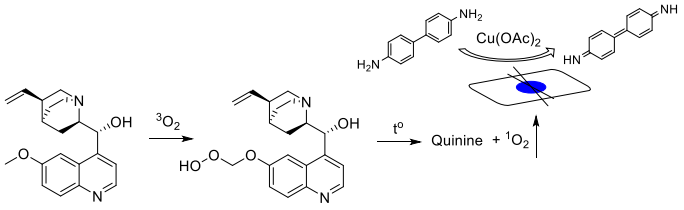
The calculations indicate that with the imposed modification, the photosensitive system composed in this way is able to register a generated photocurrent with a resistance of the order of 4000 ÷ 1500 MΩ; resistance which is very difficult to determine with conventional measuring equipment.

As soon as the quinine solution is introduced to the CAS solution deposited in the "dark cell", the appearance of a photocurrent is registered (Figure 6). The maximum recorded value is 9 millivolts. The obtained result is in full accordance with our expectations. However, realistically, the losses occurring as a result of quinine oxidation are extremely low and would not affect its general behavior

in processes where it acts as a sensitizer. This side process should have a negative impact on those analytical reactions that aim to detect the presence of analytes in ppt (*parts-per-trillion*).

In order to assess the actual quantitative losses of quinine molecules, further studies with quantum probes should be performed - studies that go beyond the scope of the current investigation.

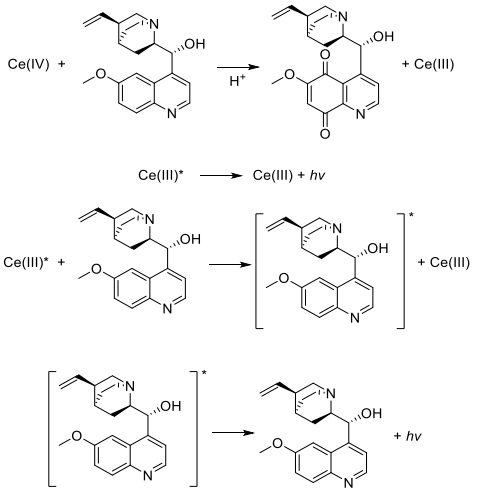
As a representative of the mixed ethers, quinine should exhibit a tendency to form hydroperoxides when exposed to atmospheric oxygen. Due to the fact that singlet oxygen released could be a possible cause of the appearance of a false-positive chemiluminescence result, an additional investigation has been conducted regarding the presence of peroxide impurity in the composition of the analyzed sample. When heated, hydroperoxides usually release singlet oxygen and restore their structure. Therefore, a study was conducted with approximately 100 milligrams of quinine base.



Scheme 4. Predicted reactivity of quinine towards atmospheric oxygen and subsequent analysis of its peroxide forms with benzidine-copper acetate-impregnated filter paper.

The presence of singlet oxygen is usually detected using filter paper impregnated with copper acetate and benzidine (Scheme 4). Using one, however, we found no indication of the presence of peroxide in the composition of the analyzed sample.

For the same purpose, an additional analysis aimed at studying the chemiluminescent behavior of the CAS solution itself was carried out. It is logical to assume that impurities may be present in the composition of the solvents used to promote the reduction of Ce(IV) ions. However, no such indication is reported. The process can be described by the following equations:



Scheme 5. Series of presumed interactions leading to the appearance of a chemiluminescent response.

Considering all these results, we decided to conduct an additional analysis with 4-aminophenol - an expected impurity of the medicinal substance paracetamol.

The result of the analysis is presented in Figure 7.

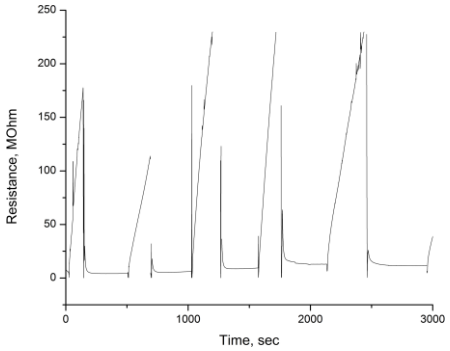


Figure 7. Photoemission spectrum reflecting the interaction of 4-aminophenol with CAS in the presence of quinine. The five emission bands obtained in a consecutive, fivefold repetition of the experiment are reposted as changes in the resistance of the system from the generated photocurrent.

The obtained data conclusively prove the reactivity of the used analyte towards the Ce(IV)/quinine system. The recorded chemiluminescence responses to the aminophenol in question are clearly distinct and reproducible (SD 6.7%). We associate the reported higher value in this analytical parameter with the uncertainty in the sample preparation process in the preparation of the three working solutions and with the impossibility of selecting absolutely equivalent volumes of them. This is also the probable reason why the flow-injection method is imposed in chemiluminescent analysis. However, the proposed method can be used in its modifications as a basic tool in the analysis of numerous (oxidizable with the oxidizing agent in question) accompanying substances.

The proposed chemiluminescence method is economical and easy to implement. The methodology allows the use of small quantities of commonly available reagents, which reduces not only the cost of the analysis, but also its impact on the environment. The relatively high

repeatability and low detection limit achieved make the method particularly suitable for the routine analysis of 4-aminophenol.

5. SORPTION BEHAVIOR OF QUININE-IMPRINTED QUARTZ CRYSTAL MICROBALANCE TOWARDS (+)- AND (-)-CARVONE.

There are also avant-garde analytical approaches for assessing the presence of various substances, combining the features of the newly introduced nano(molecular)imprinted materials and the capabilities of the so-called sensory techniques. Although the ability to create molecular imprints has long been known, today it is a cornerstone in the creation of many highly specialized materials for solid-phase extraction, drug delivery, etc. New analytical devices/techniques characterized by extremely high sensitivity are constantly being refined and developed. The technique of the Quartz Crystal Microbalance (QCM) can be presented as such.

By combining elements of these methods, we managed to organize a quinine-imprinted polymer layer on the surface of a QCM plate (QI-QCM; quinine-imprinted QCM). Considering the fact that the quinine molecule is chiral, we should expect that its molecular imprints in the "frozen" polymer layer should also be chiral. For this reason, the two carvone enantiomers – R-(+)-carvone and S-(-)-carvone – are used as chiral probes.

The obtained results of the sorption analysis of (+)-carvone are presented in Figure 8.

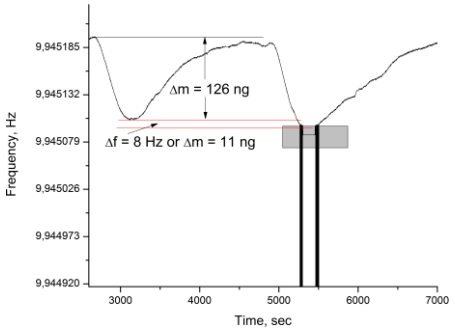


Figure 8. QI-QCM frequency response to (+)-carvone.

From the graph presented, it can be seen that the affinity of the investigated enantiomer to the QI-QCM surface is insignificant. It can be reasonably concluded that the created contacts have a physical nature and would rather be related to capillary condensation of the analyte in available microdefects in the created polymer layer.

Data from another sorption experiment revealed the higher adsorption potential of QI-QCM surface relative to (-)-enantiomer (Figure 9). Comparing the data from both experiments, it is clear that the sorption capacity of the thus created layer exceeds approximately 7 to 10 times that of the (+)-enantiomer.

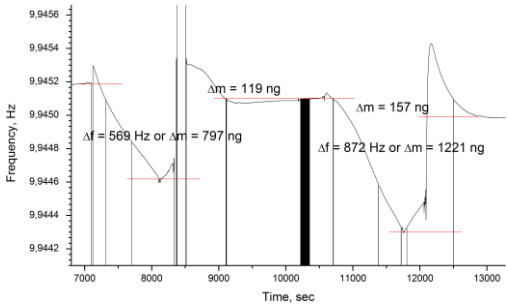


Figure 9. Sorption behavior of QI-QCM towards (-)-carvone.

Furthermore, in this particular case, along with the changes in the mass of the sample, changes in the temperature of the sample are also registered. Usually, these changes are inherent to most thermodynamically consistent processes. The reported exothermic reflexes upon the contact of (-)-carvone with the QI-QCM surface unambiguously confirm that the interaction between them is directional, configurationally consistent, and is accompanied by the release of a minimal amount of heat. However, a much larger release of thermal energy is observed in the process of (-)-carvone desorption from the QI-QCM surface. The latter should be conditioned by the general conformational changes in the deposited polymer layer. The reported changes are significantly larger than those observed in the adsorption regions of this process, which can also be interpreted as a measure of the presence of chain-volume transmitted changes in the quinine-imprinted polymer layer.

Relying on the obtained results, we can confirm that the polymer layer created is able to account for the subtle spatial/configurational differences in the two carvone isomers. We can use the reported data as an additional indication of the successfully realized quinine imprint in the thus deposited polymer layer on the QCM surface. The obtained results encouraged us to explore the response of this layer to other drug molecules, but in a liquid layer.

V. CONCLUSIONS

1. The reactivity of the quinine hydroxyl group towards the mild oxidizing agent - molten elemental sulfur – has been analytically evaluated.

2. The photoinitiated synthesis of herapathite has been realized.

3. The ability of the alkaloid quinotoxine to form a phenothiazine dye in the presence of elemental bromine has been established.

4. The inertness of the alkaloid quinine, towards the oxidizing agent octaammonium dicerium octasulfate, has been determined using an original photosensitive cell.

5. A quinine-imprinted polymer layer has successfully been organized on the surface of a quartz resonator and its sorption behavior towards (+)- and (-)-carvone has been determined by the Quartz Crystal Microbalance method.

VI. CONTRIBUTIONS

1. A new microanalytical algorithm has been introduced in the qualitative analysis of the secondary alcohol quinine group with molten sulfur, allowing the determination of the limit of detection parameter.

2. The optimal composition of a photoreactive analytical mixture for the synthesis of herapathite has been established.

3. A new phenothiazine-based dye from the alkaloid quinotoxine has been obtained.

4. The inertness of quinine towards octaammonium dicerium octasulfate used in the chemiluminescent analysis has been established with the help of an original photosensitive cell.

5. The optimal conditions for the deposition of a sensor-active, quinine-imprinted polymer film on the electrode surface of a quartz resonator have been established.

VII. LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

In publications referenced and indexed in world-renowned databases of scientific information:

IV Vasileva , IN Kolev . A sulfur-based qualitative test for determining the presence of the secondary alcohol functional group of (-)-quinine and (+)-quinidine . *Bulgarian Chemical Communications* , Volume 54, Issue 2 (pp. 147-151) , 2022. doi : 10.34049/bcc.54.2.5466

In non-refereed peer-reviewed journals:

I valina Vasileva , Iliyan Kolev . Phenothiazine dye labeling test for quinotoxine . *Scripta Scientifica Pharmaceutica ,* 2022; 9(2): 36-41. doi : 10.14748/ssm.v54i4.8662

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