PHARMACEUTICAL SCIENCES

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ISOLATION AND ANALYSIS OF CHELIDONIUM MAJUS L CELANDINE ALKALOIDS

ABSTRACT

Complex of experimental studies of the basic chemical and electrochemical processes that take place at the analysis and isolation of the amount of alkaloids bases on the cathode by electrolysis is carried out. The theory of influence of additives of indifferent electrolytes at electrochemical decomposition of which some OH--ions are isolated on the rate of cathode isolation of celandine alkaloids (Chelidonium majus L).

Keywords: *Chelidonium majus L, celandine, alkaloids, isolation, gravimetric, amperometric determination, 12-molybdophosphoric acid.*

Alkaloids are complex organic nitrogen-containing compounds of basic character, which are mainly produced by vegetable organisms. In herbs they are in the form of salts of organic acids such as oxalic, acetic, lactic, apple, citric. Alkaloids have high physiological activity: at low concentrations they show a therapeutic effect, and at large - a toxic effect. Alkaloids gained wide application as active components of cosmetics, food supplements and various bioactive drugs. They exhibit astringent, antiseptic, sedative and anesthetic properties, promote rapid healing of minor skin lesions [1-5].

One of the most promising sources of these funds is herbal raw material of celandine (Chelidonium majus L.) family of Papaveraceae [1-9]. Herbal material of this kind is multifaceted pharmacological activity and is widely used in scientific and traditional medicine. Drugs based on celandine alkaloids cause delayed growth of malignant tumors; they have fungistasis, bacteriostatic action regarding tuberculous mycobacteria. With the internal use of this plant causes a slight slowing of heart rate and lowers blood pressure, has choleretic properties. Also it is used for cardiac angina, hypertension, at various diseases involving muscle spasm; at gastric cancer it is a little pain reliever, it is successfully used with liver and gall bladder [1-3,9].

Celandine alkaloids that isolated from herbal material are a number of dosage forms - extracts, broth, ointments, vials anticancer drug «Ukrain», «Amitozyn» suppositories «Livareks» and others. In medical practice, ointment from celandine powder on lanolin and Vaseline is used, entitled «Plantazan B», for the treatment of early forms of cutaneous tuberculosis, as well as psoriasis, skin cancer, lupus and corns. Celandine infusion (Infusum herbae Chelidonii majoris) is used as a diuretic, choleretic, laxatives, painkillers, and in cosmetics. Juice of celandine is used for cauterization of warts, warts with red lupus, periodontal disease. Some clinicians recommend celandine for angina pectoris, asthma, chronic cholecystitis and rheumatism [1-9].

Thus, the most important components of celandine are alkaloids.

The structure of celandine consists of alkaloids of 4 groups:

I. Group of chelidonine (derivatives of benzophenontridine, α-naphthophenontridin):

1. α- and β - Chelidonine $C_{20}H_{19}O_5N \cdot H_2O$;
2. α- homochelidonine $C_{21}H_{23}O_5N$;
3. Methoxychelidonine $C_{21}H_{21}O_6N$;
4. Oxychelidonine $C_{20}H_{17}O_6N$;
5. Sanguinarine $C_{20}H_{13}O_4N \cdot H_2O$;
6. Oxysanguinarine $C_{20}H_{13}O_5N$;

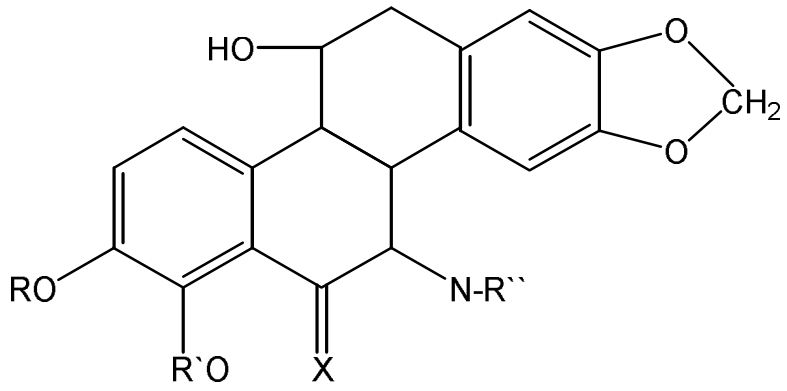
7. Chelerythrine $C_{21}H_{17}O_4N \cdot H_2O$;

8. Chelilutine $(C_{23}H_{24}O_5N)OH$;

9. Chelirubine $(C_{20}H_{18}O_5N)OH$.

Alkaloids of Ist group can be of 2 types:

a) Alkaloids containing in their structure hydrogenated benzophenontridine skeleton:

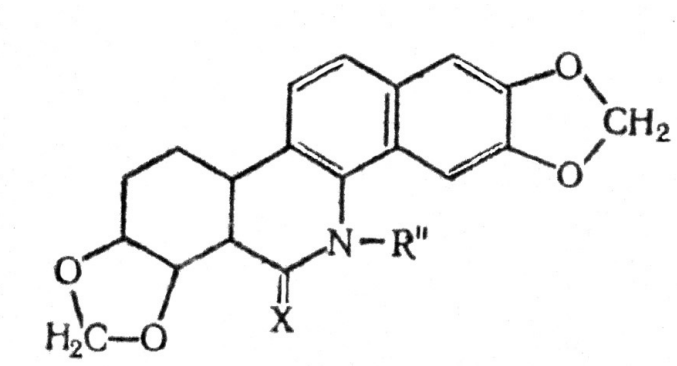


where,

chelidonine: R + R` = CH_2, R`` = CH_3, X = H_2;

oxychelidonine: R + R` = CH_2, R`` = CH_3, X = O;

homochelidonine: R = R` = R`` = CH_3; X = H_2.



where,

oxysanguinarine: X = O, R`` = CH_3.

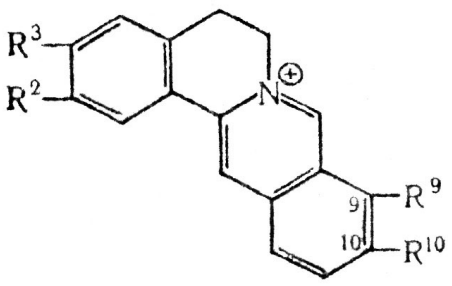
b) Alkaloids containing in their structure dehydrated benzophenontridine skeleton, where sanguinarine: $R+R'=CH_2$, $R''=CH_3$; chelerythrine $R=R'\quad CH_3$, $R''=CH_3$.

II. Group of berberine (protoberberine basis):

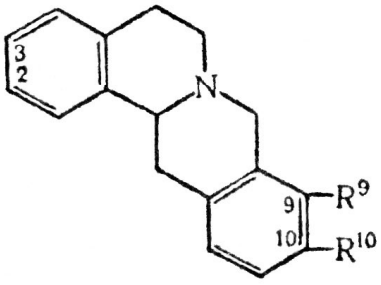
10. Berberine (chelidoxantine) $C_{20}H_{19}O_5N$;

11. d -, l - Stylopin $C_{19}H_{17}O_4N$;

12. Cuptisine $C_{19}H_{15}O_5N$.

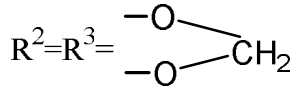


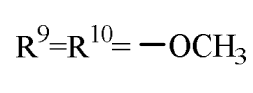
1. Protoberberine



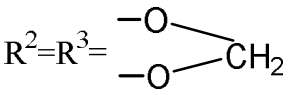
2. Tetrahydroprotoberberine

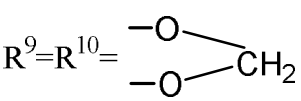
Formula of berberine I





Formula of cuptisine I





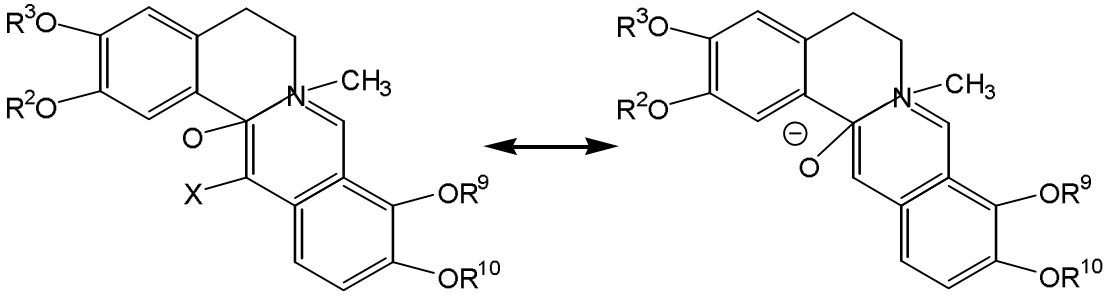
Formula of stylopine II

III. Group of protopine:

13. Protopine $C_{20}H_{19}O_5N$;

14. α - allocryptopine $C_{21}H_{23}O_5N$;

15. β - allocryptopine $C_{21}H_{23}O_5N$.

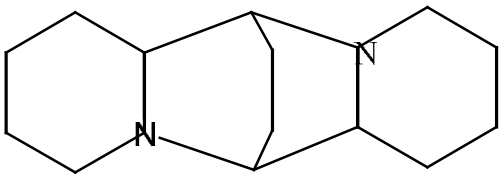


where,

protopine: $R^2 + R^3 = CH_2$, $R^9 + R^{10} = CH^2$, X = H;

α - and β - allocryptopine: $R^2 + R^3 = CH_2$, $R^9 + R^{10} = CH_3$, X = H.

IV. Group of quinolizidine:



16. d- Sparteine $C_{15}H_{26}N_2$;

17. l- Sparteine $C_{15}H_{26}N_2$;

18. dl - Sparteine $C_{15}H_{26}N_2$;

19. Chelidamine $C_{19}H_{19}O_4N$;

20. Chelimidine $C_{21}H_{23}O_6N$;

21. Tetrahydrocorisamine $C_{20}H_{19}O_4N$;

22. Chelidimerine $C_{43}H_{38}N_2O_9$.

1. Isolation of amounts of celandine alkaloids bases (Chelidonium majus L.) by electrolysis

Most existing methods for the extraction and determination of bioactive herbal drugs [7-10] in the way of toxic and valuable organic solvents extraction are time consuming, sensitive not enough and selective, don't guarantee the required reliability of the results. Therefore, the development of new methods of quantitative isolation and determination of bioactive agents, such as celandine alkaloids, is an urgent task. Solving these problems is possible by using the processes of water-acid extraction (boiling off) salts of alkaloids from herbal products with further electrodeposition of alkaloid bases amount on the surface of the metal cathode and quantitative analysis of isolated bases of alkaloids by gravimetric and amperometric methods using heteropolyanions (HPA) of Kehhin's structure as an analytical reagent on nitrogen-content biologically active substances [11-14].

Known method of isolation of celandine alkaloids amounts described in the monograph of A.I. Potopalsky [2] is based on the following methods: 1.0 kg of dry celandine is loaded in percolator. Extraction is carried out by countercurrent. Ekstrahet is a mixture of 70% acetone and 30% distilled water, acidified 0.5% acetic acid. The process of extraction and infusion lasts 8 hours. Then extract is drained off from percolator (volume 6.0 l) in round-bottom flask and on water bath acetone is distilled (~ 4.0 L). Water stillage residue (2.0 liters) is transferred to a conical flask and maintained at a temperature of 2-3°C within 10-12 hours. Sustained aqueous solution of salts of alkaloids (clear, dark-cherry color) is carefully decanted into a clean flask and alkalizing with concentrated ammonia solution to pH 10-11. These alkaloids precipitate as yellow-orange residuals. Bases of alkaloids are extracted with chloroform (3×2l). Chloroform extract (6.0 l), which contains the sum of celandine alkaloids bases, is kept for 5-10 hours and the negligible aqueous phase is isolated, and then extract is distilled to 40-50ml. The concentrate is added to 10.0 mL of distilled water and 10%-aqueous solution of HC1 to pH 3.0. It is evaporated on a water bath until the full isolation of CHC13. The resulting aqueous solution of hydrochlorides of alkaloids is cooled and thus precipitated with mass of 3.26g of amount of alkaloids in the form of hydrochloric salts. Filtered aqueous solution is evaporated to dry salts which recrystallized from water. Overall from 1kg of dry celandine we can get 4.16 g amount of alkaloids or 0.416% of the mass of herbal raw material with purity grade of 95%.

Analysis of scientific literature proves [1-7] the content of alkaloids in herbal raw material of Chelidonium Majus L. varies within 2%. Thus according to Potopalskiy methodics yield of alkaloids is up to 21% of their total content in dry herbal material. The disadvantages of this method of isolation of celandine alkaloids amount are also: the use of large quantities of expensive, highly toxic and volatile organic solvents (acetone, chloroform), a large number of stages of methods, time consuming isolation of product and quality instability.

The aim of this paper is to develop a universal complex technology of isolation the amount of alkaloids from herbal raw material of celandine as acid extracts and further electrocrystallization of amounts of alkaloids basis with purity grade not less than 98% by electrolysis.

Defined goal is achieved through integrated technology, which consists of two stages:

1) Isolation of celandine alkaloids amount from herbal material using aqueous solutions of mineral acids with weight part of the last 1-3%. Acid steaming up in a water bath for 4 hours provides a concentrated broth (extract) of water-soluble salts of celandine alkaloids amount.

2) Isolation (Electrocrystallization the cathode) of amounts of alkaloids bases in pure form from an aqueous solution of acid broth by electrolysis.

As a result of the consistent implementation of the two stages is an isolation of amount of celandine alkaloids bases

from herbal raw materials with full isolation ~ 40-45% with almost no pigments, flavanoids, lipases, which are prerequisite for further purification of alkaloids, isolation. As a result, you can drastically reduce the number of stages in the technological process of obtaining alkaloids, by 7-8 times to reduce the time of alkaloids isolation, completely exclude from the technological process usage of highly toxic and volatile organic solvents, increase the purity and quality of the finished product.

Because of the structural diversity of alkaloids there is no single method of isolating them from natural raw materials. Most methods are based on the use of the fact that the foundations of the alkaloids are usually readily soluble in organic solvents and poorly soluble in water and salt - on the contrary. When allocating alkaloids in the form of salts, herbal material is treated with 1-3% aqueous solution of hydrochloric acid. After this treatment, all alkaloids pass into the acid extract as hydrochloric salts. Water and acid extraction is carried out by heating in a percolators or on water bath.

Paying attention on the good solubility as hydrochloric salts AlkCl of celandine in water (solubility ~ 325-375 g / l) [11-13], we can assume that they will dissociate into ions:

$$AlkCl \rightarrow Alk^{+} + Cl^{-}$$

In this the cathode will provide the following electrochemical reactions:

1. Hydrogenisolation reaction by restoring hydronium ions:

$$H_3O^{+} + e \leftrightarrow \frac{1}{2} H_2 + H_2O$$

2. Restoration of water molecules on the reaction:

$$2H_2O + 2e = H_2 + 2OH^{-},$$

that is accompanied by a sharp rise in the current and change in pH of the solution at the cathode (pH = 14).

Thus, instead of ions Alk^{+} at the cathode water molecules will recover. In this case the two electrons coming from the cathode react with two molecules of water forming hydrogen molecule and two hydroxyl-ions. Reduced hydrogen is released from solution in the form of gas bubbles. Reduction of H^{+} ions is compensated by migration in katolit of Alk^{+} cations and leaving it with an equivalent amount of Cl^{-} ions. Near the cathode Alk^{+} and OH^{-} ions are accumulated. In this case, the OH-ions are the end product of the electrochemical reaction and their concentration in the electrode surface and reach some mol / l. In connection with this, the near-electrode layer chemical reaction runs:

$$Alk^{+} + OH^{-} \rightarrow AlkOH \downarrow$$

Thus, alkaloids are deposited on the cathode due to positive adsorption as finely dense residuals amounts of AlkOH alkaloids bases of «Chelidonium majus L.».

Based on these experimental data a method of getting celandine alkaloids bases by electrolysis has been developed and phenomenon indifferent electrolyte additive effect on the release rate of alkaloids is thoroughly investigated.

Examples of acid extraction fulfilling (steaming up) and electrolysis of salts of alkaloids from herbal material: 50.0 grams of dry and finely dense celandine is poured with 400 ml of 1% aqueous solution of HC1 in heat-resistant chemical flask on 1000ml and steamed up on a water bath within 4-5 hours. Acid broth is filtered through paper filter «red tape», transferred to a flask and diluted to volume of 400 ml. pH of the resulting solution is 3-4.5.

100ml of getting acid broth is transferred into electrolyzer with system of electrodes: cathode with area 2000mm2 made of steel grade; anode with area 2000mm^2, made of aluminum, which is in minicapsule with parchment paper, which provides purity of acid broth from pollution with compounds of aluminum. The distance between the electrodes is 30 mm, the voltage at the electrodes is 2.5 V, the current in the electrolyzer is I=100mA. Electrolysis of acid broth was carried out in four phases of 15 minutes, in general electrolytic isolation of alkaloids occurred during 60 minutes. Crystals of alkaloids basis amount, formed on a steel cathode were washed with d water, ethyl hydroxide and dried in baker at t=50^{0}C.

The influence of various factors on the extent of isolation of celandine alkaloids amount is studied. The degree of alkaloids isolation is mostly affected by temperature increase and introduction of additives, the final product of transformation on the electrode in which is OH^{-} ions and the equilibrium potential of which is more negative than equilibrium potential for restoration of water molecules. Table 1 shows the examples of stages of electrochemical decomposition of indifferent electrolyte additives that can influence the isolation of the amount of alkaloid bases in electrolysis.

Table 1.

Examples of stages of electrochemical decomposition of indifferent electrolyte additives

№ in n.o.	Electrochemical stages	E0, B
1	$SbO_2^{-} + 2H_2O + 3e \rightarrow Sb + 4OH^{-}$	- 0,660
2	$SO_3^{2-} + 3H_2O + 6e \rightarrow S^{2-} + 6OH^{-}$	- 0,610
3	$2SO_3^{2-} + 3H_2O + 4e \rightarrow S_2O_3^{2-} + 6OH^{-}$	- 0,580
4	$N_2O_3^{2-} + 6H_2O + 4e \rightarrow 2NH_2OH + 6OH^{-}$	- 0,730
5	$MoO_4^{2-} + 4H_2O + 6e \rightarrow Mo + 8OH^{-}$	- 1,050
6	$WO_4^{2-} + 4H_2O + 6e \rightarrow W + 8OH^{-}$	- 1,050
7	$SO_4^{2-} + H_2O + 2e \rightarrow SO_3^{2-} + 2OH^{-}$	- 0,900
8	$ClO_3^{-} + H_2O + e \rightarrow ClO_2 + 2OH^{-}$	- 0,450
9	$SeO_3^{2-} + 3H_2O + 4e \rightarrow Se + 6OH^{-}$	- 0,366

Two OH–ions are isolated at decomposition of $KClO_3$ 6OH–ions are isolated at decomposition of Na_2SO_3, and 8 OH–ions are isolated at the decomposition of Na_2MoO_4. This is clearly seen from Fig. 1. The angles of inclination of

curves correspond as 1:3:4 respectively. This fact indicates that the isolation of alkaloids bases on the cathode in the cell is a chemical reaction that occurs at electrochemical decomposition of water and inorganic additives listed.

The obtained results allowed to find optimal conditions for the quantitative amount of isolation of alkaloids bases from the broth: amperage - 100 mA, electrolysis time - 15-20 min after inclusion of current, temperature rise (from 35 to 45 °C) and addition of additives such as chlorate potassium with concentration of 0.001 M, 0.01 M and 0.1 M, selenite lead - 0.001 M, sodium sulfite - 0.001 M, 0.01 M and 0.1 M, sodium tungstate - 0.001 M and 0.01 M, sodium molybdate - 0.001 M and 0.01 M. In this case, more optimal additive is sodium sulfite.

Table 2 shows the results of indifferent electrolyte additives influence on yield of amount celandine alkaloids basis AlkOH $n = \frac{m_\partial}{m_r}$ where m_∂, m_r mass amounts of alkaloids bases isolated by electrolysis from solutions with or without the addition of additives, respectively. The growth of yield of the product depending on the nature and concentration of additives associated with increased volume of the near-electrode layer, where the chemical reaction of precipitate formation occurs:

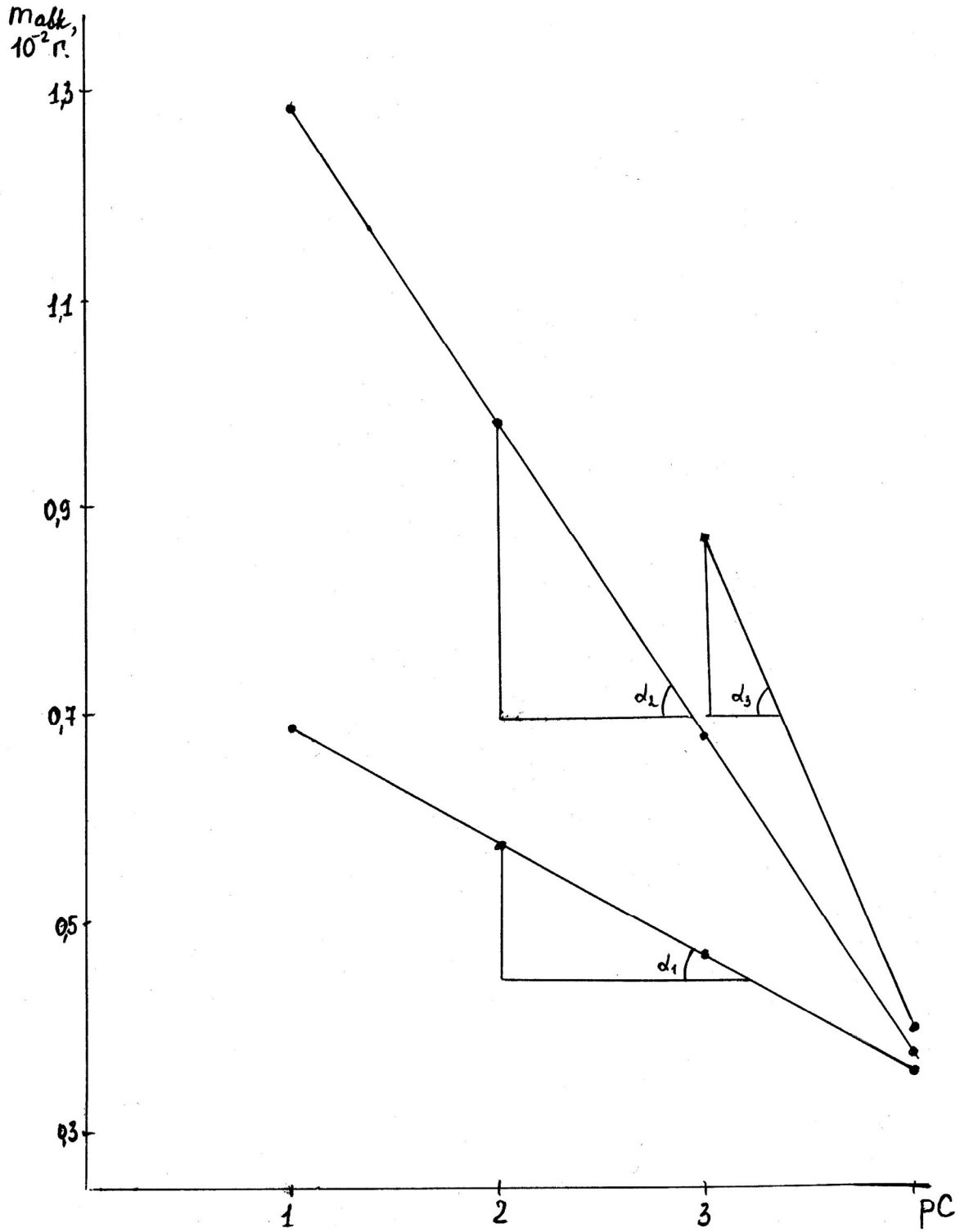


Fig.1. Dependence of isolation of alkaloids bases amount from additives of different concentrations adding: 1 - adding $KClO_3$, 2 - adding Na_2SO_3, 3 - adding Na_2MoO_4. The angles of inclination are related as 1:3:4 respectively.

Table 2.

Indifferent electrolyte additives influence to increase yield of useful product – the amount of celandine alkaloids bases

C, M	$KClO_3$	$PbSeO_3$	Na_2SO_3	Na_2WO_4	Na_2MoO_4
0,1 M	Yield grew by 1,3 times	-	Yield grew by 3,36 times	-	-
0,01 M	Yield grew by 1,24 times	Yield grew by 1,5 times	Yield grew by 3 times	Yield grew by 1,88 times	Yield grew by 1,8 times
0,001 M	Yield grew by 1,18 times	Yield grew by 1,55 times	Yield grew by 2,08 times	Yield grew by 2,22 times	Yield grew by 2,39 times

Table 3.

Conditions for the technological process of celandine alkaloids isolation

Conditions of isolation			Yield %
Time of extraction, hour	Power of current I, mA	Time of electrolyse, min	
3,5	85	50	35-40
4,0	95	55	40-45
4,5	105	60	40-45

Table 3 shows the optimal conditions for the process of celandine alkaloids isolation.

Analytical control of the content of alkaloids in acid broth before and after electrolysis was performed by amperometric titration [13.15]. The results of analytical monitoring of the content of alkaloids in acid broth before and after electrolysis by method of amperometric titration are given in Table 4.

Table 4.

Results of analyses of the content of alkaloids in acid broth before and after electrolysis by method of amperometric titration. Conditions of electrolysis of celandine acid broth: I = 100mA, E = 2.3-2.5V; t = 60 min, pH 3.5. Volume of solution for electrolysis, 100 ml; (n = 5, P = 0.95).

№ in n.o.	Content of alkaloids in 100 ml of broth, gram m0	Mass of isolated alkaloids from 100 ml of broth, gram мел	% $yield = \frac{m_{EA}}{m_o} \cdot 100\%$	Average %	Metrological chracterisitics
1	0,1386	0,0603	43,51	43,49	Sr=0,005 (x±δ)=43,49±0,28%
2	0,1386	0,0602	43,43		
3	0,1386	0,0608	43,87		
4	0,1386	0,0600	43,29		
5	0,1386	0,0601	43,36		

Thus, the developed method of isolation of celandine alkaloids amount by electrolysis reduces the time of carrying out the process in 34 hours (6.8 times) compared to A. Potopalsky method [2], enables to obtain pure substance of the amount of alkaloids bases from herbal raw material without the use of volatile and toxic organic solvents. Also important advantage is increase of alkaloids isolation % (A.I. Potopalsky method ~ 21%; developed method ~ 43%) with purity not less than 98%.

Generalization of experimental data of processes of cathodic deposition of alkaloids bases amount of «celandine» allows us to formulate the basic laws of the process [11-14]:

1) When passing current through aqueous solutions of alkaloids salts alkaloid bases are isolated;

2) Rate of alkaloids isolation depends on the voltage and time of electrolysis;

3) Stirring of electrolyte increases the value of the limit current, i.e. electroanalytical processes occurring under diffusion control;

4) A significant influence of temperature on the yield of celandine alkaloids bases amount is observed;

5) A significant influence of hydrogen ions activity on mass of alkaloids bases isolation on the electrode, due to shear of equilibrium toward the formation of hydrogen molecules;

6) When adding alkaloids of indifferent electrolyte to salts solution, dramatically increases the rate of alkaloids bases isolation;

7) The method of electrolysis can provide pure mixture of alkaloids bases, without using alkali and organic solvents.

Thus, isolation of celandine alkaloids amount from herbal raw material by described in the scientific literature methods [7-10] has some drawbacks: long-term processes, multiple-stagesness, use of large quantities of toxic and expensive organic solvents, so the process of isolation «celandine» alkaloid bases amount from herbal material by methods of acid brothing and electrolysis.

2. Analytical control of clandine alkaloids amount

The total amount of alkaloids content in acid broth and purity of alkaloids, which have been isolated by electrolysis, were carried out using amperometric titration [11, 15].

Amperometric titration of alkaloids organic cations Kat+ by solutions 12-molybdophosphoric acid $H_3PMo_{12}O_{40}$ (MPA) is based on the reaction of deposition:

$$3Kat^+ + PMo_{12}O_{40}^{3-} = (Kat)_3\, PMo_{12}O_{40}$$

Celandine alkaloids contain in their structures five membered heterocycle with nitrogen atom, which has distinct proton

acceptor abilities. While interaction of alkaloids with heteropoly acids, slightly soluble in water sediments with ion-associative nature of communication are formed [15], the stoichiometric ratio heteropolyanion (HPA) MPA – organic cation (OC) of alkaloids amount HPA:OK is equal 1:3.

During voltammetric study of the electrochemical behavior of organic cations Kat+ of alkaloids it was found that with cathode polarization in the range from +0.5 V to -0.5 V they are non-electroactive chemical compound. In the same conditions heteropolyanion of 12- molybdophosphatic acid when 0.1 M sodium sulphate solution used as a background electrolyte gives a clear wave of two atoms of molybdenum electroreduction [15]:

$PMoVI_{12}O_{40}^{3-}+2e \rightarrow PMoV_2MoVI_{10}O_{40}^{5-}$

On the basis that between the substance, which is being determined and the titrant reaction with the formation of slightly soluble compound takes place, and titrant is electroactive substance, amperometric titration of alkaloids organic cations Kat+ from aqueous solution MPA with indication of the equivalence point on the strength of diffusion current of heteropolyanion electroreduction is possible.

During amperometric titration after adding a separate portion of the reagent amperage was noted, at a voltage that corresponds to the sustaining diffusion current. For these data amperometric titration curves in coordinates were built, amperage – the volume of titrant and the equivalence point was graphically found.

Thus, the amperometric titration of aqueous solutions of alkaloids amount by aqueous solution HPC with indication of the equivalence point in strength of the diffusion current by electroreduction HPA is possible. When performing titration system is imposed by voltage which is chosen according to the results of the analysis of current-voltage curves reduction HPA.

Amperometric titration was performed as follows: from broth 10 ml aliquot was taken and placed in an electrochemical cell of 100 ml, 1 ml of background electrolyte (0.01 M sodium sulphate Na_2SO_4) was added. In cell system electrodes system (reference electrode – saturated silver-silver chloride half-cell, working electrode – graphite) then applying voltage +0.01V and in 60-90s with value of «zero» current was fixed. Titrated with 10-2 M solution of 12-molybdophosphatic heteropoly acid (MPA) H3PMo12O40 is made by portions of 0.5ml. Value fixation of sustaining current force – is made within 10s after each addition of titrant. Amperometric titration is completed after a sharp increase in sustaining current. Volume of titrant that was used for titration is determined graphically by curve of amperometric titration.

Alkaloids amount which defined is calculated by formula:

$$m = \frac{3 \cdot V_{ГПК} \cdot C_{ГПК} \cdot M}{1000}$$

where,

3 - number of alkaloid mole, that react with 1 mole of MPA;

$V_{ГПК}$ - volume of titrant титранту, ml;

$C_{ГПК}$ - concentration of MPA M/l;

M – average alkaloids molecular mass, g/M.

To calculate the quantity of content of alkaloids amount in filtrate solution it was assumed that medium molecular weight is 349.75 g/M (molecular weight of different alkaloids is in the range from 234 g/M to 383 g/M).

Amperometric titration showed that the method of acid brothing allows to remove from 1.5 to 2.0% alkaloids in the form of salts, depending on the degree of herbal material reduction.

In the process of amperometric titration after adding a separate portion of the reagent amperage is noted at a voltage corresponding to the value of sustaining current. Accordint to this data amperometric titration curve is built in coordinates, amperage – the volume of titrant and the equivalence point is graphically found (Fig. 2) [15].

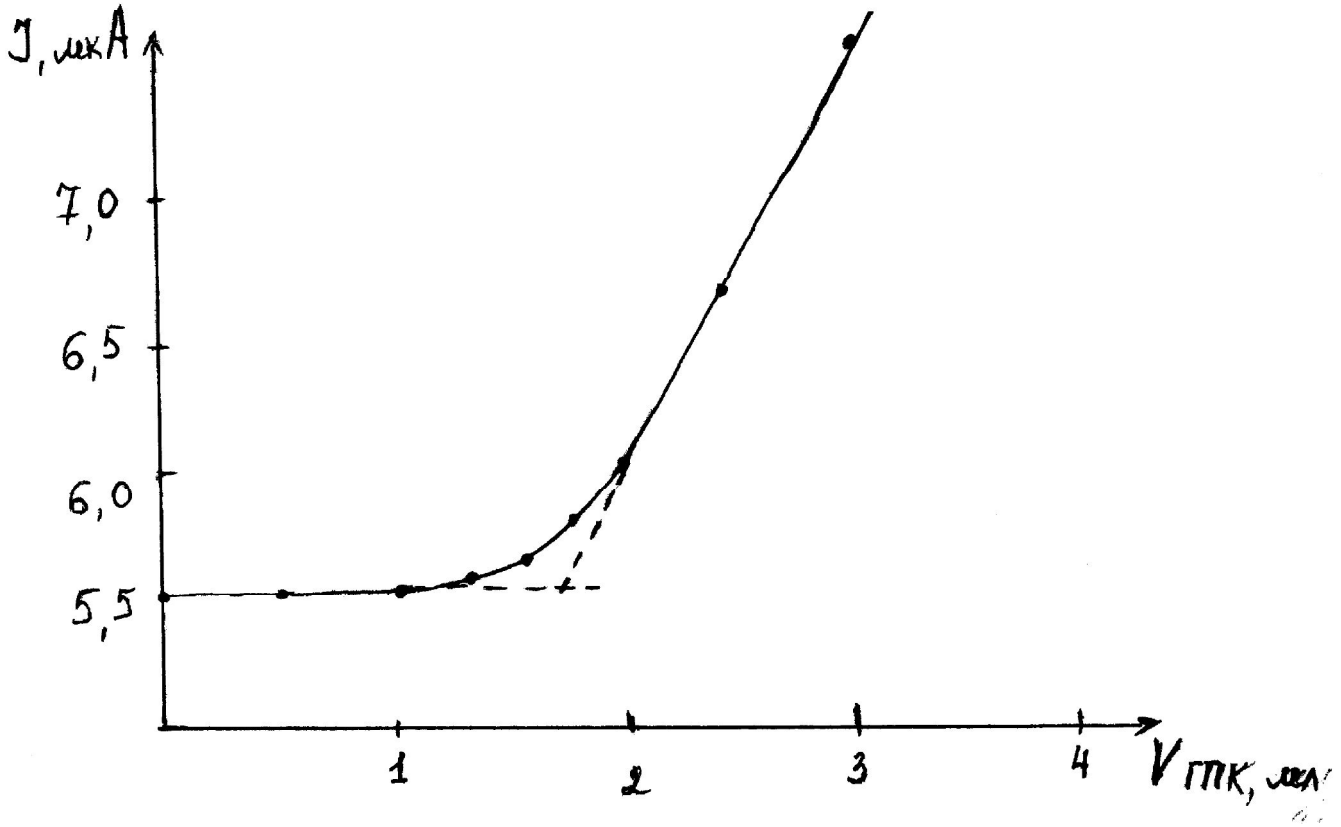


Fig. 2. The curve of amperometric titration of celandine alkaloids amount (in broth), with solution $H_3PMo_{12}O_{40}$. $C_{tit.}=10^{-2}$ M/l. E=+0,1V. pH =3,5.

For the gravimetric determination of celandine alkaloids amount was used the reaction:

$$3Kat^{+} + PMo_{12}O_{40}^{3-} = (Kat)_3\, PMo_{12}O_{40}$$

between organic cations of studied alkaloids and heteropolyanion $PMo_{12}O_4 0^{3-}$[15], which leads to the formation of slightly soluble stable compounds of general formula $(Kat)_3PMo_{12}O_{40}$, which are the optimal deposition and weight forms (reaction (1)).

Stoichiometric relationship of organic cation: heteropolyanion = 3:1. Gravimetry was performed as follows: to 30ml of filtrate obtained by acid brothing on water bath an excess of 10ml 10^{-2} M solution of MPA was added. The formed deposition has been equilibrating for two hours, then was filtrated through pre-weighed filter «blue ribbon.» The deposition was flushed out three times with distilled water, dried in a drying oven at a temperature of 70-80 °C. The mass of isolated alkaloids malk was calculated by the formula:

malk=m•F

where,

m – mass of weight form;

F – gravimetrical factor of recalculation.

F is defined by formula:

$F=\nu_1 M$ (defined alkaloids)/$\nu_2 M$ (gravimetrical form)

where,

ν_1 – number of alkaloid bases moles;

ν_2 - number of moles of MPA.

$(Kat)_3PMo_{12}O_{40}$ - gravimetrical form

Gravity confirmed the amperometric titration data, i.e. by method of acid brothing to 2.0% alkaloids can be isolated, depending on the conditions of the isolation process.

The developed methods of gravimetric and amperometric determination of the celandine alkaloids amount using heteropolyanion (HPA) of Kehhin's structure as analytical reagents were adapted to real objects of analysis:

A) Quantification of the alkaloids amount in aqueous-alcoholic extracts of celandine by gravimetric method.

Maceration of alkaloids in the form of water-alcohol extracts were performed using 96, 70 and 48% ethyl hydroxide in the long process of long-infusion (7 days).

In a 250ml flask 25g of dry reduced celandine and 200ml of 96, 70 or 48% ethyl hydroxide are added and infused for 7 days.

The results of research are given in Table 5.

Table 5.

Quantitative determination of alkaloids amount in celandine water-alcoholic extracts by gravimetric method

Objects of analysis	Content %Alk		
Extragent	96% C_2H_5OH	70% C_2H_5OH	48% C_2H_5OH
Celandine root	0.517	0.885	0.721
Celandine herb	0.333	0.565	0.440

B) Dependence study of % alkaloid content of the celandine vegetation period by amperometric titration.

According to the literature [1-9], the study of plants on the content of bio-active components it is necessary to study their different parts separately. Therefore, we have examined separately celandine roots and its herb. There is data that the % content and quality composition of alkaloid mixture can vary throughout the year [1-9], they depend on the stage of plant development (some alkaloids can be transformed into other during plant growth).

Data on dependence of % alkaloid content on vegetation period were obtained. They are presented in Table 6.

Table 6.

Dependence of % alkaloid content on celandine vegetation period

Vegetation period	Content % Alk Method of amperometric titration					
Acid	H_2SO_4		HAc		HCl	
Concentration	3%	2%	3%	2%	3%	2%
Before blossoming	1,8886	1,3430	1,0072	0,4197	0,3462	0,3357
While blossoming	1,2471	0,9134	0,8120	0,3619	0,2516	0,2278
After blossoming	1,2005	0,8975	0,8005	0,3202	0,2301	0,2114

The results shown in Table 6, allow to make conclusions that 70% ethyl hydroxide is also sufficiently optimal extragent of alkaloids of dried celandine.

The results indicate that in the process of acid brothing isolation of alkaloids decreases by 2-3 times compared to extraction with ethyl hydroxide. At the same time expressivity of acid brothing (isolation time is reduced by 30-35 times) should be noted and acid brothing is relatively cheap method. Acids nature used in the study almost do not influence the process of alkaloids isolation from herbal material. It is preferable to use in acid broths hydrochloric acid with weight 3%, which provides a complete isolation of alkaloids as hydrochloric salts, as well as appears an opportunity to remove hydrochloric acid excess due to its volatility in the process of acid brothing.

C) The results of gravimetric and amperometric determination of celandine alkaloids amount in other real objects of analysis.

The method of quantitative determination of alkaloids amount by amperometric titration in a fairly wide range of concentrations and pH solutions is developed. The correctness of the results was tested by varying of sample weight (Table 7). Results of testing developed method to determine the alkaloids amount on model solutions and real objects – celandine acid broths and pharmaceutical form "Celandine tincture" demonstrate sufficient sensitivity of the method and reproducibility. The minimum detectable concentration of celandine alkaloids amount is 0,035 mg/ml.

Reaction of $PMo_{12}O_{40}^{3-}$ and OK alkaloids with forming slightly soluble in water compounds of stable composition is used for gravimetric determination of celandine alkaloids amount (C_{min} – 1mg/ml) with sufficient analytical and metrological

characteristics. Statistical analysis of the content control of celandine alkaloids amount in herbal material by gravimetric method has showed satisfactory results (Table 8).

Table 7.

The results of determination of celandine alkaloids in model solutions (n=5, P=0,95)

Defined substance	Method	Added, mg	Found ($\bar{x}\pm\delta$) mg	S_r
Celandine alkaloids amount	Amperometric titration	5,00	5,04±0,12	0,02
	Test by additives method	5,00+0,30	5,31±0,10	0,02
		5,00+0,50	5,49±0,11	0,02
	Gravimetry	50,00	50,31±0,72	0,01
	Test by varying of sample weight method	75,00	75,22±0,50	0,01
		100,00	100,53±1,71	0,01
	Amperometric titration	5,00	5,05±0,10	0,02
	Test by varying of sample weight method	6,00	6,01±0,12	0,02
		7,00	7,02±0,20	0,02

Table 8.

The results of determination of celandine alkaloids in real objects – herbal material (100g of dry herbal material in 800ml of 2% solution H_2SO_4) and pharmaceutical forms (n=7, P=0,95)

Object	Method	Found ($\bar{x}\pm\delta$),г	S_r
Acid broth of dry herbal material	Amperometric titration	0,780±0,016	0,02
	Gravimetry	0,784±0,004	0,01
Tablets “Berberine Bisulphate”	Amperometric titration	4,970±0,181	0,03
	Ionometry	4,981±0,107	0,02
Pharmaceutical form “Celandine tincture”	Amperometric titration	0,770±0,020	0,03
	Gravimetry	0,771±0,011	0,01

Conclusions

In this paper, on the example of certain electroanalytical processes occurring at the cathode in obtaining alkaloids by electrolysis in electrodes system (steel cathode and aluminum anode), the research of the occurring conditions of spatio-temporal self-organization of electrochemical system phenomena and its impact on the growth rate of alkaloids isolation on the electrode surface and yield of useful product. Based on the results following conclusions:

1. It is found that by electrolysis the most complete alkaloids isolation from broths is observed when using 1% solution of hydrochloric acid, which is connected with less intense hydrogen isolation, which leads to destruction of the working electrode active surface and in accordance with alkaloids bases deposition;

2. The generalized reaction diagram of chemical and electrochemical processes of formation of celandine alkaloids bases amounts from acid broth on a steel cathode during electrolysis is proposed, according to which the chemical stage of alkaloids bases isolation is carried out after the stage of electrochemical decomposition of water and indifferent electrolyte additives;

3. On the basis of carried out experimental studies, it was concluded that addition of indifferent electrolyte decomposition potential of which is to the left of the expansion potential of water increases the yield of alkaloids bases;

4. The methods of gravimetric and amperometric determination of celandine alkaloids amount using heteropolyanions (HPA) of Kehhin’s structure as analytical reagents, which are highly sensitive (10^{-3}–10^{-4} M/l), simple equipment, expressivity and were adapted to real objects of analysis.

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