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RHENIUM-PICRIC ACID COMPLEX AND SOME CNDO -CALCULATIONS ON TNB, TNT AND PICRIC ACID.

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(Received November 9, 1992)

SUMMARY

Via CNDO-SCF calculations, the charge densities, ionization potentials, electron affinities and dipole moments were calculated for trinitrobenzene, TNB, trinitrotoluene, TNT, and picric acid using the closed shell system eigenvectors. Also, the electronic energies, total energies and binding energies of ground state of these compounds have been calculated.

Rhenium-Picric acid complex was prepared and characterized by HPLC.

Key WORDS: Rhenium, Picric acid, trinitrobenzene, trinitrotoluene, CNDO.

INTRODUCTION

Nitrocompounds play a vital role in organic chemistry. They are used as solvents, dyes, perfumes, analytical reagents and explosives. Picric acid has bactericidal activity and was formerly used in treatment of burns. It is used as a laboratory reagent for characterization of organic bases and polynuclear hydrocarbons. As explosive materials, TNB exhibits more explosive power than TNT as expressed by their shocke sensitivity of the impact[1] .

During the studies of Raman spectra of phenyl azide and its derivatives by El-Shahawy[2], it has been noticed that phenyl azide as an explosive compound was disnitegrated during its exposure to the ionized argon laser. Spectral studies have been done on the substituted phenols dealing with the structural point of view[3]. Few studies were reported for picryl ethers[4]. Spectral studies on some picrylphenyl ethers were discussed by Etaiw[5], to reveal the effect of the dielectric constants and the hydrogen bond formation capacity of the solvents on the displacement of the CT band positions. The dramatic difference in the impact sensitivity of some picric acid-triazole derivatives was studied by Storm[6]. In relation to the thermal decomposition of TNT, the ESR coupling constants and geometries of ten nitrobenzyls were computed using Gaussian 82 program package by Hameka[7]. Molecular orbital calculations of impact indexes and shock induced reactivity were studied by Owens[8] for trinitroaromatic molecules. Semiemperical calculations have been reported[9], in the areas of basic chemistry USA-Air Force research explosives, propellants, electrochemistry and the O_2-I laser system. These MO calculations have given useful insights in each of these areas especially on the progress of understanding TNT (118-96-7) thermochemical decomposition.

Technetium and rhenium are widely used in the nuclear medicine due to the favorable nuclear properties of these two elements which allow images of high resolution to be obtained with a low radiation dose to the patient and the ability of technetium and rhenium to combine chemically with a variety of legands to produce radiopharmaceuticals of high organ specifity[10].

In the present work CNDO calculations have been done to shed some light on the electronic features of the studied molecules, especially picric acid in comparison with TNB and TNT molecules. Also, complexation between Re and picric acid has been carried out in order to find out information on the complex formation and to prepare a new picric acid-rhenium complex which can be of potential use in clinical studies.

EXPERIMENTAL

All common laboratory chemicals were of reagent grade. Rhenium as NH_4ReO_4 of high purity was given as a gift from KFA, Germany.

The uv-vis absorption spectra were recorded on a Shimadzu uv 200 S double beam spectrophotometer using a 1 cm matched silica cell.

Rhenium complexation with picric acid was carried out by mixing an aqueous solution of NH_4ReO_4 with ethanolic solution of the legand, picric acid. A slightly acidic solution of $NaBH_4$ as a reducing agent was added dropwise to the reaction mixture with good stirring. Immediately a red colour appears, then after a few minutes the colour is changed to the permanent brown. High pressure liquid chromatography, HPLC, analyses were performed using L-6000 high pressure liquid chromatography apparatus (Hitachi, Ltd. Tokyo) with a variable wavelength monitor in the range from 190 to 6000 nm. Analysis was performed on Lichomosorb RP-18 (7 mu) coloumn in a mixture of methanol-water 60/40 (v/v) as a mobile phase. The flow rate was 2 ml min^{-1}. All absorption was made at 254 nm.

RESULT AND DISCUSSION

From the obtained self-iterative eigenvectors, by the aid of CNDO program in the text[12], of picric acid, TNB and TNT, their charge densities have been calculated in the singlet electronic ground state configuration, Figs 1-2 . It is clear that the increase of the positive charge on the nitrogen atoms and the negative charge on the oxygen atoms decreases the sensitivity of the compound to the impact. This means that the introduction of a donor group to TNB molecule such as a hydroxyl or methyl group decreases the sensitivity of the parent explosive compound, TNB. Dealing with picric acid, its phenolic hydrogen atom bears a positive charge which is higher than that of the

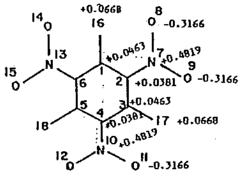

Fig. 1: TNB — molecule

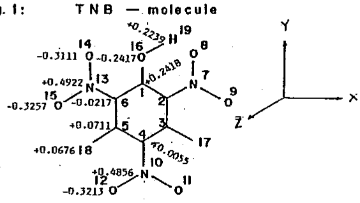

Fig. 2a: Picric acid molecule

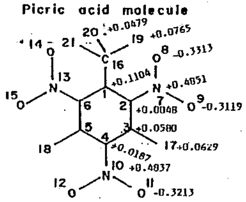

Fig. 2b: TNT — molecule

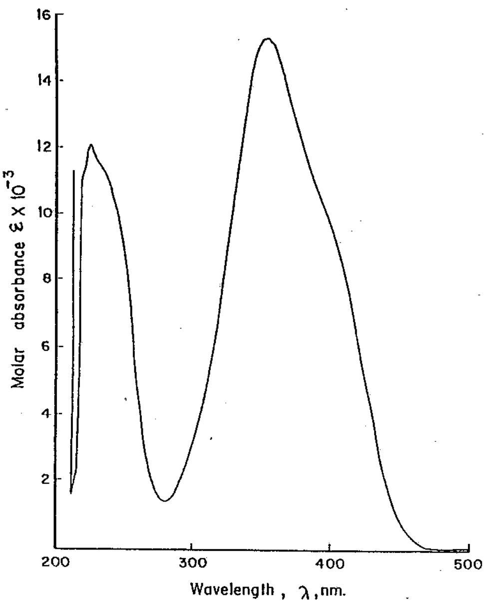

Fig. 3: Electronic absorption spectrum of picric acid in MeOH.

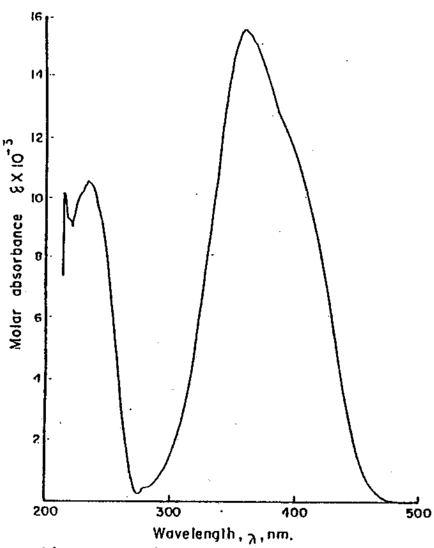

Fig. 4. Electronic absorption spectrum of picric acid in isopropyl alcohol.

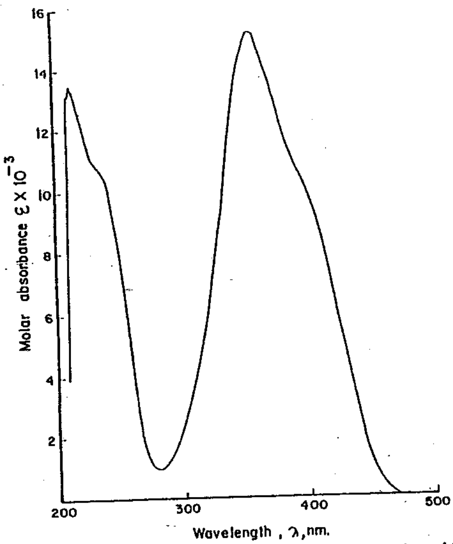

Fig. 5: Electronic absorption spectrum of picric acid in EtOH.

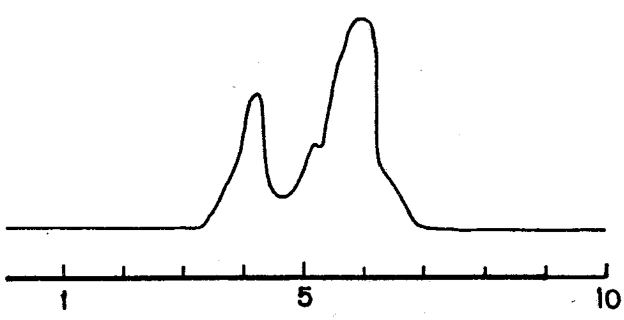

Fig. 6: HPLC chromatogram of Rhenium – picric acid complex.

carboxylic hydrogen atom in N- acetylanthranilic acid[11], + 0.1656. This means that this phenolic hydrogen atom behaves as an acidic carboxylic hydrogen atom in the organic acids. Therefore, this phenolic oxygen atom can be involved in the complexation with rhenium as a central matal ion.

Extension our CNDO calculations of the studied molecules, dipole moments were calculated in the singlet electronic ground state and it was found that it has a value which is equal to zero in TNB molecule. Picric acid molecule has a dipole moment, 2.134 D, which is higher than that of TNT molecule, 0.7664 D. This may give an impression that the increase of the dipole moment decreases the impact sensitivity.

The ionization potentials and the electron affinities of TNB, TNT and picric acid were calculated according to pople[13].

$$E_m = \sum_u \sum_v C_{mn} C_{mv} F_{uv}$$

It has been found that the ionization potenital of TNB, 13.826 eV, is higher than those of picric acid, 13.823 eV, and TNT, 13.436 eV. On the other hand the electron affinity of TNT, 0.101 eV, is the lowest among those of TNB, 0.220 eV and picric acid, 0.297 eV.

Via the CNDO-SCF calculations, the self-iterative eigenvectors of the closed shell system, in the studied molecules, were used to calculate the electronic energy and the total energy according to the following equations respectively[11,12].

$$\varepsilon_{elec.} = 2 \sum_i^n H_{ii} + \sum_i^n J_{ii} + \sum_i^n \sum_{j \neq i}^n (2 J_{ij} - K_{ij})$$

$$\varepsilon_{Total} = \frac{1}{2} \Sigma_{uv} \; P_{uv} \; (H_{uv} + F_{uv}) + \sum_{A<B} Z_A Z_B R_{AB}^{-1}$$

It has been noticed that the electronic energy of picric acid, -780.222 a.u., is the lowest among those of TNT, -768.66o a.u., and TNB, -684.070 a.u. Also picric acid has a lower total energy, -209.019 a.u., than those of TNT, -199.248 a.u., and TNB, -190.542a.u. Of course, the equilibrium molecular geometries of these molecules are defined as the geometries corresponding to the minimum total energy. The theoretical calculation of the equilibrium geometry for a molecule involves systematically minimizing the total energy with respect to all independent internal displacement coordinates. The binding energy of each molecule is then the difference between the total energy in equilibrium geometry and the sum of the atomic energies of the component atoms. The binding energy calculated of TNB, -9.913 a.u., is the lowest with respect to those of TNT, -11.176 a.u., and picric acid, -10.309 a.u.

HPLC chromatogram of the complex solution, Fig 6, shows a number of peaks. The first beak eluted before 5 min. retention time was due to unreduced NH_4ReO_4. The remaining unresolved peaks were due to the complex and may be attributed to the formation of different complex species with different rhenium oxidation states. The solution was set a side for two weeks in air and another HPLC was performed to test the stability of the complex. It has been found that almost no change in the position and the intensity of the peaks which indicates to a quite stability of the complex.

ΠΕΡΙΛΗΨΗ

ΣΥΜΠΛΟΚΑ ΡΗΝΙΟΥ - ΠΙΚΡΙΚΟΥ ΟΞΕΩΣ ΚΑΙ ΜΕΤΡΗΣΕΙΣ CNDO ΕΠΙ ΤΩΝ TNB, TNT ΚΑΙ ΠΙΚΡΙΚΟΥ ΟΞΕΩΣ

Μέσω μετρήσεων CNDO-SCF, υπολογίσθησαν οι πυκνότητες φορτίου, τα δυναμικά ιονισμού και οι ηλεκτρονοσυγγένειες και διπολικές ροπές των τρινοτροβενζολίου TNB, τρινιτροτολουο-

λίου TNT και πικρικού οξέος, χρησιμοποιώντας τα ιδιοανύσματα του συστήματος κλειστής στιβάδας. Επίσης υπολογίσθησαν οι ηλεκτρονικές ενέργειες, οι ολικές ενέργειες και οι δεσμικές ενέργειες της θεμελιώδους καταστάσεως των ενώσεων αυτών. Το σύμπλοκο Ρηνίου - Πικρικού Οξέος παρασκευάσθηκε και χαρακτηρίσθηκε με την μέθοδο HPLC.

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INHIBITION OF PAF-INDUCED PLATELET AGGREGATION, BY VITAMIN C (ASCORBIC ACID), IN VITRO.

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(Received November 28,1992)

SUMMARY

The inhibitory effect of vitamin C, on PAF-induced platelet aggregation was tested in vitro. Tests were performed in rabbit washed platelets and human PRP,pretreated by ASA and CP/CPK,by the use of an aggregometer .
Results were expressed as percentage of inhibition of maximum platelet aggregation induced by 50 pg of PAF.
Dehydroascorbic acid (DHAA) was also tested at various concentrations.
Oxidation curve of vitamin C in presence of platelets activated by PAF was also monitored spectrophotometrically.
Lipoxygenase-linoleic acid reaction was performed in vitro in presence of various concentrations of vit.C and DHAA.
Vitamin C, but not DHAA, inhibited PAF-induced aggregation of human and rabbit platelets and exhibited a disagregating effect when added 2 minutes after the initiation of PAF-induced aggregation. Vitamin C inhibited also,l ipoxygenase-linoleic acid reaction at low concentrations and was oxidized during platelet activation by PAF.
Results indicate that inhibition of PAF-induced platelet aggregation by vitamin C, could probably be attributed to the inactivation of platelet lipoxygenase, due either to the reduction of Fe^{3+} at the active site of the enzyme or to the scavenging ,by the vitamin C, of free radicals neccessary for the enzymic activation,or both.

Key words: Platelet aggregation, PAF, vitamin C, linoleic acid, lipoxygenase, free radicals, antioxidants.

INTRODUCTION

Platelets play a fundamental role in hemostasis and thrombosis and aggregation is the major step of the their contribution to this process.Platelets aggregate in vivo and in vitro by various physiological and synthetic agonists.(1)
Platelet Activating Factor (PAF, 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine), is a naturally occuring phospholipid,that is a potent mediator of inflammatory and allergic reactions and has a variety of pathophysiological actions in vivo and in vitro. Among

these actions,PAF is known to exhibit a very potent pletelet aggregatory activity (2,3,4).

We have recently reported that PAF-induced platelet aggregation is probably mediated by the intercellular generation of free oxygen radicals derived mainly from lipoxygenese activity and that free radical scavengers and antioxidants can inhibit platelet aggregation in vitro.(5)

Vitamin C (ascobirc acid) is a known reducing agent with antioxidant and free radical scavenging properties.(6)

Thus,the ability of vitamin C to inhibit PAF-induced platelet aggregation in vitro and the possible mechanisms of such an action,are investigated in the present study.

MATERIALS AND METHODS

Female New Zeland white rabbits and healthy male human volunteers aging 30-40 years old,were used as sources for platelets.

Substances tested : Ascorbic Acid(La Roche) and dehydroascorbic acid (DHAA-La Roche).

Buffers and reagents : Tyrode's-gelatin-EGTA solution(TG-EGTA) KCL 2,6 .nM, $MgCl_2$ 1nM,NaCl 137 nM,glucose 1g /L,gelatin (Merck) 0,25% and ethylenoglycol-tetraacetic acid 0,2 nM,pH 7,4.

Tyrode's-gelatin-Ca^{2+} - buffer solution (TG-Ca^{2+}),pH 7,4 .EGTA with $CaCl_2$ 12nM and Tris hydroxymethyl-aminomethane(fluka),10 nM.

Creatine phosphate(CP),creatine phosphokinase(CPK),diluted in saline(Sigma) and acetylsalicylic acid as a lysine soluble salt (Egicalm-Galenica).

EDTA(Merck) 0,2 M solution in saline,pH 7,2 and ACD solution(citric acid trisodium citrate,D-glucose,1M each one)were used as anticoagulants.

Synthetic PAF(Bachem) diluted in BSA(Bovine Serum Albumin),2.5 mg/ ml.

Platelet preparations :

a.Washed rabbit platelets (rPRP)

Whole blood collected from rabbits into polyethylene tubes with EDTA(1:10 v/v) was centrifuged at 375 g,for 20 min at room temperature,to obtain PRP(Platelet Rich Plasma).The upper 2/3 were removed and centifuged at 1400 g and platelets were restored to 40 ml in volume with TG-EGTA buffer solution and by successive centrifugations and dilutions according to the method af Ardlie et al as modified by

Benveniste et al (7), washed rabbit pletelets were obtained.The remaining blood was centifuged at 1400g for 15 minutes to obtain PPP(Platelet Poor Plasma). Platelet counts were determined in a Coulter Counter(Coulter Electronics,Ltd) and the washed platelets were suspended in the appropriate volume of TG-EGTA to yield a concentration of $2,5X10^9$ cells/ml.

b. Human Platelet Rich Plasma.(hPRP)

Human whole blood collected into polyethylene tubes with ACD(1:9 v/v)was centrifuged at 164 g,for 10 min. to obtain PRP.
The 2/3 of the upper phase of platelet rich plasma was removed (PRP) and the remaining was centifuged for 30 min. at 3000 g to obtain PPP.Platelet counts of PRP were determined in Coulter Counter and adjusted with homologus PPP to $0.25X10^9$ cells per ml.

Platelet aggregometry

Platelet aggregation was monitoret by a Chronolog single channel aggregometer (model 330),under constant stiring of 1200 rpm at 37 ^{o}C.
PRP was treated with ASA 1nM,15 min. before aggregation test.
Aggregation of washed rabbit platelets was performed as follows :
100 μl of platelet suspension were transfered into the cuvettes of the aggregometer diluted 1:5 with $TG-Ca^{2+}$ buffer solution.In this buffer CP 0,7nM and CPK 13,9 u/ml were added for PAF aggregometry..
Human platelet aggregation was measured in aliquots of 0,5 ml of hPRP.The same as above combination of CP/CPK was added into the cuvettes before testing PAF.
All substances tested,except PAF,were added as $TG-Ca^{2+}$ solutions at pH 7,4 to volumes of 1 to 5 μl.
Results were expressed as percentage(%) of inhibition of the maximum irreversible aggregation obtained by 50 pg of PAF.

Disaggregation tests.

In washed rabbit platelets,aggregated by PAF,the tested substances,were added into the cuvetes of the aggregometer 2 to 3 minutes after the initiation of aggregation ,at final concentrations of $10^{-3}M$ for vit.C and $10^{-2}M$ for DHAA. and the
result was recorded.

Lactic dehydrogenase determination(LDH)

100 ml of washed rabbit platelets were suspended into 15 cuvettes containing 400ml of TG-Ca^{2+} each.In five of them vitamin C at final concentration 10^{-2}M,and in five dehydroascorbic acid(10^{-2} M),was added.Five of them were used as controls.All cuvettes were incubated at 37 ^{o}C for 1 hour, centrifuged to 1500 rpm and LDH of the supernatant was estimated photometricaly ,by Monotest of LDH ott (Boehringer). Results were expressed as U/L.

Oxidation of ascorbic acid(vitamin C) test :

Into 20 cuvettes containing 2 ml of TG-Ca^{2+} the following were added ,in groups of five of cuvettes.:

-Vitamin C at final concentration of 10^{-7} M.

-Vitamin C at the above concentration and washed rabbit platelets($5X10^{6}$ cells).

-Vitamin C at the above concentration and PAF at concentration of 10^{-7} M.

-Vitamin C,washed rabbit platelets and PAF at concentrations described above.

Samples were centrifuged for 30 seconds at 1500 rpm and the supernatant was transferred into the cuvettes of a spectrophometer (Hittachi Mod.100-4006).
Vitamin C spectra were monitored in each sample for 5 minutes at 265 nm and results were expressed as absorbance difference(ΔMA) in relation to time.

Lipoxygenase-linoleic acid reaction

Lipoxygenase-linoleic acid reaction was performed as previously described (9) by a spectrophotometer(Hittachi,Mod 100-4006).
Into the cuvettes of the spectrophotometer lipoxygenase-linoleic acid reaction absorption curve was monitored for 3 minutes.
Vitamin C at concentrations of 10^{-4} M,10^{-5} M and 10^{-6} M was suspended into the cuvettes,(five cuvettes at each concentration),into a final volume of 3 ml of the appropriate mixture of buffer reagents solution,neccessary for the reaction(9).Then lipoxygenase solution was added and absorption curves at 234 nm were monitored for 3 minutes.
Results were expressed as absorbance difference (ΔMA) in relation to time.

RESULTS

Lactic dehydrogenase of platelets incubated with vitamin C and dehydroascorbic acid in comparison to controls are shown in table 1. Mean values of LDH were not significantly different than those of the controls ($p>0.05$).

Number	Control	vitamin C	DHAA
1	18	18	22
2	19	20	21
3	21	19	18
4	20	18	23
5	17	21	22
MV±SD	19±1.6	19.2±1.3	21.2±1.6

Table 1: *LDH values (U/L) of platelets incubated with vitamin C and dehydroascorbic acid(DHAA)*

Percentage of inhibition of PAF-induced platelet aggregation,in rabbit and human PRP, by various concentrations of vitamin C, are shown in figure 1.

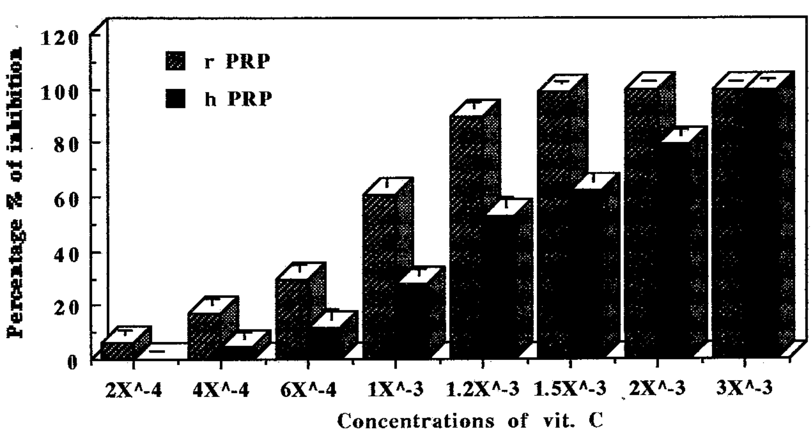

FIG. 1: *Percentance % of inhibition (mean ± SD of five estimations) of the maximum PAF-induced platelet aggregation (rabitt and human), in precence of various concentrations of vitamin C.*

Inhibition of PAF-induced maximum aggregation was complete at concentrations of $1{,}5X10^{-3}M$ and $3\ X10^{-3}M$ vit.C,for rabbit and human PRP respectively.

Dehydroascorbic acid failed to exert any inhibition of aggregation even at concentrations of 10^{-2} M(data not shown).

Vitamin C added in the cuvettes at concentration of 10^{-3} M, two minutes after the initiation of aggregation by PAF, resulted in disagregation of platelets,whereas dehydroascorbic acid failed to exert any disaggregating effect,even at concen-trations of 10^{-2} M (data not shown).

Results concerning the oxidation of vitamin C in presence of platelets and PAF,are demonstrated in figure 2.No absorbance differences were recorded in TG-Ca^{2+} solution in absence of platelets and/or PAF, at 265 nm.Activation of platelets suspension by PAF,resulted in significant differences of absorption,indicative of vitamin C oxidation (8).

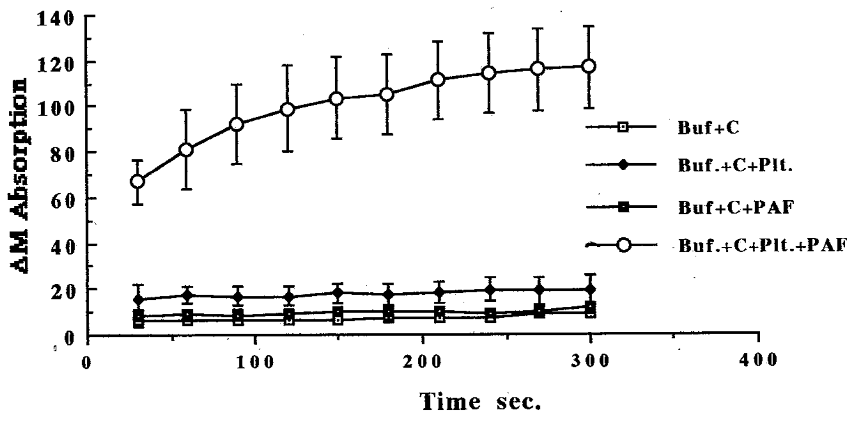

FIG. 2: *Oxidation curve of vitamin C during platelet aggregation by PAF (Mean ±SD values of five estimations)*

Lipoxygenase-lionoleic acid reaction curves were markedly influenced by vitamin C at concentrations of 10^{-4} M and 10^{-5} M.

when administered 2 to 3 minutes after the initiation of platelet aggregation, induced by PAF.

Inhibition could not also be due to any interaction of vit.C with cycloxygenase, since in our experiments this enzyme was inhibited by treating platelets with acetylosalicylic acid before the aggregation test.

There are two possible mechanisms that could explain the inhibitory effect of vitamin C on PAF-induced platelet aggregation.

Vitamin C, being a potent reducing substance could reduce Fe^{3+}, in the active site of lipoxygenase,to Fe^{2+},inhibiting thus the activity of the enzyme .This is one of the ways of inhibition of the reaction by reducing substances (15,16,17). Lipoxygenase-linoleic acid reaction which is an in vitro model,for arachidonic acid-lipoxygenase reaction, which takes place in platelets in vivo,was markedly inhibited by vitamin C at concentrations of 10^{-5} M and10^{-4} M.Since in our experimental model, PAF induces platelet aggregation mainly via lipoxygenase pathway, because platelets were pretreated by ASA and CP/CPK, inhibition of the activity of this enzyme, by the mechanism described above, could explain our results.

In contrast,the oxidized form of vitamin C (dehydroascorbic acid) did not affect the above reaction even at concentrations of 10^{-2} M and failed to inhibit platelet aggregation or to exert any disaggregatory effect. The above iindicates that the inhibitory effects of vitamin C on platelet aggregation is probably exerted by the antioxidant and reducing properties of the substance, since its oxidized form (DHAA),that lacks such proerties does not manifest any of the inhibitory effects of the ascorbic acid.

It has also been reported that platelets produce free oxygen radicals,when activated by arachidonic acid, mainly via lipoxygenase pathway (18,19). We have,in addition, shown that free radicals are probably an important step for PAF-induced platelet aggregation and that platelet aggregation can be inhibited by free radical scavengers.(5, 20).

Our data previously reported indicate also,that free radical species may regulate positivelly and negativelly(down regulation) the activites of both enzymes implicated in the biochemical pathways of platelet aggegation,ie cyclo- and lipoxygenase. pathways(5).Vitamin C is also a potent antioxidant that possess properties of free radical scavenger,whereas dehydroascorbic acid does not (6).

It is therefore possible,that PAF-induced platelet aggregation to be inhibited by vitamin C, because of scavening, by the vitamin ,of free radicals that are neccessary for the lipoxygenase activation(10).Oxidation of vitamin C during platelet activation by PAF, as well as failure of its oxidized form (DHAA) to exert any inhibitory or disagregating effect, suport such a hypothesis. Furthermore vitamin C seems to inhibit

Lower concentrations(10^{-6}M) gave absorbance spectra similar to those of the reaction (control) at any time (fig.3).

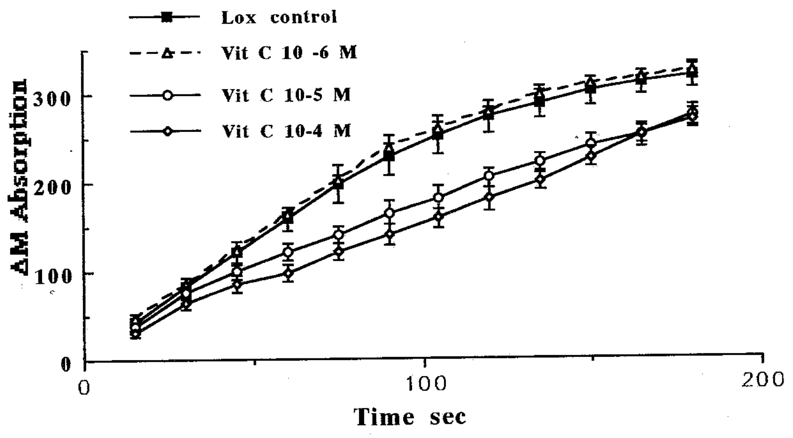

FIG. 3: *Influence of various concentrations of vitamin C on linoleic acid-lipoxygenase reaction (Mean ± SD of five estimations).*

Dehydroascorbic acid did not influence lipoxygenase-linoleic acid reaction even at concentrations of 10^{-2} M.(data not shown).

DISCUSSION

Platelets incubated with vitamin C or dehydroascorbic acid were not affected by the substances,since no difference of the control values of LDH was found (table 1).Platelets as well contain large quantities of vitamin C(1.9±0.8 mM per gr)as a physiological constituent of the cell .

Vitamin C inhibited completely PAF-induced maximum (irreversible) aggregation of rabbit(washed) and human (PRP) platelets, at conentrations from $1,5X10^{-3}$ M to $3X10^{-3}$M respectively.

The inhibition observed in our experiments was not probably due to PAF receptor antagonism by ascorbic acid, since vit.C exerted a remarkable antiaggregatory effect when administered 2 to 3 minutes after the initiation of platelet aggregation, induced by PAF.

Inhibition could not also be due to any interaction of vit.C with cycloxygenase, since in our experiments this enzyme was inhibited by treating platelets with acetylosalicylic acid before the aggregation test.

There are two possible mechanisms that could explain the inhibitory effect of vitamin C on PAF-induced platelet aggregation.

Vitamin C, being a potent reducing substance could reduce Fe^{3+}, in the active site of lipoxygenase,to Fe^{2+},inhibiting thus the activity of the enzyme .This is one of the ways of inhibition of the reaction by reducing substances (15,16,17). Lipoxygenase-linoleic acid reaction which is an in vitro model,for arachidonic acid-lipoxygenase reaction, which takes place in platelets in vivo,was markedly inhibited by vitamin C at concentrations of 10^{-5} M and10^{-4} M.Since in our experimental model, PAF induces platelet aggregation mainly via lipoxygenase pathway, because platelets were pretreated by ASA and CP/CPK, inhibition of the activity of this enzyme, by the mechanism described above, could explain our results.

In contrast,the oxidized form of vitamin C (dehydroascorbic acid) did not affect the above reaction even at concentrations of 10^{-2} M and failed to inhibit platelet aggregation or to exert any disaggregatory effect. The above iindicates that the inhibitory effects of vitamin C on platelet aggregation is probably exerted by the antioxidant and reducing properties of the substance, since its oxidized form (DHAA),that lacks such proerties does not manifest any of the inhibitory effects of the ascorbic acid.

It has also been reported that platelets produce free oxygen radicals,when activated by arachidonic acid, mainly via lipoxygenase pathway (18,19). We have,in addition, shown that free radicals are probably an important step for PAF-induced platelet aggregation and that platelet aggregation can be inhibited by free radical scavengers.(5, 20).

Our data previously reported indicate also,that free radical species may regulate positivelly and negativelly(down regulation) the activites of both enzymes implicated in the biochemical pathways of platelet aggegation,ie cyclo- and lipoxygenase. pathways(5).Vitamin C is also a potent antioxidant that possess properties of free radical scavenger,whereas dehydroascorbic acid does not (6).

It is therefore possible,that PAF-induced platelet aggregation to be inhibited by vitamin C, because of scavening, by the vitamin ,of free radicals that are neccessary for the lipoxygenase activation(10).Oxidation of vitamin C during platelet activation by PAF, as well as failure of its oxidized form (DHAA) to exert any inhibitory or disagregating effect, suport such a hypothesis. Furthermore vitamin C seems to inhibit platelet aggregation induced by arachidonic acid and adenosine diphosphate (ADP) (5).It has also been reported that vitamin C can inhibit platelet aggregation induced by rabbit atheromatic aorta,in vivo,(11) and in humans suffering from coronary heart disease((12),who exhibit increased platetlet sensitivity to the aggregatory effects of PAF (13,14).

In conclusion, although further studies are neccessary in order to elucidate the possible mechanisms of inhibition of PAF-induced platelet aggregation by vitamin C, this action may probably be due to the lipoxygenase inactivation by the ascorbic acid.Such an effect is possibly mediated, either by the reduction of Fe^{3+} at the active site of the enzyme (9), or by the scavening of free radicals neccessary for the activation of lipoxygenase pathway(10), by vitamin C, or both.

Finally,the ability of vitamin C to inhibit PAF-induced platelet aggregation, as well as the aggregation iduced by arachidonic acid and ADP, could probaly explain some of its beneficial effects in coronary heart disease(12) and may suggest its administration preventively and/or therapeuticaly in situations that thrombosis is the main event.

* Whom requests for reprints will be addressed to.

ΠΕΡΙΛΗΨΗ

ΑΝΑΣΤΟΛΗ ΤΗΣ ΣΥΣΣΩΡΕΥΣΗΣ ΤΩΝ ΑΙΜΟΠΕΤΑΛΙΩΝ ΠΟΥ ΠΡΟΚΑΛΕΙΤΑΙ ΑΠΟ ΤΟΝ ΠΑΡΑΓΟΝΤΑ ΕΝΕΡΓΟΠΟΙΗΣΗΣ (PAF),ΑΠΟ ΤΟ ΑΣΚΟΡΒΙΚΟ ΟΞΥ (ΒΙΤΑΜΙΝΗ C).

Είναι γνωστός ο ρόλος της ενεργοποίησης και της συσσώρευσηςτων αιμοπεταλίων στην φυσιολογία και την φυσιοπαθολογία της αιμόστασης. Μεταξύ των φυσιολογικών αγωνιστών της συσσώρευσης in vitro & in vivo, ο Παράγοντας Ενεργοποίησης των Αιμοπεταλίων (PAF), κατέχει σημαντική θέση λόγω των πολλαπλών δράσεών του.
Στην παρούσα μελέτη διερευνήθηκε η αναστολή της συσσώρευσης των αιμοπεταλίων που προκαλείται από τον PAF, με βιταμίνη C.
Οι δοκιμασίες συσσώρευσης και αναστολής της έγιναν σε πλυμένα αιμοπετάλια κουνελιού και πλάσμα ανθρώπου πλούσιο σε αιμοπετάλια (hPRP) αντιστοίχως.
Μετρήθηκαν επίσης φασματομετρικά , η οξείδωση της βιταμίνης C κατά την ενεργοποίηση των αιμοπεταλίων με PAF και η επίδραση της ουσίας και της οξειδωμένης μορφής της , δευδροασκορβικό οξύ, στην in vitro αντίδραση λινελαϊκού-λιποξυγονάσης.
Διαπιστώθηκε ότι η βιταμίνη C αναστέλλει την μεγίστη μη αναστρέψιμη συσσώρευση των αιμοπεταλίων από PAF, σε αιμοπετάλια κουνελιού και ανθρώπου μέχρι 100% σε συγκεντρώσεις 1.5X 10^{-3} M και 3X10^{-3} M αντίστοιχα, ενώ επίσης αποσυσσωρεύει τα αιμοπετάλια όταν προστεθεί μέχρι και 2 λεπτά μετά την έναρξη συσσώρευσής τους από τον PAF. Το δεϋδροασκορβικό οξύ αντιθέτως δεν εμφανίζει καμμία από τις παραπάνω δράσεις, ακόμη και σε συγκεντρώσεις 10^{-2} M.

Ακόμη η βιταμίνη C, οξειδώνεται κατά την ενεργοποίηση των αιμοπεταλίων από τον PAF, και αναστέλλει την αντίδραση λιποξυγονάσης-λινελαϊκού σε συγκεντρώσεις μεγαλύτερες από 10^{-5} M.
Τα ευρήματα συνηγορούν ότι η ανασταλτική δράση της βιταμίνης C στη συσσώρευση των αιμοπεταλίων από PAF,,μπορεί να αποδοθεί στην αναγωγή του Fe^{3+}, στο ενεργό της λιποξυγονάσης, σε Fe^{2+}, ή σε αναστολή της δραστηριότητας του ενζύμου συνεπεία εκκαθάρισης των ελευθέρων ριζών που είναι απαραίτητες για την ενεργοποίησή του, ή και στις δύο δράσεις.
Τα ευρήματά μας ερμηνεύουν μερικώς την ευεργετική δράση της βιταμίνης C στη στεφανιαία νόσο , όπου φαίνεται ότι ο PAF,παίζει σημαντικό ρόλο και συνηγορούν για την πιθανή προληπτική ή θεραπευτική χορήγηση της ουσίας σε καταστάσεις όπου η θρόμβωση κατέχει σημαντική θέση.

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EXTRACTION OF COBALT IONS WITH EMULSION LIQUID MEMBRANES. I. THE LIQUID MEMBRANE OBTAINING

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SUMMARY

The paper describes a lab study for Co^{2+} ion separation and concentration. Some aspects of metallic ion transport through liquid membrane are discussed and the suitable conditions for obtaining and using Kerosen membranes to Co^{2+} permeation are established.

Key words : Liquid membranes, ion transport, metal permeation, Co^{2+} permeation, obtaining of liquid membranes.

INDRODUCTION

The conventional methods for metallic ions recovery are the following: solvent extraction, precipitation, electrodialysis, ionic exchange, electrolysis [1,2,3,4]. These methods are rather expensive and imply several steps.

The solvent extraction only takes place for certain pH values. The separation of the extracted material is achieved in a second step (stripping).The kinetics of the extraction is slower owing to the small transfer surface area. At the same time a cetrtain amount of metal can be blocked in the solvent as R_nM_m (R - organic remainder, M-metal). The procedure may become inefficient because of the high content of some other organic compounds which are also extracted.

Generally, precipitation leads to the metal hydroxide formation which has but few applications [5,6], a fact which imposes its processing subsequent to its filtration, aiming at obtaining some more useful compounds.

Electrodialysis and the ionic exchange are low productivity techniques in metal recovery.

Electrolysis, a useful process for pure metal recovery, gives poor results when diluted solutions are used. They should be concentrated and this is a very expensive operation.

A procedure permitting the selective and advanced separation and at the same time the concentration of metals, is the liquid membrane separation. This up-to-date technique also offers the possibility of directly obtaining them as some preestablished chemical compounds (Fig. 1): nitrites, chlorides, sulphates (by choosing the inner phase of the W/O/W emulsion).

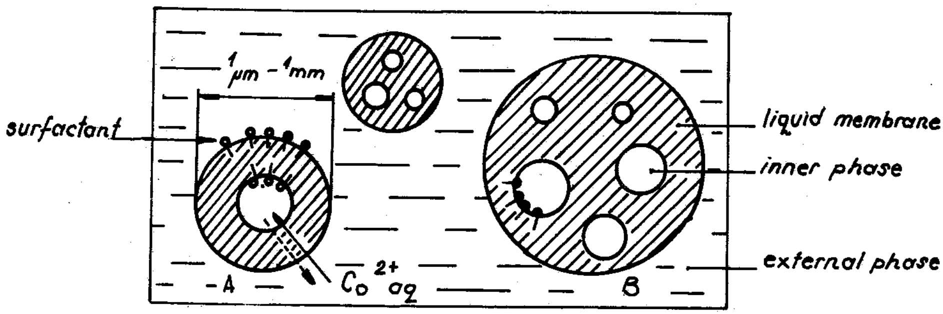

FIG.1 : *Metal ions recovery*

The metal extraction technique is based on their diffusion across the membrane, an irreversible chemical reaction associated to it, so that the flow only takes place in a selective one way [7,8]. The large transfer surface area of the emulsion liquid membranes (larger than 10^6 m^2/m^3) ensures a rapid transfer of metallic ions [1,2,5,6,9,10,11,12].

There are four steps in obtaining the liguid membrane (fig 2)

1. preparation of water-oil emulsion
2. treating the metal containing waste water and the ion permeation
3. phase separation
4. breaking-up the exhausted emulsion and metal recovery

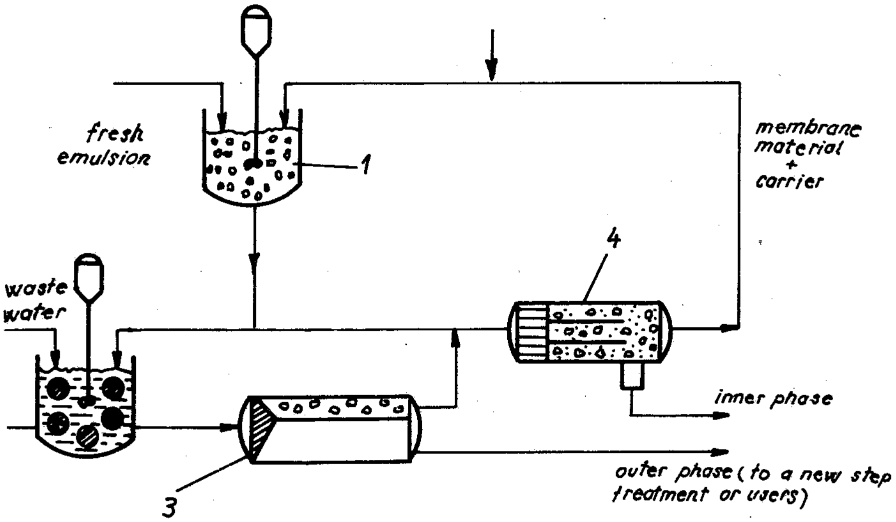

FIG. 2 : *Liquid membrane obtaining. 1: primary emulsion obtaining. 2: permeation. 3: phase separation. 4: breaking-up of the exhausted emulsion*

Metal separation by liquid membranes is done by a "carrier" facilitating the transport [13-16]. The transfer of heavy metals:Hg,Cr, Cd,V, Cu [2,13,17,18-20], Zn [1,20], was mainly studied.The technique of liquid membranes was widely applied in the field of metallurgic industry, alkaline and alkaline earth metal industry [21,22] as well as depollution of radioactive waste water

[23,24].

The carriers reversibly react with the permeating species forming an intermediate which diffuses across the membrane. At the interface, the metal is released in side the internal phase and the free carrier returns to the external interface thus restarting the cycle. The carrier and the stripping agent selection is limitative for this method. A carrier must meet the following requirements: selective, cheapness, pollution-free, easily regenerative, membrane soluble only.

The compound made up by the carrier with the cation must be stable enough to withstand the hydrating tendency of the cation at the outer membrane interface, but not stable enough to release the ion at the inner membrane interface. The releasing rate decreases with the increase of the compound stability. It controls the transport rate in the field of values of the stablity constant [22]. There are several methods of metallic ions fixing by the carrier.

The carriers having ionizable protons can transport only the metallic ions across the membrane. While releasing them into the receiving phase, they take over the protons which are transported backwards, releasing them in the source phase. The phenomenon is called "counter-transport".

Concidering the chemical structure, the carriers may be classifief into:

a) acyclic compouds: anionic or cationic tensioactif agents
b) cyclic compounds: oligomers, crownethers, cryptates, peptides and proteins

The first of them reacts with the metal, which is consequently transorted as a salt towards the internal phase, where it is released and where the protons are taken from, and transported backwards ("the proton pump"). Tertiary amines, quaternary amonium salts, etc., react according to this scheme.

The cyclic carriers coordinate the cation due to some giving polar groups. Characteristic to the macrocyclic ligands is the fact that in a certain solvent, they form complexes with the cations showing differentiated stabilities, a fact that allows a high selectivity. The complexes with macrocyclic ligands diffuse across the membrane together with the associated anions; the strong influence of the nature of the anion on cation transport might be explained considering the differences of their free energy: the anions having the lowest free energies release more easily the metallic ion [12,25-28].

The liquid membrane permeation is influenced by the nature of the solvent used: an aliphatic solvent usually gives better results than an aromatic one (e.g.n-heptan vs. toluen) because it acts differently over the liquid membrane (swelling,breaking-up,etc) [12,14,21,25,26].

THE EXTRACTION MECHANISM

As it was mentioned above the transport of the metallic ion compounds may be, depending on the nature of the carrier, a co-transport or a counter-transport.

Co-transport

In this case the carrier binds the metallic ion as a compound accompanied by the anion of the initial salt, during diffusion. The cation transport across the membrane only takes place accompanied by a parallel movement of the negative charges. In the case of a monovalent cation forming a compound at a ratio 1:1 with a neuter L ligand (carrier, membrane soluble only), the equililbrium between the aqueous phase containing a M^+ cation and a A^- anion, and the organic phace containing L, may be written as follows:

$$M^+_a + L_o + A^-_a \rightleftharpoons (MLA)_o \quad (1)$$

$$K_e = \frac{[(MLA)_o]}{[M^+_a]\,[L_o]\,[A^-_a]} \quad (2)$$

were the indexes "a" and "o" indicate the aqueous phase and the organic phase respectively. Under these conditions the equilibrium constant (Ke) is given by Eq. (2).

The low ligand hidrophobicity and the low resulting compound stability lead to the increased metallic ion extractability [22]

The monovalent metallic ion co-transport mechanism is shown in Fig. 3 [21] according to these steps:

1. M^+ cation and the carrier form a compound at the I interface
2. The compound and the A^- anion diffuse across the membrane
3. M^+ and A^- are released in the receiving phase (interface II)
4. The free carrier diffuses backwards through the membrane to restart the cycle.

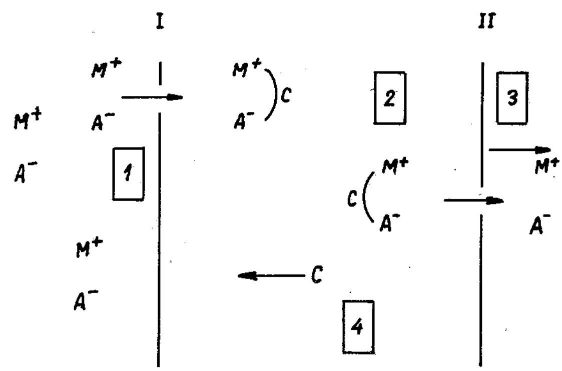

FIG. 3. *Co - transport mechanism. M^+ - metallic ion. A- - anion. C - carrier. MCA - complex*

Counter-transport

It is characteristic for this mechanism the presence of an aiding component (e.g.H^+) at the inner interface, that is taken at the moment when the metallic cation is released. This component crosses the membrane in the opposite sense vs. permeate.The transport takes place as long as there is a concentration difference of aiding component, between the inner and outer phases. The general mechanism scheme is shown in Fig.4 , e.g.Zn^{2+} [29].

Liquid membranes enable the concentration of valuable metals in the form of some desired combination in a small amount of liquid. Thus, one can achieve waste water treatment together with the metal recovery.

For instance in the liquid-phase oxidation process for obtaining: phenol, cyclohexanol, acetic acid, terephtalic acid, phtalic anhydride, etc., in the presence of Co and Mn salts as catalysts, waste water containing Co^{2+} and Mn^{2+} results. In these cases, the application of the liquid membrane process for the recovery of Co^{2+} leads to some promising results.

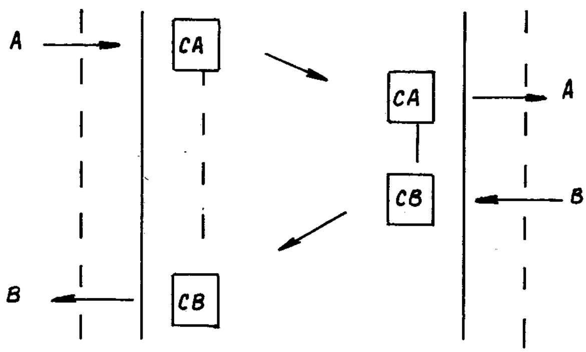

FIG. 4. *Counter - transport mechanism. A- permeate component. C- carrier. B -aiding component. CA- carrier-permeate complex or salt. CB- carrier-aiding component compound.*

EXPERIMENTAL PART

Reagents Used

The membrane tested materials were Kerosene (C_{11}-C_{13}) and some fractions of alkanes (C_{11}-C_{14} ; C_{15}-C_{20}) and isoalkanes (C_{11}-C_{14}; C_{15}-C_{20}). The C_{11}-C_{14} and C_{15}-C_{20} fractions of alkanes and isoalkanes have been obtained by the gas oil processing according to the following steps:

a. refinement with concentrated H_2SO_4 for aromatic hydrocarbon separation
b. adduct-formation with urea for alkane isolation
c. distillation of the isoalkane fractions (containing cycloalkanes as well) (Tab. I.)
d. adduct decomposition and fractional distillation of alkanes (tab.I)

Solutions of various concentrations of nitric acid, hydrochloric acid, sulphuric acid, EDTA (Reactivul-Bucuresti) and PEG (polyethylenglycol with the average mole wt. 20.000-Fluka) have been used as internal phases.

The experimentally tested carriers were analytically pure reagents such as: phosphoric acid esters (D2EHPA and PC88A-Merck, pyridine (Fluka), stearic acid (Stela-Bucuresti), silicone oil (Merck), acetylacetone (Merck), naphtenic acids from crude oil (theirs characteristics are presented in TableII).

TAB. I. *Characteristics of membrane*

Membrane	Composition Fraction	%	Density Kg/m^3	Visc. . 10^{-3} N.s/m^2	Inflam. point ^oC	Temp. interva ^oC
Kerosene	C_{10}	40	804	1.83	48	–
	C_{11}-C_{12}	50				
	C_{13}	5				
alkanes	C_{11} - C_{14}		845	4.87	59	196-253
	C_{15} -C_{20}		845	4.80	101	270,5-344
isoalkanes	C_{11}-C_{14}		845	5.70	50	170-230
	C_{15}-C_{20}		845	5.69	95.5	250-320

TAB II. *Characteristics of naphtenic acids*

Characteristics	Value
Composition	alkanic acids alkyl cyclopentanic acids alkyl cyclohexanic acids
density, Kg/m^3	826
viscosity, N.s/m^2	725.79 . 10-3
acidity value, mg KOH/g	243.06
saponification value, mg KOH/g	247.13

$CoCl_2$ (Reactivul-Bucuresti) solutions having a 625 mg/l concentration and 300 mg/l (for source phase) respectively have been used in order to study the Co^{2+} transport across the membranes.PH adjustment of source phase has been carried out by using a 0.1N NaOH solution (Reactivul-Bucuresti).

Analyses

Metallic ion permeation was falllowed by determination of the residual Co^{2+} concentration after a liquid membrane treatment. Analyses have been performed with a Carl Zeiss Jena flameabsorption spectrophotometer AAS-1.

Primary Emulsion Preparation

The primary emulsions were prepared in a discontinuous system using some thermostatical cells and a seven branched pellet stirrer RW47 with a continuous adjustable rotation speed ranging from 100 to 1300 r.p.m. The surfactant (SPAN 80) and the carrier are mixed with membrane material and thus, the internal phase is added. The following working conditions are required :

composition : membrane material, vol.	25
carrier, vol.	25
internal phase, vol.	50
surfactant, SPAN 80, % wt.	1- 8
internal phase : organic phase ratio	1:1
stirrer rotation speed, r.p.m.	1200
stirring time, min .	15
temperature, ^{o}C	20 ± 2

Multiple Emulsion Preparation

When a primary emulsion is dispersed in the Co^{2+} containing solution, by stirring, a multiple W/O/W emulsion is obtained. The dispersing process has been carried out according to the following condition :

stirrer rotation sped, r.p.m.	200-300
stirring time, min.	3- 5
primary emulsion/solution ratio	1: 3
temperature, ^{o}C	20 ± 2

Droplets having an average diameter ranging from 0.1 to 3 mm were obtained. They have encapsulated many small inner phase droplets with a 7-50 μm diameter.

RESULTS AND DISCUSSION

Membrane

For the study of Co^{2+} transport across the membrane some notations such as :

pH_i = inner phase initial pH value
pH_e = outer phase initial pH value
η = transport yield, %

$$\eta = \frac{c_i - c_f}{c_i} \cdot 100$$

were used, where :

c_i - initial concentration of the Co^{2+} solution mg/l

c_f - final concentration of the Co^{2+} solution after treatment, mg/l

In order to test the liquid membranes thus obtained, emulsions having the organic/aqueous phase ratio 1:1 have been prepared using the materials and techniques (mentioned above). The kerosene membranes showed the best results (Table III)

TAB. III. *Membrane selection*

Membrane	Initial. conc. Co^{2+} mg/l	Final conc. Co^{2+} mg/l	pHi	pHe	η %
Kerosene		300	3.5	8	52
n-alkanes	625	500	3.5	8	16.6
isoalkanes		525	3.5	8	12.5

Internal phase

When selecting the best suited internal phase, one takes into account the transport mechanism, the stability of the compound obtained by blocking the Co^{2+} ions released by the carrier and membrane stability (depending on the ionic strength of the internal and external phase, respectively). Co^{2+} ions can be blocked either as salt compounds or complex compounds, the internal phase acting as proton donor in the former case and non-donor in the latter one.

The experiments show that, by choosing different internal phases: proton donor or proton non-donor, the Co^{2+} transport occurs by the "counter - transport" mechanism only, for many tested carriers. This can be easily observed in Table IV. Even the inner phase forms a stable complex with Co^{2+} (e.g. the Co^{2+} +EDTA complex, having a stability constant of the 10^6 order), the yield of co-transport process is always poor.

TAB. IV. *Influence of inner phase nature on the extraction yield*

Inner phase	Initial conc Co^{2+}, mg/l	Final conc. Co^{2+}, mg/l	pH_i	pH_e	n %	Obs.
sulphuric acid		575			8	
nitric acid		495			20.8	carrier
	625		3.5	8		used :
chlorhidric acid		130			79.8	naphte-
						nic
PEG 5% solution		440			29.6	acids
EDTA 0.05 M solution		420			32.8	

Carrier

The carrier reacts with the Co^{2+} ions (Fig.5) following the equilibrium reactions.

$$Co^{2+} + 2HX \underset{K_2}{\overset{K_1}{\rightleftharpoons}} 2H^+ + CoX_2 \qquad (3)$$

where the ratio $K_1/K_2 = K_e$ is the equilibrium constant. The formed intermediate CoX_2 diffuses across the membrane according to the first Fick law :

$$J = - D \frac{d_c}{d_x}$$

where : J - the permeate flux, mole . $m^{-2} . s^{-1}$

D - diffusion coefficient

$\frac{d_c}{d_x}$ - gradient of concentration

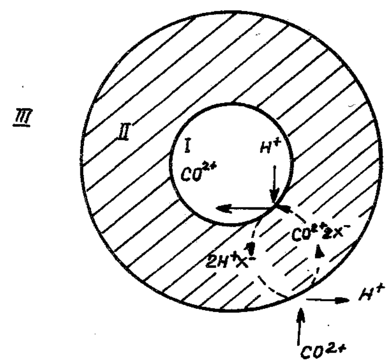

FIG. 5. *Co^{2+} permeation . I- inner phase. II- membrane phase. III- outer phase.*

Many carriers were tested and for the internal phase which was a HCl solution, the naphtenic acids gave the best yields (TableII, Table V)

Co^{2+} ion transport

The driving force of the "counter - transport" is the difference between the proton concentration of the internal (I) and the external (III) phase.

While subjecting the $CoCl_2$ solution ($pH_e = 6$, $c_1 = 625$ mg Co^{2+} / l) to the liquid membrane treatment, no change in the Co^{2+} concentration is noticed. In this particular case, $CoCl_2$ is totally dissociated and the metallic ion is hydrated with six water moles [30]. The addition of alkaline solutions determines a change in the pHe and lead to the destruction of the aqueous Co^{2+} form (by partial replacement of water molecules with OH^-). The Co^{2+} ions can be taken over the more efficiently, the greater the pH_e - pH_i difference is ; the transport yield also increases (Fig.6).

Experiments were carried out for pH_i values ranging from 1 to 5.5 and for pH_e values ranging 7 to 10. Though a maximum yield is expected for the maximum pH_e - pH_i difference, practical data show that for pHi ranging from 1 to 3, the membrane does not withstand the treatment conditions (the membrane is swelling or breaking).

The maximum transport yield is obtained for $pH_i = 3.5$ and $pH_e=10$ (Fig. 6). For more diluted solutions (c_2 = 300), high yields were obtained for pH_i ranging from 3 to 5.5 and $pH_e=10$.

The ion transport on the source phase is achieved using a very efficient stirring system.

The $CoCl_2$ is completely dissociated. The $pH_e = 6$ and the pH_i = 3- 3.5, but no transport occurs, even it the proton pump conditions are established and a well -strirring system is used.

Some authors have shown that hexaqueous Co^{2+} complex is kinetically inert. Its extraction from aqueous to organic phase is limited (or stopped in this case) by a slow releasing of water molecules [31, 32].

The adding of ligands to the system (propionate, acetate, salicylate, formate, succinate) enhances the rate of extraction process by replacing the water molecules with the ligand ones [33]. A thermodynamically less stable and kinetically more labile complex is obtained (Fig. 7). It can react more quickly with the carrier and tends to populate the aqueous - organic interface more than the hydrated ion does.

TAB.V. *Carrier selection* $(pH_i + 3.5, pH_e = 10)$

Carrier	Intermediate form	Initial conc. Co2+ mg/l	Final conc. Co2+ mg/l	η %	Notes
naphtenic acids	salt		25	96	cheap
D2EHRA	salt		385	38.4	costly, toxic
PC 88A	salt	625	480	23.2	costly, toxic
pyridine	complex		315	49.6	costly, toxic
acetyl acetone	complex		500	20	costly
silicone oil	complex		550	12	costly
stearic acid	salt		550	12	

D2EHRA - Di (2-ethylhexyl) phosphoric acid
PC88A - 2 - Ethylhexylphosphoric acid mono-2-ethylhexyl ester

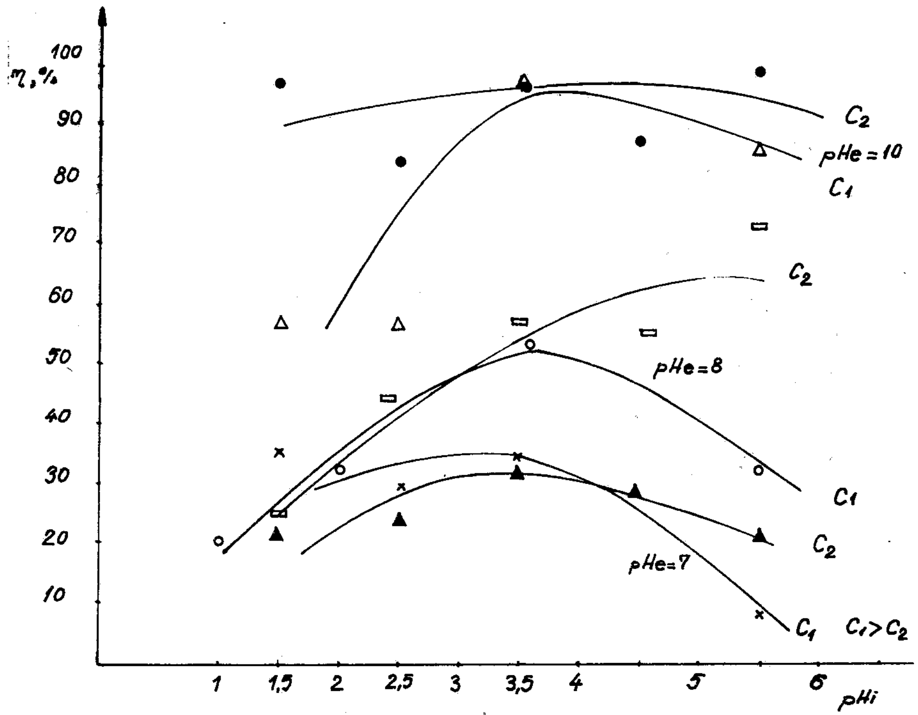

FiG 6. *Transport yield dependence vs.* pH_i *and* pH_e

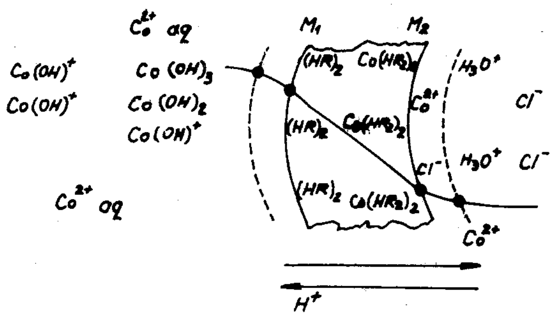

FIG. 7. *Profile concentration of Co^{2+} ions*

In this work the hexaqueous Co^{2+} complexes are modified by adding the NaOH solution. The water molecule replacement and the obtaining of suitable condition for proton pump function are achieved. The $Co(OH)^{+}$, $Co(OH)_2{}^0$, $Co(OH)_3{}^-$ (characterized by the stability constants $pK_1 = 4.4$, $pK_{1,2} = 4.6$, $pK_{1,2,3} = 10.5$ [34] respectively) Co^{2+} aq and $Co(OH)_2$ (s) are main species formed in the source phase (at pH=10). The soluble complexes facilitate the metallic ion up-taking in to 96% yields.

For the Co^{2+} transport study the multiple emulsion is photographed in a transparent graduate (cm) vessel). In the photographs, a few thousand particles were counted and their diameters were estimated with help of the graduate scale (Fig. 8).

We can suppose :

a. the photographed surface is small enough to be considerated flat (the curved wall of the vessel must not affect the particle diameters):

b. the particle distribution is uniform in the entire volume of the source phase :

c. the particles are spherical :

d. the dimensions of a large enough number of particles are estimated so that the average calculated diameter is the nearest to the real one :

e. the particle dimensions do not obviously modify by breaking-up, swelling and coalescence processes, an external average transfer area (A) may be calculated.

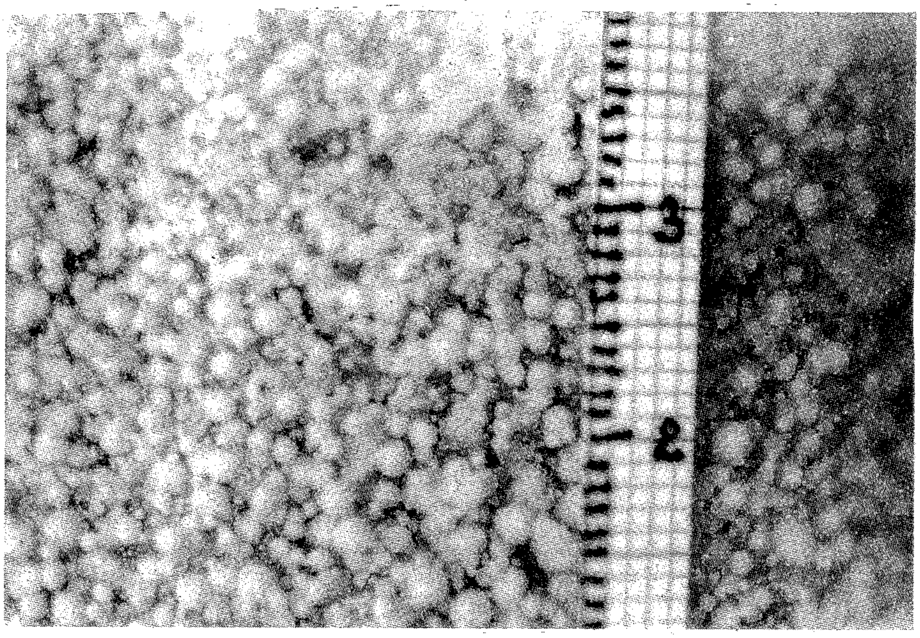

FIG. 8. *Multiple emulsion W/O/W*

If the following are be known:

a. the dispersed volume of primary emulsion (V_{em} = 40 cm^3)

b. the droplet volume : $V_d = 4\pi r^3 / 3$ (r=average radium)

c. the droplet area : $A_d = 4\pi r^2$

The particle number (N_d) and the external average transport area (A) could be determined :

$$N_d = V_{em} / V_d$$

$$A = N_d \cdot A_d$$

The average diameter (D_A) determined by counting and measuring of four thousands particles is :

$$D_A = 0.0776 \text{ cm}$$

One can calculate :

$$N_d = \frac{40.3}{4\pi.\ 0.0388^3}$$

$N_d = 163\ 484$

$A = 3092\ cm^2$

The decrease of Co^{2+} concentration in he source phase is observed by taking and analyzing samples, at equal intervals of time.

The application of Fick's first law leads to the following equation :

$$\ln \frac{C_i}{C_t} = \frac{D_i\ .\ A}{\Delta_x}\ .\ t$$

where : C_i = initial concentration, $mgCo^{2+}/l$

C_t = momentary concentration, at t time , $mgCo^{2+}/l$

D_i = diffusion coefficient

The function : $\ln \frac{C_i}{C_t} = f(t)$ (Fig. 9, Tab.VI) is plotting and its slope is calculated to determine the apparent diffusion coefficient D1.

$$D_1 = D_i / x$$

$$tga = D_1\ .\ A$$

$$tga = 0.119$$

$$D_1 = 3.85.10^{-5}\ cm$$

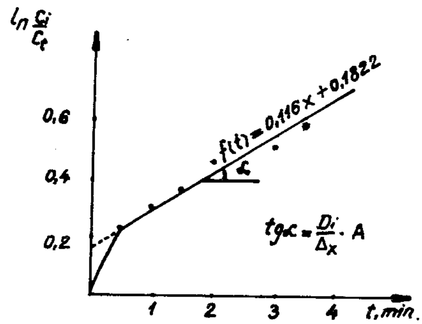

FIG. 9. *Apparent diffusion coefficient (D_1) determination*

There is a certain difference between the theoretical line and the experimental one. The presence of an intercept (f (0) = 0.1822) may be due to experimental errors, but the repeated testes lead to the same results.

TAB. VI. *Co^{2+} momentary concentration in source phase*

No.	pH_i	pH_e	Init conc. mg/l	Time min	Mom. conc.	$\ln(\frac{C_i}{C_t})$
157a	3.5	10	625	0.5	500	0.223
157b				1	462.5	0.301
157c				1.5	437.5	0.357
157d				2	400	0.446
157f				3	375	0.520
157g				3.5	350	0.580

This intercept suggests that initially (t=0 - 0.5 min.), the process is limited by the up-taking of metallic ions performed by the carrier.

When the carrier is "charged" the diffusion occurs and governs the transport process through the membrane.

The carrier is a good solvent for the Co^{2+} salts. At M_1 interface, it receives only Co^{2+} ions, without ligand (OH^-) and at the same time releases protons : the protons react rapidly with the disposable OH^- ions.

$$Co^{2+} + 2\,(HR)_2 \rightleftharpoons Co(HR_2)_2 + 2H^+ \qquad (4)$$

$$2H^+ + 2OH^- \rightleftharpoons 2H_2O \qquad (5)$$

This assertion is supported by the following arguments :

a. the naphtenic acids are liquid ion exchangers and they have the same behavior as the phosphorous acids (D 2EHPA, similar PC-88A) and the versatic acids (C10), with belong to the acidic extractant group too : they form dimers in non-polar solvents, they generate the proton pump in the transport mechanism, etc. [35].

b. by RMN methods, it was demonstrated for pure acids that they take-out only metallic ions, without ligand molecules [33] :

c. the values of the chemical equilibrium constant are :

$$K_1 = \frac{[Co^{2+}]\ [OH^-]}{[CoOH^+]} \ ; \qquad K_1 = 3.98 \cdot 10^{-5} \qquad [34]$$

$$[CoOH^+] = \frac{[Co^{2+}]\ [OH^-]}{K_1}$$

$$K_{1,2} = \frac{[Co^{2+}]\ [OH^-]^2}{[Co(OH)_2^0]} ; \qquad K_{1,2} = 2.51 \cdot 10^{-5} \quad [34]$$

$$[Co(OH)_2^0] = \frac{[Co^{2+}]\ [OH^-]^2}{K_{1,2}}$$

$$K_{1,2,3} = \frac{[Co^{2+}]\ [OH^-]^3}{[Co(OH)_3^-]} ; \qquad K_{1,2,3} = 3.16 \cdot 10^{-11} \quad [34]$$

$$[Co(OH)_3^-] = \frac{[Co^{2+}]\ [OH^-]^3}{K_{1,2,3}}$$

The total Co^{2+} concentration from solution is :

$$[Co^{2+}] = [Co^{2+}]_{aq} + [CoOH^+] + [Co(OH)_2^0] + [Co(OH)_3^-]$$

One notes : $[OH^-] = L$ (ligand)

$$\frac{1}{K_1} = \beta_1$$

$$\frac{1}{K_{1,2}} = \beta_2$$

$$\frac{1}{K_{1,2,3}} = \beta_3$$

$[Co^{2+}] = Co$

C_{Co} = total Co^{2+} concentration from solution , mol /l (analytical concentration)

The following equation is obtained :

$$C_{Co} = Co + CoL\beta_1 + CoL^2\beta_2 + CoL^3\beta_3$$

$$C_{Co} = Co\,(1 + L\beta_1 + L^2\beta_2 + L^3\beta_3)$$

At pH=10 when the process is starting, the L concentration is : $L=10^{-4}$ mol/l . One can know the proportion of the species :

$[Co^{2+}] = a_0 \cdot C_{Co}$

$[Co(OH)^{+}] = a_1 \cdot C_{Co}$

$[Co(OH)_2{}^{0}] = a_2 \cdot C_{Co}$

$[Co(OH)_3{}^{-}] = a_3 \cdot C_{Co}$

where : $$a_0 = \frac{1}{1 + \beta_1 L + \beta_2 L^2 + \beta_3 L^3} = 0.282$$

$$a_1 = \frac{\beta_1 L}{1 + \beta_1 L + \beta_2 L^2 + \beta_3 L^3} = 0.709$$

$$a_2 = \frac{\beta_2 L^2}{1 + \beta_1 L + \beta_2 L_2 + \beta_3 L^3} = 1.1 \cdot 10^{-4}$$

$$a_3 = \frac{\beta_3 L^3}{1 + \beta_1 L + \beta_2 L^2 + \beta_3 L^3} = 8.9 \cdot 10^{-3}$$

For the precipitation reaction the solubility product is known :

$$Co(OH)_2 \rightleftharpoons [Co^{2+}]\ [OH^-]^2 \qquad (6) \quad PS = 6.3 \cdot 10^{-15} \quad [34]$$

$$[Co^{2+}] = \frac{PS}{[OH^-]^2}$$

$$[Co^{2+}] = \frac{6.3 \cdot 10^{-15}}{10^{-8}} = 6.3 \cdot 10^{-7} \text{ mol/l}$$

and :

$$C_{Co} = \frac{[Co^{2+}]}{a_0}$$

$$C_{Co} = \frac{6.3 \cdot 10^{-7}}{0.282}$$

$$C_{Co} = 2.23 \cdot 10^{-6} \text{ mol / l}$$

So the concentrations are :

$[Co^{2+}]$ = $2.23 \cdot 10^{-6}$

$[Co(OH)^+]$ = $0.709 \cdot 2.23 \cdot 10^{-6}$ = $1.58 \cdot 10^{-6}$ (mol / l)

$[Co(OH)_2^0]$ = $1.1 \cdot 10^{-4} \cdot 2.23 \cdot 10^{-6}$ = $2.45 \cdot 10^{-10}$ (mol / l)

$[Co(OH)_3^-]$ = $8.9 \cdot 10^{-3} \cdot 2.23 \cdot 10^{-6}$ = $1.98 \cdot 10^{-8}$ (mol / l)

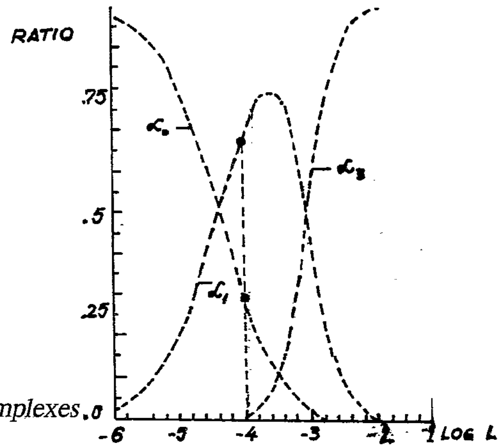

FIG. 10 *Stability range of complexes.*

Fig. 10 and the { [Co^{2+}] and [Co(OH)+] } concentration computation show the possibility that both species react with naphtenic acids. Co^{2+} ions are hydrated with six water molecules and present an octahedral structure and a dissipated charge on a big volume. The $Co(OH)^{+}$ complex has a tetrahedral structure and presents a directed charge. In all probability, this complex react with naphtenic acids and release the Co^{2+} ion.

As the Co^{2+} ions are taken over by the membrane and as the HO^{-} ions are consumed, the equilibrium (6) shifts completely to $Co(OH)_2$ solubilization.

The carrier concentration in the system was preferred be higher than the stoechiometric one because it favors the more rapid taking over of Co^{2+} ion. At the same time, the membrane diffusion could be considered not limitative in the transfer process.

The releasing of Co^{2+} ions at the M_2 interface is achieved by a chemical reaction.

The liquid membrane treatment procedure represents an advanced method of waste water cleaning, usually applied after water treatment by classical methods, when the resulting water has an about 8 pH value. The two step waste water treatment with liquid membranes leads to the Co^{2+} removal down to a residual concentration of 9 - 10 ppm (Table VII)

The step treatment process implies mixing of the already treated solution with a new quantity of fresh emulsion maintaining the same processing conditions.

TAB. 7. *Two step treatment*

Step	Initial conc. Co^{2+} mg/l	Final conc. Co^{2+} mg/l	pHi	pHe	η %
I	625	300	3.5	8	52
II	300	10	3.5	8	98.4

Conclusion

Co^{2+} ion permeation through liquid membranes could be carried out in a one-step process, in a 96% yield, or in a two-step process, under the conditions given below with increased yields up to 98%:

$pH_i = 3.5$ or $pH_i = 3.5$

$pH_e = 10$ $pH_e = 8$

membrane	: Kerosene
carrier	: naphtenic acids
inner phase	: HCl solution
treatment contact time, min	: 5
strirrer rotation speed, r.p.m.	: 200-300
temperature, °C	: 20

The liquid membrane permeation is a modern and selective recovery process for metallic ions.

The technological simplicity makes this method superior to that of solvent extraction, electrodialysis, ionic exchange.

ΠΕΡΙΛΗΨΗ

ΕΚΧΥΛΙΣΗ ΙΟΝΤΩΝ ΚΟΒΑΛΤΙΟΥ ΜΕ ΓΑΛΑΚΤΩΜΑΤΑ ΤΥΠΟΥ ΥΓΡΩΝ ΜΕΜΒΡΑΝΩΝ. Ι. ΠΑΡΑΣΚΕΥΗ ΥΓΡΩΝ ΜΕΜΒΡΑΝΩΝ.

Η εργασία περιγράφει μια αναλυτική μελέτη για τον διαχωρισμό των ιόντων Co^{+2}, εκφράζονται και συζητούνται απόψεις για την μεταφορά του μεταλλικού αυτού ιόντος δια μέσου της υγρής μεμβράνης.

Επίσης καθορίζονται οι συνθήκες παρασκευής και χρήσης υγρών μεμβρανών από κεροζίνη, οι οποίες χρησιμοποιήθηκαν για τον διαχωρισμό του Co^{+2}.

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SHORT PAPER

SELECTIVE LEACHING OF MAGNESITE WITH HCl ACID SOLUTIONS

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ABSTRACT

In this paper the possibility of selective dissolution of magnesite ore was investigated in order to recover magnesia and separate the undesirable admixtures of mixed oxides from the solution instead of two in one stage. The process is based on the great difference in the solubilities. The partial leaching of magnesite with an amount of HCl acid corresponding to 85% of the stoichiometric value, necessary for the complete dissolution, led to a final solution containing 98.8% MgO and 0.18% R_2O_3 (R: Fe, Al). In addition, the influence of temperature on dissolution rate of magnesite constituents was examined.

Key words : magnesite, selective dissolution, hydrochloric acid solutions

INTRODUCTION

Many fine mineral particles are currently deposited or discarded in the mine area, usually due to the unavoidable use of economically attractive technological processing methods. Apart from a hydrometallurgical route, flotation often constitutes an alternative solution. Certain flotation techniques suited for fines recovery were reviewed.[1] Magnesite belongs to salt-type minerals and its selective processing is generally difficult. The main separation problems are found with the crypto-crystalline (amorphus) type, such as that existing in Greece (Halkidiki, Evia). The ore contains Mg and Ca as carbonates (including dolomite), admixtures of silicates (seprentine, quartz etc.) and trivalent metals compounds (mainly Fe and Al).

The hydrometallurgical process for the recovery of magnesia, MgO, is usually realised in two stages.[2,3] In the first stage, an excess of hydrochloric acid at 50-80°C is added for the complete dissolution of the magnesite ore constituents, which consequently are converted to chlorides ($MgCl_2$, $CaCl_2$, $AlCl_3$, etc.); Chlorine gas is also added as an oxidising agent. In the second stage, the trivalent ions are converted to hydroxides, by increasing the pH of the solution; the hydroxides and insoluble silicates are separated after filtration. The production of magnesia from magnesium chloride is accomplished by roasting.[4,5]

The study of a selective, dissolution of magnesite ore or tailings and separation of mixed oxides (R: Fe, Al), from the solution in the stage is based on the great difference of solubilities between $MgCO_3$ and $CaCO_3$ and on the other hand, of the hydroxides of Fe and Al, referring to the conditions used during disolution. A reduction of added amount of hydrochlorid acid under the theoretically required for complete dissolution, would lead in only one stage to the production of a solution with low content in R_2O_3 (gangue). This exactly is the scope of the present paper.

For instance, the solubility product of magnesium carbonate is $6.82X10^{-6}$, of calcium carbonate is $4.96X10^{-9}$, while the values for ferric hydroxide and aluminium hydroxide are respectively $2.64X10^{-39}$ and $5X10^{-33}$.[6] Salt-type minerals are known to dissolve in aqueous solutions, with their ions undergoing various types of hydrolysis or complex formation reactions, which for the magnesite are coming up to eleven.[7]

MATERIAL, METHODS AND RESULTS

The natural magnesite ore used in the experimental part came from Gerakini in Halkidiki (Northern Greece) and had the chemical composition[8] and fraction size analysis shown in Table I; an x-ray crystallographic analysis follows (Fig. 1 and Table II). The specific surface of material was found by the Blaine method to be 5252 cm^2/gr.

TABLE I

Chemical analysis and particle size analysis of material (weight %)

moisture	0.72	+50	mesh	(+300 μm)	0.0
silicates	3.86	-50+70	"	(-300+212 ")	44.5
R_2O_3 (R: Fe, Al)	0.63	-70+100	"	(-212+150 ")	28.0
MgO	44.24	-100+200	"	(-150+75 ")	21.5
CaO	0.47	-200	"	(-75 ")	6.0
I.o.i.	50.08				

TABLE II

X-Ray crystallographic analysis data of magnesite[9]

D (A)	I/I_1
2,742	100
2,102	43
1,700	34
2,503	17
1,939	12
1,338	8
1,354	7
1,488	5
2,318	4
1,510	4
1,426	4

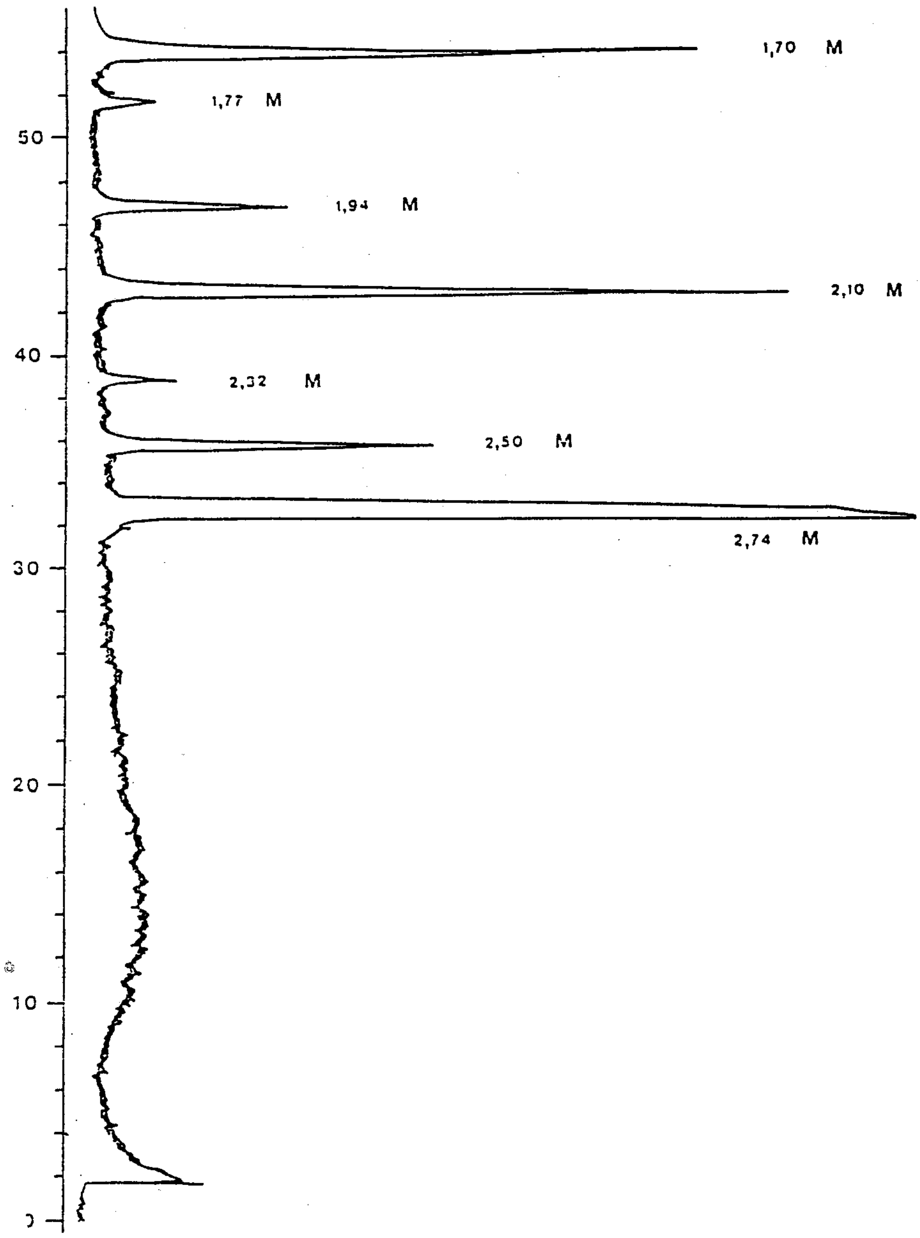

Figure 1. X-Ray crystallographic analysis of magnesite ore.

All the experiments were realized with 100 gr samples and 1:1 aqueous solutions of hydrochloric acid 37%. The theoretically required amount of the acid for complete dissolution of the material was calculated from the quantitative analysis and the chemical reactions of the magnesite constituents with hydrochloric acid. Leaching was carried out in open vessels and the solution was stirred in a thermostatic device. Two series of experimental tests were conducted.

In the first series, the influence of the amount of hydrochloric acid on the dissolution of the various constituents of the magnesite ore was investigated. The dissolution time was kept constant for 24 hours (time sufficient for the completion of the dissolution reactions), the temperature was also kept constant at 25° C and the amount of added hydrochloric acid was varied corresponding respectively to 85, 100 and 110% of the theoretical stoichiometric value required for the complete dissolution. The results are shown in Fig. 2. In this figure the curve (1) presents the dissolved fraction in % and the curve (2) presents the content in % of the solution in each constituent (weight in solution of each constituent / total weight of solution) after the completion of the dissolution reactions.

In the second series, the dissolution rate of the magnesite ore constituents at various temperatures was studied. The hydrochloric acid amount was kept constant at 85% of the stoichiometric value, the temperatures varied at 25, 35 and 45° C and the dissolution time was varied from 1 to 24 hours. The results are shown in Figures 3, 4 and 5.

In each of these figures the results of the dissolution of the various constituents of magnesite ore correspond to the dissolved fractions in % and they are representative of the dissolution rate.

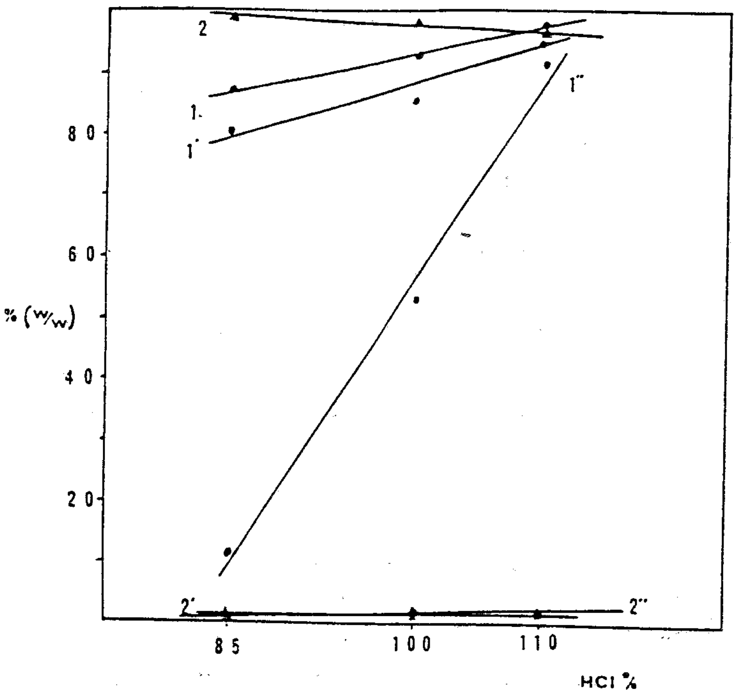

Figure 2. Influence of HCl acid amount on MgO, CaO, R_2O_3 dissolution. Temperature: 25° C, Leaching time: 24h.

(1) MgO weight in solution/initial MgO weight in magnesite (%)
(2) MgO weight in solution/total weight of solution (%)
(1') CaO weight in solution/initial CaO weight in magnesite (%)
(2') CaO weight in solution/total weight of solution (%)
(1'') R_2O_3 weight in solution/initial R_2O_3 weight in magnesite (%)
(2'') R_2O_3 weight in solution/total weight of solution (%)

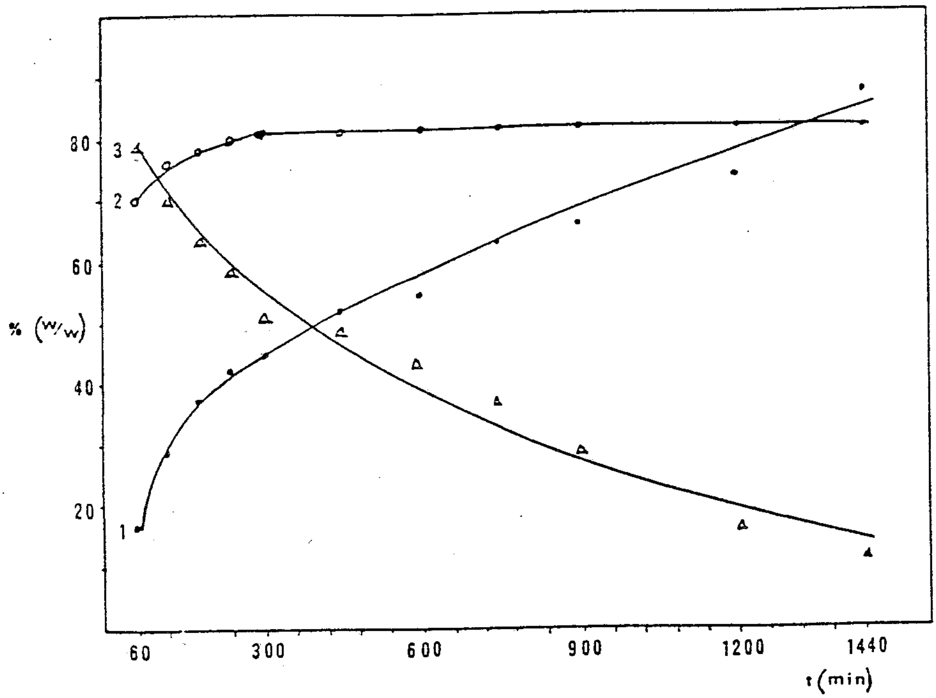

Figure 3. Influence of dissolution time on dissolution rate of magnesite constituents. Temperature: 25° C, HCl acid amount 85% of the stoichiometric value.

(1) MgO weight in solution/initial MgO weight in magnesite (%)

(2) CaO weight in solution/initial CaO weight in magnesite (%)

(3) R_2O_3 weight in solution/initial R_2O_3 weight in magnesite (%)

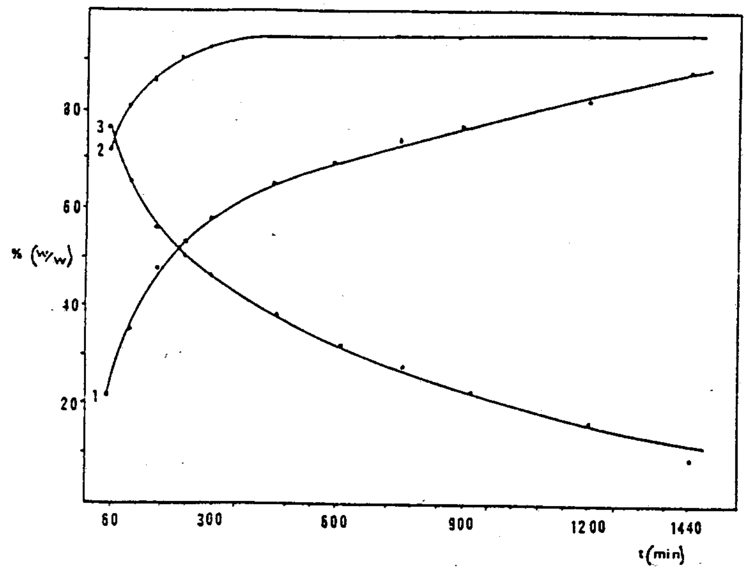

Figure 4. Influence of dissolution time on dissolution rate of magnesite constituents. Temperature: 35° C, HCl acid amount 85% of the stoichiometric value; as in Fig. 3.

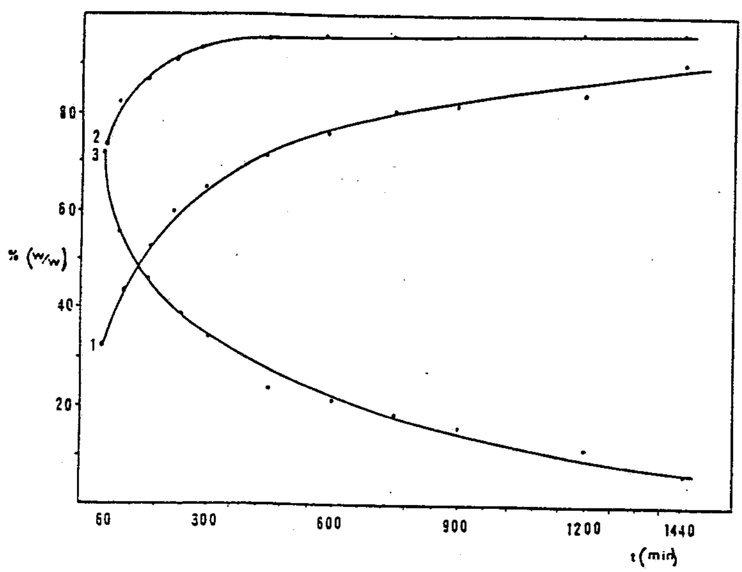

Figure 5. Influence of dissolution time on dissolution rate of magnesite constituents. Temperature: 45° C, HCl acid amount 85% of the stoichiometric value; as in Fig. 3.

DISCUSSION

From the experimental results showing the effect of HCl acid concentration on magnesite ore dissolution it follows that the effect was different for the various constituents of the ore. Increasing the HCl amount from 85% to 110% of the stoichiometric value for complete magnesite dissolution, it was noticed that :

a) the dissolution of MgO increased from 87.45 to 97.95% whereas the MgO content of the solution after filtration was greater (98.77%) for the HCl amount corresponding to 85% of the stoichiometric value (Fig. 2)

b) the dissolution of CaO increased from 80.95 to 95.24 (Fig. 2)

c) the dissolution of R_2O_3 increased from 11.11 to 91.49%, whereas after filtration the R_2O_3 content of the solution correspondimgly increased from 0.18 to 0.97% (Fig. 2).

These results show that for the HCl amount corresponding to 85% of the theoretically required, a greater part of $MgCO_3$ and only a small part of R_2O_3 had been dissolved. By increasing the HCl amoumt to 100% of the stoichiometric value the dissolution of $MgCO_3$ increased a little, but at the same time a great part of R_2O_3 remained in solution.

The obtained results were exactly as foreseen when programming this work, according to the great difference of solubilities. During the leaching of magnesite ore in hydrochloric acid solutions, the more important reactions taking place are the following :

$$M^{2+} + CO_3{}^{2-} \rightleftharpoons MCO_{3(s)} \quad (1)$$

$$M^{2+} + 2Cl^- \rightleftharpoons MCl_2 \quad (2)$$

$$R^{3+} + 3Cl^- \rightleftharpoons RCl_3 \quad (3)$$

$$CO_3{}^{2-} + 2H^+ \rightleftharpoons H_2O + CO_{2(g)} \quad (4)$$

$$R^{3+} + 3OH^- \rightleftharpoons P(OH)_{3(s)} \quad (5)$$

In these reactions we are denoting M for Mg^{2+}, Ca^{2+} and R (as usual) for Fe^{3+}, Al^{3+}

From the above equations it follows that: the augmentation of the hydrochloric acid concentration increases the dissolution of both magnesium carbonate and oxides of Fe and Al. Therefore, the equilibrium of equation (1) is moved to the left and in equation (2) to the right. Owing to the low pH of the solution, the equilibrium of the irreversible reaction (4) moves to the right. This acidic pH also moves equation (5) to the left. In other words, M^{2+} and R^{3+} are initially transferred to the solution as chlorides (in fact, calcium and ferric ions are the first reacting).

A diminution of the hydrochloric acid concentration moves the equilibria of the equations (1) and (5) to the right; hence, precipitation of MCO_3 and $R(OH)_3$ occurs, but this influence depends on solubilities. The difference in the solubility products shows that the diminution of the hydrochloric acid concentration is favourable for the separation of the trivalent metals. It is therefore concluded that, the dissolution of magnesite ore in hydrochloric acid of concentration lower than the stoichiometric will lead to an increase of the ratio M/R in solution. These assumptions were confirmed in the experimental study.

When the 85% concentration of the stoichiometric value was used, the Cl^- ions that existed in solution were not sufficient to convert all the constituents (except the silicates) to soluble chlorides. The initial dissociation of magnesium carbonate and R_2O_3, and the formation of chlorides led to an increase in pH of the solution. In this way, at a pH around 3.5 the precipitation of Fe^{3+} and later of Al^{3+}, in the form of hydroxides, is started. A small part of magnesium carbonate certainly remained insoluble.

Upon time, an ion exchange occured in the pulp between the ferric and magnesium ions, as chlorides. Hence, the increase of leaching time is increasing the MgO and CaO contents of the solution and respectively is decreasing the R_2O_3 (Fig. 3, 4, 5). It was also observed that, the dissolution rate was increased with the temperature (Fig. 3, 4, 5).

CONCLUSIONS

The partial dissolution of magnesite with an amount of hydrochloric acid corresponding to the 85% of the theoretically required for complete dissolution, led to selective dissolution of $MgCO_3$ and separation of the underisable admixtures (R_2O_3) from the solution in one stage. Under these conditions, 87.5% of the MgO contained in magnesite and only 11% of R_2O_3 are dissolved; the resulting solution contained 98.8% MgO and 0.18% R_2O_3. This result was attributed to the great difference in the solubilities.

Acknowledgements

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ΠΕΡΙΛΗΨΗ

ΕΚΛΕΚΤΙΚΗ ΔΙΑΛΥΤΟΠΟΙΗΣΗ ΤΟΥ ΜΑΓΝΗΣΙΤΗ ΜΕ ΔΙΑΛΥΜΑΤΑ HCl

Στην εργασία αυτή εξετάζεται η δυνατότητα εκλεκτικής διαλυτοποίησης, σε ένα στάδιο του μαγνησίτη με στόχο την ανάκτηση της μαγνησίας και την απομάκρυνση των ανεπιθύμητων προσμίξεων. Η διεργασία βασίζεται στην μεγάλη διαφορά των διαλυτοτήτων. Η μερική διαλυτοποίηση του μαγνησίτη με ποσότητα HCl που αντιστοιχεί στο 85% της στοιχειομετρικής, αναγκαίας για πλήρη διαλυτοποίηση, οδηγεί στην παραλαβή τελικού διαλύματος που περιέχει 98.8% MgO και 0.18% R_2O_3 (R:Fe,Al). Μελετάται επίσης η επίδραση της θερμοκρασίας στην ταχύτητα διαλυτοποίησης των συστατικών του μαγνησίτη.

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EXTRACTION OF COBALT IONS WITH EMULSION LIQUID MEMBRANES II. ELECTRIC BREAK-UP OF LIQUID MEMBRANES

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SUMMARY

The exhausted liquid membranes which contain Co^{2+} ions can be breaking-up in electric field. Some conditions and some considerations about the process are presented.

Key words: Liquid membranes, extraction of Co^{+2}, break-up

INTRODUCTION

Liquid membranes, invented by N.N.Li in 1968, are made from an emulsion of two immiscible phases (O/W or W/O) and then by dispersing the emulsion into a third phase (the continuous or "feed" phase) [1].

The compounds' separation by a membrane permeation process is particularly useful whenever conventional separation techniques cannot occur with good results (e.g. when the compound to be extracted is in a very low concentration)

This process was used to recover Co^{2+} ions from waste water resulting from the liquid phase oxidation reactions [2] .

The breaking-up of liquid membranes involeves separation of the inner phase from the organic phase. The inner aqueous phase contains Co^{2+} ions as $CoCl_2$. The organic phase contains the carrier and the surfactant which have to be recycled.

There are many methods for breaking-up the emulsions [3, 4, 5]: chemical ones (by heating or freezing), mechanical ones (by using ultrasound or by usin high stirring combined with phase inversion) and electrical methods (the cleanest and the most rapid).

The electrical breaking-up of Co^{2+} ion liquid membrane is studied.

EXPERIMENTAL PART

The process implies the emulsion (Tab. I, Fig.1) exposing in a pulsing, continuous or alternating electric field having controllable characteristics [6, 7, 8, 9, 10, 11]: shape field, voltage and frequency.

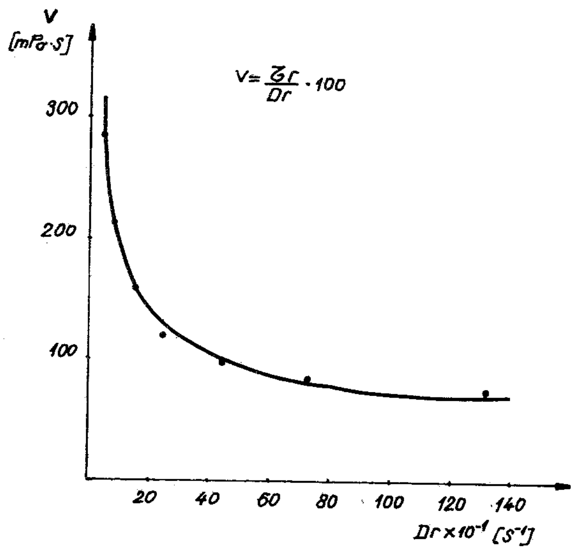

FIG. 1. *Emulsion viscosity, V- viscosity, D_r-speed gradient , τ_r - shearing stress*

The paper describes the exhausted membrane break-up in an alternating field.

A cylindrical cell having an inner vertical insulated electrode and outer one (coating the cell) was used for the membrane break-up study.

The alternating voltage applied to the system ranges from 0 V to 5000 V and the field frequency ranges from 500 to 2000 Hz

TAB. I. *Characteristics of the emulsion*

Emulsion characteristics	Values	Observations
particles dimension, μm	7 - 50	non uniform field
density, Kg/m^3	1024	
viscosity, mPa.s*	Fig.1	non newtonian fluid
centrifugal stability, ** ratio water/emulsion height layers	2/3	

* tested by Rheotest RV.
** determinations made by Janetzki T 23 centrifuge, at 4000 Rpm, 10 min.

DISCUSSION AND RESULTS

An emulsion droplet contains a lot of hydrated ions hexaqueous Co^{2+} and tetraqueous Cl^-. Immersed in a viscous continuous phase, the droplet has a "natural oscillation frequency" [10], depending on : composition and size, physical properties of the two phases and the interfacial tension.

In an electric alternating field, the droplet undergoes polarization and a two time per cycle deformation; at the same time, an amount of energy is accumulated (higher applied voltages lead to higher energy accumulation) and the amplitude change of droplet oscillation caused by field frequency is achieved. The system instability performed by the increasing of droplet collision intensity and number, which leads to coalescence, and finally to layer separation, is the result of these changes.

The time lag of th break-up process, the splitting time and the remaining water content in organic phase were studied.

The break-up process was observed (visual) by the change in the H/H_0 ratio versus time (t) (H-the level of separated aqueous layer,H_0 - entire height of emulsion layer).

The experimental data were fitted means of IBM/PC/XT computer according to the equation below:

$H/H0 = b_1 + b_2\, e^{-b3.t}$

where : H - the level of the separated aqueous layer [mm]

H_0 - the entire height of the emulsion layer at the initial time [mm]

t - time [s]

b_1, b_2, b_3 - parameters (Tab. II)

TAB. II *b_1, b_2, b_3, parameters determination by nonliniar regression*

voltage V	Frequecy Hz	b_1	b_2	b_3	Corel. coef.	Mean square deviation
	500	0.48057	-0.60787	-0.00667	0.9916	0.00413
3000	1000	0.51324	-0.51943	-0.00408	0.9982	0.00029
	2000	0.43494	-0.48075	-0.00319	0.9820	0.00268
	4000	0.25611	-1.44541	-0.00808	0.9541	0.00162
	500	0.56378	-0.61503	-0.00755	0.9979	0.00170
4000	1000	0.56383	-0.66049	-0.00427	0.9976	0.00104
	2000	0.52136	-0.59962	-0.00732	0.9829	0.00860
	500	-	-	-	-	-
5000	1000	0.59345	-0.59406	-0.00757	0.9844	0.00585
	2000	0.01251	-0.37641	-0.001688	0.9892	0.00894

This equation offers the possibility for the correct plotting of experimental data. At the time value t=0, one can extrapolate the time lag of breaking - up process:

$t = 0,\ H/H_0 = b_1 + b_2$ (2)

and one can find the splitting time of process (time for the half initial amount breaking-up of exhausted membrane).

Fig. 2 a, b, c shows that the higher the frequency, the lower the separation efficiency (H/H_0) is at constant value of applied voltage.

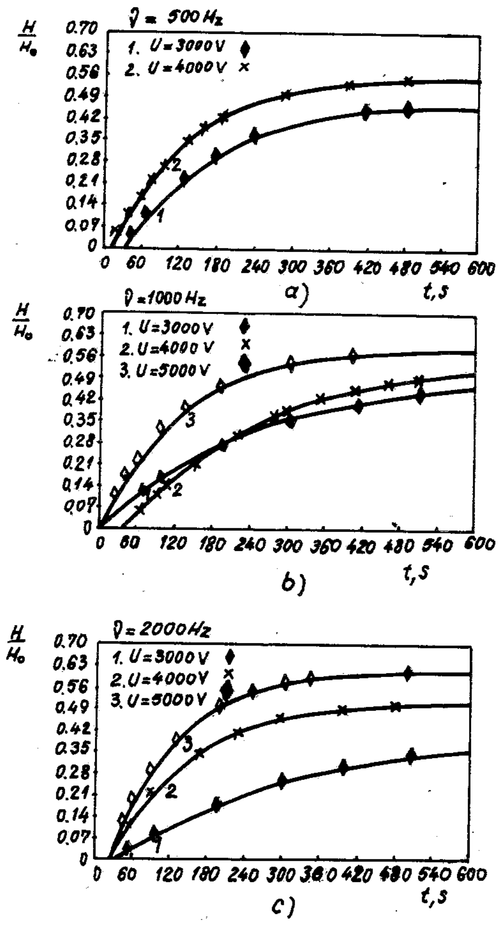

FIG 2. *Frequency influence on break - up process. a. at v=500 Hz, b. at v=1000 Hz, c. at v=2000 Hz.*

At constant values of working frequencies, at low alternating voltages applied (ranging from 0 V to 2000 V), the break-up process does not occur. Higher applied voltages lead to a higher break-up efficiency (Fig. 3 a, b, c). At 3000 V (Fig. 3 a) one can notice that the higher the frequency (ranging from 500 Hz to 4000 Hz) the slower the breaking process is (Tab. III).

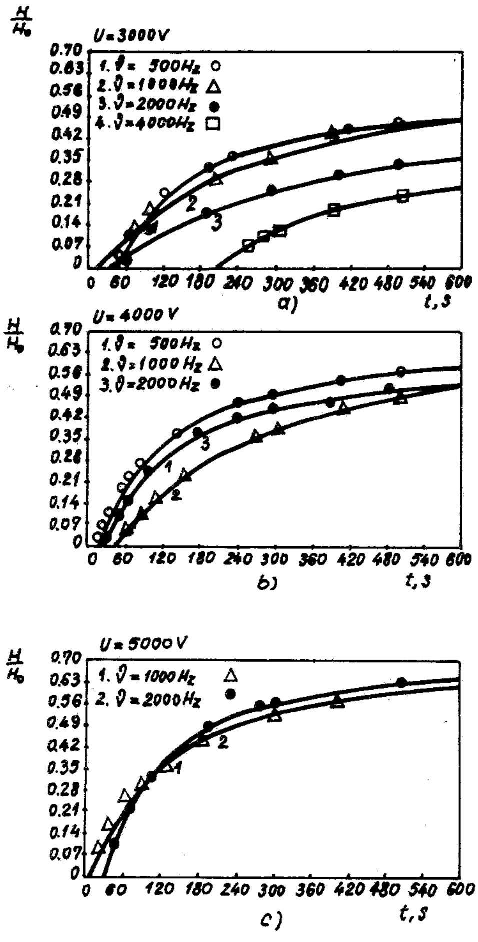

FIG. 3. *Voltage influence on break-up process. a. at U=3000 V, b. at U = 4000 V, c. at U = 5000 V*

TAB. III *Liquid membrane break - up in electric field*

Ctr. nr.	Voltage V	Frequency Hz	Remaining water content in organic phase %	Splitting time s	Time lag s	Breaking time s
1.	3000	500	1.5	139	35.2	
		1000	1.2	172.8	2.9	
		2000	1.13	248.7	31.3	
		4000	1.12	300	214.1	
						600
2.	4000	500	1.3	103.3	11.5	
		1000	1.14	199.4	37.0	
		2000	1.19	113.8	19.1	
3.	5000	1000	0.8	91.7	0.1	
		2000	1.19	242.4	22	

At frequencies about 20000 Hz, the emulsion do not break-up.

The emulsion has a large particle diameters dispersion. Most of them have large dimension and they begin resonated at low frequencies. At high frequencies only a few small particles can do it; so the emulsion remains unchahged under these conditions.

The remaining water content in the organic phase, as a consequence of the break-up process, was established using the Karl Fischer method (Tab. III). The higher the applied voltages, the lower the water membrane content is.

After the breaking-up process finished, some aspects about Co^{2+} ion transport through liquid membrane and real efficiency of the transport could be discussed. There are the following steps in Co^{2+} permeation:

1. The metallic ion transport into solution (source phase);

2. The metallic ion transport through a bonded layer at the M_1 interface;

3. The up-taking of metallic ions and H^+ releasing into source phase at M_1 interface;

4. The diffusion through membrane (including surfactant layers);

5. Chemical reaction for stripping metallic ion at M_2 interface and the up-taking of two H^+;

6. The metallic ion diffusion through a bonded layer at M_2 interface;

7. The diffusion into receiving phase.

1,2. The ion transport on the source phase is achieved usion a very efficient strirring system.

The $CoCl_2$ is completely dissociated and the hexaqueous Co^{2+} are Kinetically inert. The adding of ligands to the system (e.g. OH^-) enhances the rate of extraction process by replacing the water molecules with the ligand ones [12]. A Kinetcally and thermodynamically less stable complex is obtained. It reacts more quickly with the carrier, the diffusion occurs and governs the transport process through the membrane.

3. The carrier is a good solvent for the Co^{2+} salts. At M_1 interface, it receives only metallic ions, without ligand (OH^-).

At the same time it releases protons; the protons react rapidly with the disposable OH^- ions.

4. The carrier concentration in the system was preferred be higher than the stoichiometric one because it favors the more rapid taking over of Co^{2+} ion and increases the viscosity of membrane, so its stability. The membrane diffusion could be considered not rate limitative in the transfer process.

5. The releasing of Co^{2+} ions at the M_2 interface is achieved by a chemical reaction. The receiving phase is a HCl solution of a 3.5 pH (HCl is stronger than naphtenic acids), corresponding to a $3.2.10^{-4}$ mol hydrogen. The calculated hydrogen necessary for exchange the whole quantity of metallic ions demonstrate that this concentration does not suffice (75 mg Co^{2+} is up-taking in exchange of $1.27.10^{-3}$ mols of hydrogen; that correspond to pHi=2.9).

Tests for values of pHi=1, pHi=2 and pHi=3 were made (this values ensuring a higher or equal proton concentration comared to the stoichiometric one), but the results obtained were fluctuated regarding the transport yield, the swelling and breaking-up of the membrane.

6. After the exhausted emulsion breaking-up, only 175 mg Co^{2+}/l remain in the inner phase (the pH = 5.5-6). This is 5.4 times smaller quantity than the stoichiometric possible one.

It is generally known that in a W/O/W emulsion droplet, the inner transfer area M_2 is much larger than the outer one because there are many small incapsulated water droplets. The unusual low concentration of metallic ions in the inner phase (compared to the other studied species) leads to the supposition that in the second bonded layer, a "stagnation" of diffusion process is achieved.

7. The big metallic ions and chloride aqueous ions slowly diffuse. This is probably, the rate determining step. The phenomenon is mentioned in literature under the name of "concentration polarization".

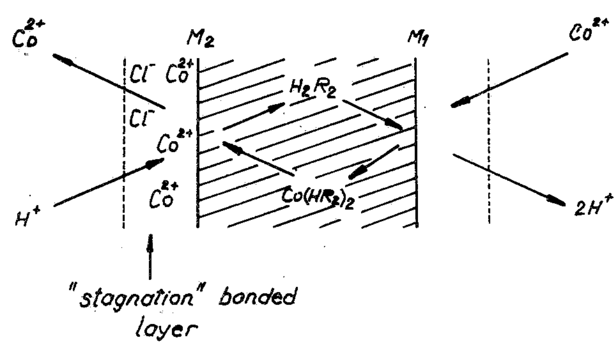

FIG. 4. *Co^{2+} permeation*

CONCULISION

The liquid membranes (contain Co^{2+} ions) can easily, but not completely (H/H0 = 0.6) be break - up at 4000 - 5000 V and 500 - 1000 Hz. The membrane material keeps an important Co^{2+} quantily due to slowly diffusion of ions in the second bonded layer. It is necessary to "wash" the membrane with diluted HCl solution for recyclated it.

The electric method may used with best results both on continuous and discontinuous systems.

ΠΕΡΙΛΗΨΗ

ΕΚΧΥΛΙΣΗ ΙΟΝΤΩΝ ΚΟΒΑΛΤΙΟΥ ΜΕ ΓΑΛΑΚΤΩΜΑΤΑ ΤΥΠΟΥ ΥΓΡΩΝ ΜΕΜΒΡΑΝΩΝ. II. ΚΑΤΑΣΤΡΟΦΗ ΥΓΡΩΝ ΜΕΜΒΡΑΝΩΝ ΣΕ ΗΛΕΚΤΡΙΚΟ ΠΕΔΙΟ.

Οι χρησιμοποιημένες (εξαντλημένες) υγρές μεμβράνες οι οποίες περιέχουν ιόντα Co^{+2} μπορούν να υποβληθούν σε καταστροφή ("break-up") υπό την επίδραση ηλεκτρικού πεδίου.

Στην εργασία αυτή παρουσιάζονται συνθήκες και θεωρητικά δεδομένα, σχετικά με τη διαδικασία καταστροφής των μεμβρανών.

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A SIMPLE COLORIMETRIC METHOD FOR ACCURATE QUANTIFICATION OF PARAQUAT IN BIOLOGICAL TISSUES AND FOODSTUFFS

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SUMMARY

The present work describes a new approach to the colorimetric quantification of paraquat in biological tissues and foodstuffs, overcoming all the technical problems of previous procedures due to turbidity, pigmentation and/or unsuitable ionic strength of solutions. This is effected by measuring the background absorption of each individual sample after decolorization of the blue paraquat radical by vigorous shaking or addition of 1 drop of dilute hydrogen peroxide. In addition, the necessary reference solutions are prepared by a spiking procedure under similar conditions.

Key words: Paraquat, colorimetric assay

INTRODUCTION

Paraquat (dichloride salt of the 1,1'-dimethyl-4,4'-bipyridinium ion) is a powerful herbicide used worldwide as a non-selective, contact weedkiller. It was first synthesized in the last century[1] as a redox indicator known as viologen and later on, in 1959, this and other bipyridyl compounds like diquat, were found to possess potent herbicidal properties[2]. Since then a large number of fatal intoxications have been reported and all aspects of paraquat poisoning have been reviewed by several authors[3].

Quantitative determination of paraquat is effected by several methods, which utilize either colorimetry[4-14], gas chromatography[15-20], TLC[21,22], HPLC[23-28], or bioassay and immunoassay procedures[29-31]. The most widely used technique is colorimetric measurement of the intense blue radical derived by reduction of paraquat with alkaline dithionite, after concentration by cation-exchange chromatography[11,15]. Developments in the extraction of cations into organic solvents as ion-pairs[28,32] have opened the way for improvements, i.e. replacement of the time consuming cation-exchange technique by extraction of paraquat from alkaline media into organic solvents followed by re-extraction by a minimal volunme of 1 M H_2SO_4.

It is largely recognized that the difficulty of obtaining true blank values in the colorimetric assay decreases the accuracy of the method[27]. This paper describes a technique effectively overcoming the above problem, thus increasing the accuracy and validity of the colorimetric quantification of paraquat in biological samples and foodstuffs.

MATERIALS AND METHODS

Materials

Paraquat dichloride from Aldrich Chemical Co. (Milwaukee, WI) was used as authentic sample for standarization of solutions of a commercial product (Gramoxon, 20% paraquat), which were used thereafter.

Cultures and homogenates of *Tetrahymena pyriformis* were prepared as previously described[33]. Rat tissues were obtained from the Department of Experimental Pharmacology, School of Medicine, University of Athens.

Preparation of samples

Rat livers and lungs were spiked with paraquat (1-10 μg/g), homogenized in ten volumes of 10% trichloracetic acid and centrifuged. The precipitate was resuspended in 5 volumes of the acid, centrifuged and the combined supernatants were chromatographed on a cation-exchange column (Permutit Zeo-Garb 225, 52-100 mesh, 8% DVB, or Dowex AG 50Xx8, 100-200 mesh) according to Calderbank and Yuen[15]. Alternatively, the tissues were homogenized in 0.2 N H_2SO_4 and treated as described below for blood and urine.

Blood and urine were spiked with paraquat (0.1-1 mg/ml) and deproteinized by acidification to pH 2-3 with 1 N H_2SO_4 followed by centrifugation. Then, either they were neutralized with 10% sodium hydroxide (and used directly in the colorimetric assay), or they were extracted with an equal volume of 2.5% (w/v) sodium dodecyl sulphate in methylisobutylketone/isobutanol, 1:1 (v/v). Paraquat was back extracted from the organic solvent with one tenth vol. 1 M H_2SO_4[28,32], and the aqueous extract was centrifuged after neutralization with 10% sodium hydroxide.

Tetrahymena cultures were administered with known amounts of paraquat and homogenized by sonication. Colorimetric assays by the new method were carried out either on total homogenates, or on subcellular fractions obtained by ultracentrifugation.

Colorimetric assay (adopted)

Take 2 ml of sample in a colorimetric tube, add 0.5 ml of distilled water and 0.5 ml of freshly prepared sodium dithionite reagent and mix gently. Within 10 min, measure and absorbance at 600 nm against distilled water. Then, decolorize the sample either by adding 1 drop of dilute hydrogen peroxide (5% v/v), or by vigorous shaking of the contents of the tube until complete decolorization (15-20 sec). Measure the absorbance of the decolorized sample (blank value) against distilled water and subtract it from the original absorbance (before decolorization). This difference is the corrected absorbance, Au, of the unknown sample.

Concommitantly, take another 2 ml of sample in another colorimetric tube and, instead of the distilled water, add 0.5 ml of paraquat standard solution (10-30 μg/ml). Then, proceed exactly as directed above to measure the corrected absorbance, As, of the second, paraquat-spiked sample.

Calculate the unknown quantity, Wu, of paraquat in the unknown 2 ml sample by using the following formula:

$$Wu = \frac{Au}{As-Au} \times Ws$$

where Ws is the quantity (μg) of paraquat added to the second tube.

Using more -than one- sample spiked with 0.5 ml of standard solutions of different paraquat concentrations (2-30 μg/ml) a standard curve may be constructed from the individual values of (As-Au) and the respective quantities (μg) of added paraquat.

RESULTS AND DISCUSSION

The main feature (and advantage) of the present method is that it allows the selective measurement of the blue color of reduced paraquat in solutions either turbid, or colored, or both, thus overcoming all the severe technical problems of previous colorimetric procedures. This advantage is effected by a suitable technique of decolorization of the blue paraquat radical (after measuring its absorbance), without affecting the pH or any other experimental variable of the sample. Consequently, subtraction of the absorbance of the decolorized sample from its original value gives an accurate measurement of the blue paraquat radical.

Initial attempts to decolorize the samples by acidification with acetic or mineral acids proved to be inadequate. Shortly after addition of the acid to non-turbid samples it was observed that they gradually developed turbidity, apparently owing to dithionite decomposition. A similar behaviour was observed after addition of perhydrol, while addition of dilute hydrogen peroxide, or simply vigorous shaking were found to be quite effective for decolorization of the blue paraquat radical without creating any other problem.

However, despite the overcoming of the above problems, subsequent recovery experiments showed that this was not sufficient for an accurate quantification of paraquat in turbid (and some colored) solutions. Namely, the increase of absorbance of a turbid sample due to added paraquat was lower than that due to the same quantity of paraquat added to a non-turbid sample. The difference between these two values, corresponds to the well-known "obscured" concentration[34] resulting from two phenomena. First, the flattening of the absorption spectrum of suspensions, as compared to that of solutions, described by Duysens[35]; and second, the multiple scattering[36] which decreases the probability of the incident photons to be absorbed by the chromophor, due to light scattering[34,36].

This problem was successively overcome by adopting a special procedure for the preparation of the standard(s) (see Material and Methods), which permits the measurement of the absorbance of the known quantities of standard paraquat under the same conditions of the unknown sample.

Finally, it is noteworthy that in the early stages of this investigation it was found that the sensitivity, precision and accuracy of the colorimetric assay step strongly depends on the ionic strength and the alkalinity conditions of the final solutions. Optimization experiments have shown that the conditions adopted in the present method are most appropriate for maximum color intensity and stability. Contrarywise the intensity and stability of the blue color formed in the saturated ammonium chloride eluents from cation-exchange columns, or in samples deproteinized with trichloroacetic acid were substantially lower.

Under the conditions of the present method, the absorbance of the final color is about 0.050 per 1 ppm paraquat (1 μg/ml) i.e. the sensitivity of the method is about 10 times lower than by GLC or HPLC methods. However, this is not a limiting factor for analysing samples of much lower paraquat concentration since a 10-fold increase of paraquat concentration is achieved by the extraction procedure using sodium dodecyl sulphate (2.5%) in organic solvents (see Preparation of Samples). Obviously, even a 100-fold concentration is possible by repeating once more the extraction - back extraction steps.

In conclusion, the present method is quite suitable for simple and accurate quantification of paraquat in biological tissues and foodstuffs.

ΠΕΡΙΛΗΨΗ

Απλή χρωματομετρική μέθοδος για τον ακριβή ποσοτικό προσδιορισμό του paraquat σε βιολογικούς ιστούς και τρόφιμα

Περιγράφεται μια νέα προσέγγιση του προβλήματος του χρωματομετρικού προσδιορισμού paraquat σε βιολογικούς ιστούς και τρόφιμα, που αντιμετωπίζει όλα τα τεχνικά προβλήματα προηγουμένων μεθόδων, τα οφειλόμενα σε θολερότητα, χρωματισμό και/ή ακατάλληλη ιοντική ισχύ των διαλυμάτων. Αυτό επιτυγχάνεται με τη μέτρηση της μη ειδικής απορρόφησης (απόσβεσης) κάθε δείγματος ξεχωριστά μετά από

καταστροφή του κυανού χρώματος της ρίζας paraquat είτε με έντονη ανακίνηση, είτε με την προσθήκη μιας σταγόνας αραιού υπεροξειδίου υδρογόνου. Επιπλέον, τα απαιτούμενα πρότυπα διαλύματα αναφοράς παρασκευάζονται με την προσθήκη γνωστών ποσοτήτων paraquat σε δείγματα του αγνώστου διαλύματος και εφαρμογή της ίδιας διαδικασίας (με τις ίδιες συνθήκες).

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CHIMIKA CHRONIKA, *NEW SERIES*

CONTENTS

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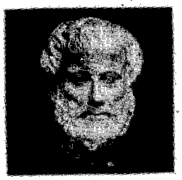
This year visit Macedonia

Macedonia

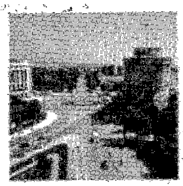
For 4,000 years* steeped in the history of Greece

Statue of Aristotle, Stagira.



Aristotle, the tutor of Alexander the Great, was born in Stagira in Macedonia in 384 BC. Together with Plato, he is regarded as one of the greatest philosophers the world has known. Aristotle was a true academic, concerned with Physics, Astronomy, Rhetoric, Literature, Political Science and History. His teachings laid the foundation for modern scientific thought.

The White Tower of Thessaloniki.

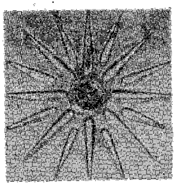


Thessaloniki, the heart of Macedonia, is a modern city with 1,000,000 inhabitants. It is strategically located at the crossroads of Europe with Asia. Having spread the Word at Philippi, the Apostle Paul continued his teachings in Thessaloniki. Its important monuments from antiquity and byzantium up to the present, provide testimony to the role that the city has played as the second capital of Hellenism.

The Bust of Alexander the Great. Acropolis Museum, Athens.

Alexander was born in 356 BC in Pella, Macedonia, established by his father Philip II, as the centre of Hellenism. Nurtured on the thoughts of his tutor, Aristotle, he rose to fame as a brilliant military leader. He influenced the course of history, rightfully earning his title as Alexander the Great. In 335 BC he became Commander in Chief of all the Greeks. By the time of his death in 323 BC he had created an enormous empire, stretching from the shores of the Adriatic to India, and from the Caucasus Mountains to Egypt. He spread the Greek spirit far and wide among nations who worshipped him as a god.

Symbol of the Greek Macedonian Dynasty from the tomb of Philip II. Archaeological Museum, Thessaloniki.



This 16 pointed star of Vergina was uncovered during the archaeological excavations at Vergina. This symbol of the Greek Macedonian Dynasty decorated the golden tomb of Philip II. The Star of Vergina, extracted from the soil of Macedonia, has since become the symbol of Hellenism.

The Olympian Aphrodite (3rd Century BC) Museum of Dion.

This statue of Aphrodite came to light during archaeological digs at the ancient sacred city of Dion. Dion, at the foot of Mt Olympus, was the most important spiritual site for the Northern Greeks, playing the same role in their lives as that of the oracle at Delphi.

St Dimitrios, detail of 7th Century Mosaic, Church of St. Dimitrios, Thessaloniki.

St Dimitrios, Protector of the city of Thessaloniki, was martyred in 305 AD defending Christianity. He is regarded a the Patron Saint of Thessaloniki and its saviour during difficult moments.

4.000 years: Post-Mycenaean ceramic relics found in Assiros and Mycenaean swords found in Grevena date back 4.000 years, evidence of Macedonia's role at the vortex of Greek history. Even in mythology Macedon, mythical founder of the Macedonian race, is the son of Aeolos (god of the winds). Throughout the years Macedonia contributed to the fountain of knowledge of the Ancient Greeks. In the 5th century BC Demokritos, father of Atomic Theory, lived and worked in Avdira.*

GREECE
Chosen by the Gods