SCIENCO

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

ISSN 0104-5431

AN INTERNATIONAL FORUM FOR THE RAPID PUBLICATION OF ORIGINAL SCIENTIFIC ARTICLES DEALING WITH CHEMISTRY AND RELATED INTERDISCIPLINARY AREAS

VOLUME SEVEN, NUMBER EIGHT

DECEMBER 1999

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
ISSN 0104-5431
VOLUME SEVEN, NUMBER EIGHT DECEMBER 1999

CONTENTS / CONTEÚDO

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

A TRIBUTE TO PROFESSOR CLIFFORD A. BUNTON ON HIS 80TH BIRTHDAY

Lavinel G. Ionescu
Departamento de Química Pura, Faculdade de Química
Pontificia Universidade Católica do Rio Grande do Sul -PUCRS
Porto Alegre, RS BRASIL 90610-900
&
Departamento de Química,Centro de Ciências Naturais e Exatas
Universidade Luterana do Brasil - ULBRA
Canoas, RS BRASIL 92420-280

ABSTRACT

On January 4, 2000 Professor Clifford A. Bunton will celebrate his 80th birthday, over fifty years of contributions to mechanistic organic chemistry and more than thirty years of work on the structure and reactivity of micelles and other association colloids. He obtained the B.Sc. Degree in Chemistry from University College, London in 1941 and the Ph.D. Degree in mechanistic organic chemistry from the same institution in 1944. He remained there as Lecturer and Reader until 1963, when he joined the faculty of the University of California, Santa Barbara,where he is at the present an Emeritus Professor. Professor C.A. Bunton's research work centers on physical organic chemistry of carboxylate and phosphate ester hydrolysis, micellar catalysis, kinetic isotope effects and reactions of ferrocenyl carbocations. He had a large number of students and collaborators from all continents and all corners of the globe and published hundreds of scientific works.

RESUMO

No dia 4 de Janeiro de 2000 o Prof. Dr. Clifford A. Bunton vai celebrar seu octagésimo aniversário, junto com mais de cinquenta anos de contribuições na química orgânica e mais de trinta anos de trabalho sobre a reatividade e estrutura de micelas e outros colóides de associação. Ele obteve o título de B.Sc. em Química no University College, Universidade de Londres em 1941 e o Ph.D. em Química Orgânica Mecanística na mesma instituição em 1944. Ocupou os cargos de Lecturer e Reader na mesma universidade até 1963 quando foi para a Universidade da California, Santa Bárbara como Professor Titular e onde ainda segue desempenhando as suas atividades na presente data como Professor Emeritus. O trabalho de pesquisa do Prof. Dr. C.A. Bunton se concentra na físico-química orgânica da hidrólise de ésteres de carboxilato e fosfato, catálise micelar, efeitos cinéticos isotópicos e reações de carbocátions de ferrocenila. Ele contou com um número grande de estudantes e colaboradores de todos os continentes e todos os cantos do globo terrestre e publicou centenas de trabalhos científicos.

KEYWORDS History of Chemistry, Carboxylate and Phosphate Ester Hydrolysis, Micellar Catalysis, Isotope Effects, Mechanistic Organic Chemistry

DOI: 10.48141/SBJCHEM.v7.n8.1999.4_1999.pdf

Professor Clifford A. Bunton was born on January 4, 1920 in England.

He obtained the Bachelor of Science Degree in Chemistry from University College, London, where he had various distinguished teachers, among them E.D. Hughes and C.K. Ingold. In 1944 he earned his Doctor of Philosophy Degree in Mechanistic Organic Chemistry from the same institution.

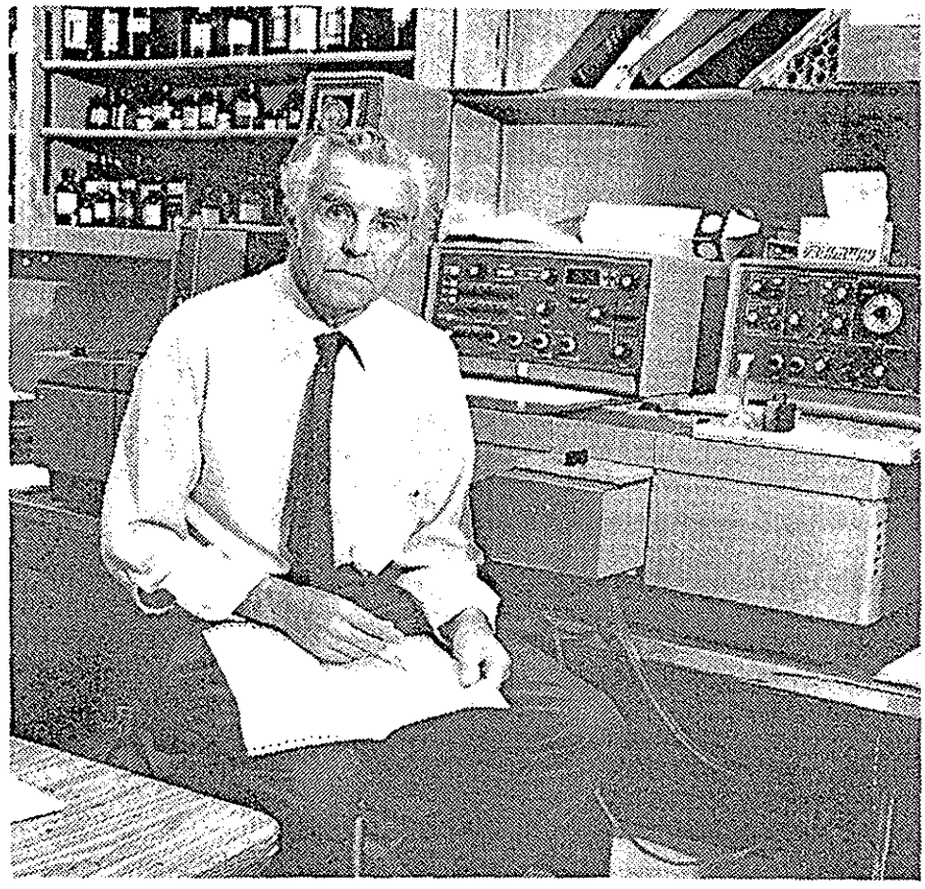
Dr. Bunton remained at University College, London as Lecturer and Reader until 1963. During parts of 1961 and 1963, he occupied positions as Visiting Lecturer at the University of California at Los Angeles and Visiting Professor at the University of Toronto, Ontario, Canada.

In 1963 he joined the faculty of the Chemistry Department of the University of California, Santa Barbara as Full Professor, a position that he held until his official retirement on May 26, 1990. On that occasion, he was honored by friends, former students and associates with a symposium entitled "*Quantitative Treatment of Organic Reactions in Solution*" that included very distinguished invited speakers, among them Joe Bunnett(University of California, Santa Cruz), Janos Fendler (Syracuse), Keith Inglod (NRC, Ottawa), Fred Menger (Emory University, Atlanta, Georgia), Larry Romsted (Rutgers University,Piscataway, N.J.) and Jack Shiner (University of Indiana, Bloomington). His formal retirement in no way hindered his scientific investigations and he continues to be very active in research as an Emeritus Professor.

During his 27-year tenure as Professor at the University of California, Santa Barbara, Dr. Bunton participated of a wide number of committees at the departmental, university, state and national levels. He was a member of the Graduate Committee, Building, Storeroom, General Education, Science and Engineering Library and Educational Policy Committees. He served as Chairman of the Science Policy Committee for the University of Chile/University of California Cooperative Program for many years. From 1967 to 1972, Dr. C. A. Bunton served as Chairman of the Chemistry Department. Together with Professors Bruce Rickborn, Glyn O. Pritchard and Thomas C. Bruice, he may be considered as one of the pillars of the Chemistry Department of the University of California, Santa Barbara.

In recognition of his outstanding research, he was named UCSB Faculty Research Lecturer. He has been the recipient of numerous fellowships and plenary lectureships. Dr. Bunton is a member of the Chemical Society (London), Institute of Chemistry and the American Chemical Society. He served as referee and was part of the Editorial Board of many journals throughout the world and also acted as referee for the Petroleum Research Fund and most Federal Granting Agencies.

Professor C.A. Bunton's research work centers on the physical organic chemistry of carboxylic esters and phosphate ester hydrolysis, micellar catalysis and reaction of ferrocenyl carbocations. He has a continuing interest in models for biological reactions, mechanisms of oxidation and the use of isotope tracers in the study of reaction mechanisms.



PROFESSOR DR. CLIFFORD A. BUNTON

His work on micellar catalyzed reactions provides a glimpse into the approaches that he has taken in terms of scientific investigations during a period of continuous effort that spans more than half a century.

Micelles were used as models for a variety of interfaces from membranes to enzyme active sites. His early work explored the effect of micelles on a wide range of reactions including spontaneous decarboxylations, acid catalyzed acetal hydrolysis, the benzidine rearrangement and aromatic nucleophilic substitutuion besides reactions of carboxylate and phosphate esters.

Later, Professor C.A. Bunton worked on kinetic models that led to quantitative interpretations of micellar effects on rates and equilibria and on probes of micelle structure that led to a better understanding of the aqueous interface.

On the more practical side, he opened the pathway for the use of functionalized micellar solutions for detoxifying people poisoned by organophosphate pesticides and nerve gases. During the period beginning in 1963 and up to very recent times Prof. C.A. Bunton had ample financial support from the Petroleum Research Fund, the National Science Foundation, National Institutes of Health, the United States Army Office of Research and other U.S. Granting Agencies.

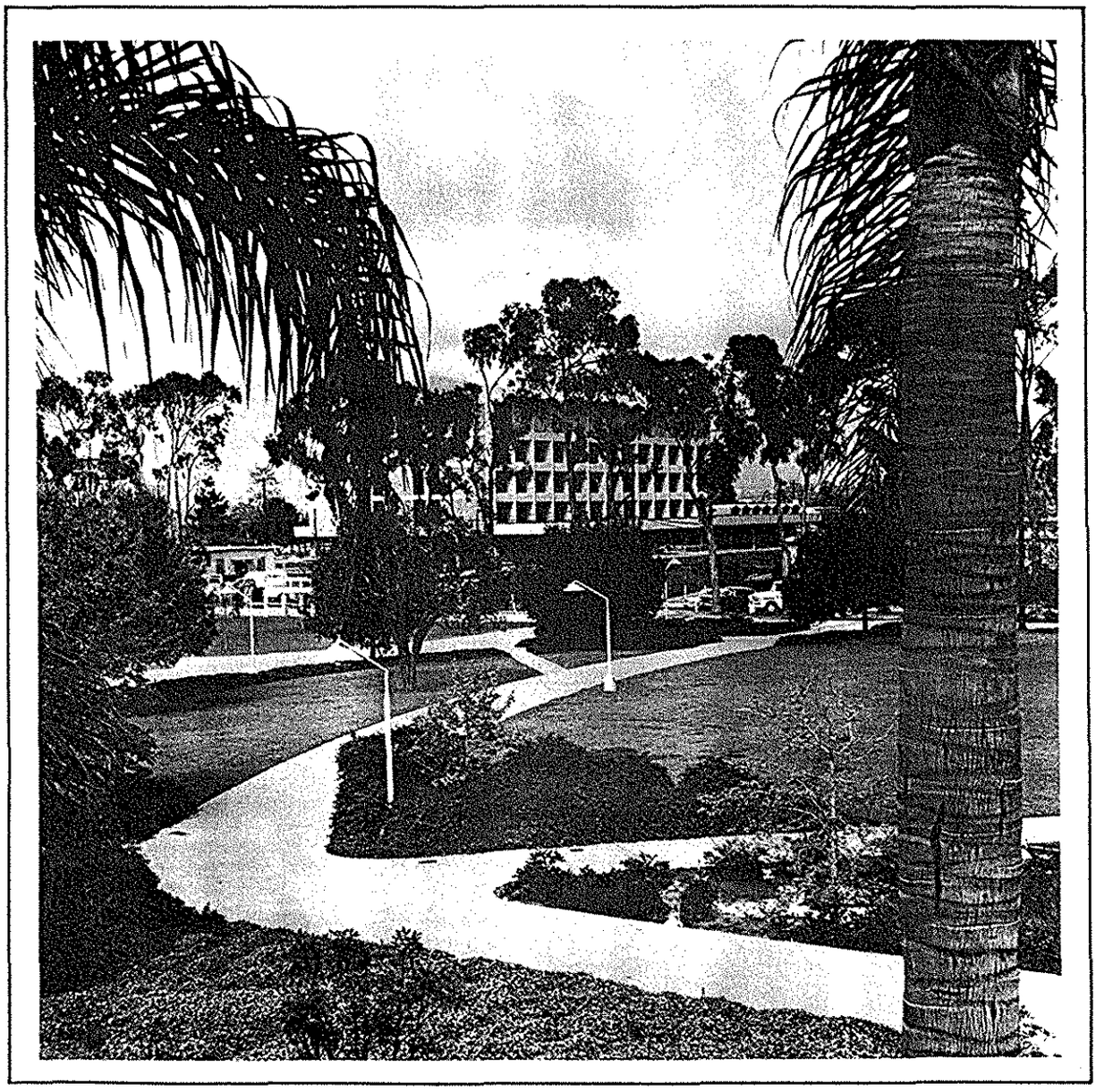
We had the privilege to work and collaborate closely with Professor C.A. Bunton on two occasions.Firstly, as a Postdoctoral Fellow during 1971-72 and secondly during 1982, while a Visiting Professor at the University of California, Santa Barbara.

Professor C.A. Bunton is a very kind, fair, considerate and hard working person and has always treated others with respect and dignity. He is called "*Bunny*" by his close friends and associates. Among his hobbies are sailing, mountain climbing and jogging. He is an excellent connoisseur of wines. During his frequent trips to Chile, where he was always treated with admiration and respect, besides the hard work opening the frontiers of chemistry, he used to do a lot of mountain climbing in the Cordillera of the Andes. During the noon hour, at times he used to go jogging on the beach in Isla Vista or Santa Barbara with friends. Among his most frequent companions was Glyn O. Pritchard, a physical chemistry professor at UCSB. When he was not jogging, he used to eat lunch in his office and spend the rest of the noon break reading journals.

Professor C.A. Bunton used to make two daily rounds in the laboratory, one around 10:00 am in the morning and the other during the latter part of the afternoon , about 4:30 or 5:00 pm and enjoyed discussing and analyzing new experimental results with his students and collaborators. One of his graduate students, who was a little bit absent minded, could not obtain the desired product in a synthesis after various attempts. All people in the laboratory were puzzled, only to discover days later that the fellow had forgotten to to add one of the reagents. On another occasion, a postdoctoral fellow that was working with cyanide wanted to order a few kilograms from a supplier. Dr. Bunton remarked that with that "*bloody quantity he could kill all the people in Santa Barbara*".

He also liked to talk about politics. He was no fan of Ronald Reagan, then Governor of the State of California, a fellow who trimmed the budget of the University of California and distributed significant sums to the state colleges.

His typical greeting was "*How is it going?*". The greeting actually had two meanings and it required two different answers. The first one is obvious and had to do with one's well being. The second one was really an implicit question about new experimental results that were obtained during the day. It was a very elegant way to remind one of his responsibility and at the same time stimulate productive research activity in the laboratory.



CHEMISTRY BUILDING, UNIVERSITY OF CALIFORNIA,
SANTA BARBARA, USA.

During our first stay in Santa Barbara as a postdoctoral fellow, Isla Vista, where the campus of the university is located was also one of the national headquarters of the Hippie Movement. Public disturbances were rather common as was the use of tear gas by the California State Police. The burning of the branch of the Bank of America and its rebuilding in a "bunker style" in the center of Isla Vista is part of the Hippie Legend. One night, a public protest against the Vietnam War turned into a riot and Isla Vista was saturated with tear gas. We did not sleep all night and cried a good part of it. Early in the morning we went to the Laboratory on the Campus where there was a smaller concentration of tear gas. For some good reason, Dr. Bunton came a little bit earlier and began his morning round with his usual greeting: " *How is it going ?*" We answered that we were not going very well because of the riot and the tear gas , that the eyes were red that we did not sleep all night and cried a good part of it and did not say anything about the new experimental results that we had botained. Professor Bunton listened carefully, shook his head and then patiently said again: "*But I mean how is it going?*". We than had an interesting discussion and made a good analysis of the fresh experimetal results.

Throughout the more than fifty years of continuous hard work, Professor Clifford A. Bunton had a large number of students and coworkers from all continents and all corners of the globe, probably more than two hundred of them.

His formal collaborations were manily with the University of Chile, Santiago, the University of Perugia and the University of Rome in Italy and the Universidade Federal de Santa Catarina and Universidade de São Paulo in Brazil. He treated all his students and coworkers as friends and many of them remember with pleasure the Christmas parties and the dinners offered in their home in Santa Barbara by Dr. and Mrs. Bunton.

Professor C. A. Bunton is the author of a very large number of scientific publications and received a large number of awards, prizes and distinctions and no attempt will be made to enumerate them at the present. He is a Member of the Brazilian and Chilean Academies of Science, Doctor Honoris Causa of the University of Perugia, Fellow of the American Association for the Advancement of Science and Recipient of the Tolman Medal. He is a very prolific scientific writer and has published almost five hundred scientific articles. We shall list ony a few representative publications at the end of this homage.

On the occasion of his 80th birthday (January 4, 2000) we pay our modest tribute to Professor Dr. Clifford Allen Bunton as a scientist, teacher, colleague and friend and congratulate him for all the accomplishments that he has achieved during more than half a century of effort, hard work, diligence and good will. We convey him the best wishes of good health, happiness and success for the days to come.

REPRESENTATIVE PUBLICATIONS

1. Univalent Electron Transfer in Aromatic Nitration?, G. A. Benford, C.A. Bunton, E.S. Halberstadt, E.D. Hughes, C. K. Ingold, G.J. Minkoff and R. I. Reed, *Nature, 156,* 688 (1945).

2. Nitration of Phenol and Aniline Derivatives, C.A. Bunton. E.D. Hughes, R.I. Reed and G.J. Minkoff, *Nature, 158,* 514 (1946).

3. A Re-Examination of the Reported meta Nitration of Phenols and Phenolic Ethers, C.A. Bunton, G.J. Minkoff and R. I. Reed, *J. Chem. Soc.,* 1416 (1947).

4. The Formation of Hydroxy Peroxides, C. A. Bunton, E.S. Halberstadt and G. J. Minkoff, *Nature, 161,* 172 (1948).

5. The Oxidation of α-β Unsaturated Ketones with Alkaline Hydrogen Peroxide, C.A. Bunton and G.J. Minkoff, *J. Chem. Soc.,* 665 (1949).

6. Oxidation of α-Diketones and α-Keto-Acids by Hydrogen Peroxide, C.A. Bunton, *Nature, 163,* 444 (1949).

7. Kinetics and Mechanism of Aromatic Nitration. VI. The Nitration of Phenols and Phenolic Ethers, C.A. Bunton, E.D. Hughes, C.K. Ingold, D. Jacobs, M. Jones, G.J. Minkoff and R. I. Reed, *J. Chem. Soc.,* 2628 (1950).

8. Walden Inversion in the Acid Hydrolysis of Carboxylic Esters by Unimolecular Alkyl Fission, C.A. Bunton, E.D. Hughes, C.K. Ingold and D. Meigh, *Nature, 165,* 680 (1950).

9. The Hydrolysis of Aryl Sulphonates. I. Phenol Toluene-p-Sulphonate, C.A. Bunton and Y.F. Frei, *J. Chem. Soc.,* 1872 (1951).

10. Alkyl Oxygen Fission in the Acid Hydrolysis of tert-Butyl Acetate, C.A. Bunton, A.E. Comyns and J.H. Wood, *Research, 4,* 383 (1951).

11. Preparation of Aryl-Alkane Sulphonic Acids, C.A. Bunton and E.A. Halevi, *J. Chem. Soc.,* 4541 (1952).

12. Oxygen Exchange between Nitric Acid and Water, I., C.A. Bunton, E. A. Halevi and D.R. Llewellyn, *J.Chem. Soc.,* 4913 (1952).

13. Isotope Effects in the Oxidation of Oxalic Acid, C.A. Bunton and D.R. Llewellyn, *Research, 5,* 443 (1952).

14. Exchange d'Oxygene entre l'Acid Nitrique et l'Eau, C.A. Bunton, *Mem. Serv. Chim. de l'Etat, 38,* 417 (1953).

15. The Hydrolysis of Methyl Glucosides, C.A. Bunton, T.A. Lewis, D.R.Llewellyn, H. Tristram and C.A. Vernon, *Nature, 174,* 560 (1954)

16. Tracer Studies in Ester Hydrolysis. I. Triphenylmethyl Acetate, C.A. Bunton and A. Konasiewicz, *J. Chem. Soc.,* 1354 (1954).

17. The Mechanism of the Hydrolysis of Cyclic Sulphites, C.A. Bunton, P.B. D. de la Mare, D.R. Llewellyn, R.B. Pearson and J.G. Pritchard, *Chem. and Ind.,* 981 (1956).

18. Kinetic Form of the Benzidine and Semidine Rearrangements, C.A. Bunton, C.K. Ingold and M.M. Mhala, *J. Chem. Soc.,* 1906 (1957).

19. Hydrolysis of Organic Phosphates by Prostatic Acid Phosphatase, C.A. Bunton, B. Silver and C.A. Vernon, *Proc. Chem. Soc.,* 348 (1957).

20. Über die Oxydation von Ascorbinsaure durch saltpetrige Saure. III. Kinetik im Gebiet der Taylor'schen Gleichung, H. Dahn, L. Loewe and C.A. Bunton, *Helv. Chim. Acta, 43,* 303 (1960).

21. "Nucleophilic Substitution at a Saturated Carbon", C.A. Bunton, Elsevier Publ., Amsterdam, 1963.

22. The Hydrolysis of Carboxylic Anhydrides. IV. Succinic and Tetramethyl Succinic Anhydrides in Acid, C.A. Bunton, J.H. Fendler N.A. Fuller, S.G. Perry and J. Rocek, *J. Chem. Soc.,* 5631 (1963).

23. The Acid Catalyzed Hydrolysis of Pyrophosphoric Acid, C.A. Bunton and H. Chaimovich, *J, Inorg. Chem., 4,* 1763 (1965).

24. The Hydrolysis of Bis(2,4-dinitrophenyl) Phosphate, C.A. Bunton and S.J. Farber, *J.Org. Chem., 34,*767 (1969).

25. The Mechanism and Micellar Catalysis of the Acid Cleavage of α-Phenylvinyl Diethyl Phosphate, C.A. Bunton and L. Robinson, *J. Amer. Chem. Soc., 91,* 6072 (1969).

26. Stereospecific Micellar Catalyzed Ester Hydrolysis, C.A. Bunton, L. Robinson and M.F. Stam, *Tetrahedron Letters,Nº2,*121 (1971).

27. Micellar Catalyzed Decarboxylation of 6-Nitrobenzioxazole-3-Carboxylate Ion, C.A. Bunton and M.J. Minch, *Tetrahedron Letters, Nº 44,* 3881 (1970).

28. Catalysis of Nucleophilic Substitution by Micelles of Dicationic Detergents, C.A. Bunton, L. Robinson, J. Shaak and M.F. Stam, *J. Org. Chem., 36,* 2346 (1971).

29. Hydrolysis of Di-and Trisubstituted Phosphate Esters by Nucleophilic Surfactants, C.A. Bunton and L.G. Ionescu, *J. Amer. Chem. Soc., 95,* 2912 (1973).

30. Micellar Effects on the Acid-Catalyzed Decomposition of Monoalkyl Xanthates, C.A. Bunton, J.E. Salame and L. Sepulveda, *J. Org. Chem., 39,* 3128 (1974).

31. Effects on Changes in Surfactant Structure on Micellar Catalyzed Spontaneous Decarboxylation and Phosphate Hydrolysis, C. A. Bunton, A.A. Kamego, M.J. Minch and J. L. Wright, *J. Org. Chem., 40,* 1321 (1975).

32. Micellar Effects upon the Reaction of 2,4-Dinitrophenyl Phosphate and Ethyl p-Nitrophenyl Phosphate with Amines, C.A. Bunton, S. Diaz, J.M. Hellyer, Y. Ihara and L.G. Ionescu, *J. Org. Chem., 40,* 2313 (1975).

33. Micellar Catalysis and Inhibition, C.A. Bunton, *Pure & Appl. Chem., 49,* 969 (1977).

34. Micellar Catalysis and Inhibition, C.A. Bunton, *La Chimica e L'Industria, 60(1),* 20 (1978).

35. Tests of the Pseudophase Model of Micellar Catalysis. Its Partial Failure, C.A. Bunton, L.S. Romsted and G. Savelli, *J. Amer. Che. Soc., 101,* 1253 (1979).

36. Reactions in Micelles of Cetyltrimethylammonium Hydroxide. Test of the Pseudophase Model for Kinetics, C.A. Bunton, L.H. Gan, J.R. Moffatt and L.S. Romsted and G. Savelli, *J. Phys. Chem., 85,* 4118 (1981).

37. Critical-Like Behavior Observed for a Five-Component Microemulsion, R. Dorshow, F. de Buzzaccarini, C.A. Bunton and D.F. Nicoli, *Physical Review Letters, 47(18),* 1336 (1981).

38. Quantitative Treatment of Reaction Rates in Functional Micelles and Comicelles, C.A. Bunton, F.H. Hamed and L-S. Romsted, *J. Phys. Chem., 86,* 2103 (1982).

39. Oil-in-Water Microemulsions as Reaction Media, C. A. Bunton and F. de Buzzaccarini, *J.Phys. Chem., 85,* 3139 (1981).

40. A Quantitative Treatment of the Deprotonation Equilibria of Benzimidazole in Basic Solutions of Cetyltrimethylammonium Ion (CTAX) Surfactants, C.A. Bunton, Y.S. Hong and L.S. Romsted, in "*Solution Behavior of Surfactants*", K.L. Mittal and E.J. Fendler, Editors, Plenum Publishing Corporation, New York, USA, Vol.2, 1982, pp.1137.

41. Nucleophilic Aromatic Substitution in Microemulsions, V. Athanassakis, C.A. Bunton and F. de Buzzaccarini, *J. Phys. Chem.*, *86*, 5002 (1982).

42. Dephosphorylation in Cationic Micelles and Microemulsions, Effects of Added Alcohols, C.A. Bunton, F. de Buzzaccarini and F.H. Hamed, *J. Org. Chem.*, *48*, 2457 (1983).

43. Quantitative Treatment of Micellar Effects upon the Nucleophilicity of Halides Ions, H. Al-Lohedan, C.A. Bunton and J. R. Moffatt, *J. Phys. Chem.*, *87*, 332 (1983).

44. Comparative Study of Intermicellar Interactions Using Dynamic Light Scattering, R.B. Dorshow, C. A. Bunton and D. F. Nicoli, *J. Phys. Chem.*, *87*, 1409 (1983).

45. Micellar Reactions of Hydrophilic Ions: A Coulombic Model, C.A. Bunton and J. R. Moffatt, *J. Phys. Chem.*, *89*, 4166 (1985).

46. Ionic Competition in Micellar Reactions: A Quantitative Treatment, C. A. Bunton and J. R. Moffatt, *J.Phys.Chem.*, *90*, 538 (1986).

47. In Memoriam - Professor Luis Sepulveda Araneda (1940-1989), C. A. Bunton, *Bol. Soc. Chil. Quim.*, *35*, 1 (1990).

48. Do Aqueous Micelles Catalyze Bimolecular Ionic Reactions?, C. A. Bunton, G. Cerichelli and G. Savelli, *Bol. Soc. Chil. Quim.*, *35*, 121 (1990).

49. Effects of 1-Butanol Modified Micelles on S_N2 Reactions in Mixed-Ion Systems, C.R.A. Bertoncini, M.F.S. Neves, F. Nome and C.A. Bunton, *Langmuir*, *9*, 1274 (1993).

50. Effect of 1-Butanol on Micellization of Sodium Dodecyl Sulfate and on Fluorescence Quenching by Bromide Ion, D. A. R. Rubio, D. Zanette, F. Nome and C. A. Bunton, *Langmuir*, *10*, 1151 (1994).

The SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY (ISSN: 2674-6891; 0104-5431) is an open-access journal since 1993. Journal DOI: 10.48141/SBJCHEM.
http://www.sbjchem.com.
This text was introduced in this file in 2021 for compliance reasons.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

EQUILIBRIUM AND HYDROLYSIS STUDIES OF PHOSPHATE ESTERS MODEL MOLECULES AND DNA CATALYZED BY OBISDIEN-Zn(II) COMPLEXES.

Marcia M. Meier[1], Patrícia Karloh[1], Hernán Terenzi[2], Bruno Szpoganicz[1*],
[1]Departamento de Química
[2]Departamento de Bioquímica,

Universidade Federal de Santa Catarina

88040-900 Florianópolis-SC
bruno@qmc.ufsc.br

*Corresponding author. Tel.: +55-48-331-9219; fax: +55-48-3319711

ABSTRACT

Dinuclear Zn(II)-OBISDIEN complexes catalyze hydrolysis of DNA and a phosphate ester model molecule: bis(2,4-dinitrophenyl)phosphate (BDNPP). The increase in the rate of hydrolysis of BDNPP at p[H] values above 8.0 is attributed to the presence of a ternary hydroxide species, in which intramolecular catalysis is favored by the proximity of the hydroxide group coordinated to the bimetallic center of the receptor complexes, in agreement with equilibrium results of OBISDIEN-Zn(II)-ATP (1:2:1 molar ratio) system. In the treatment of pBR322 circular DNA with OBISDIEN-Zn(II) complexes, the results suggest that these complexes cleave DNA in a random and nonspecific manner, since no distinguishable low molecular weight bands were observed after treatment.

RESUMO

Complexos dinucleares Zn(II)-OBISDIEM catalisam a hidrólise do DNA e de uma molécula modelo: fosfato de bis(2,4-dinitrofenil) (BDNPP). O aumento da velocidade de hidrólise do BDNPP em valores de p[H] maiores do que 8,0 pode ser atribuído a presença de uma espécie ternária hidróxida, na qual catálise intramolecular é favorecida pela proximidade do grupo hidróxido coordenado ao centro bimetálico do complexo receptor, em acordo com os resultados de equilíbrio do sistema OBISDIEN-Zn(II)-ATP (razão molar 1:2:1). No tratamento do DNA circular pBR322 com os complexos OBISDEIN-Zn(II), os resultados sugerem que estes complexos hidrolisam o DNA de uma maneira aleatória e não específica, pois não foram observadas bandas de pesos moleculares baixos após o tratamento.

KEYWORDS: hydrolysis, DNA, bis(2,4-dinitrophenyl)phosphate, ATP

DOI: 10.48141/SBJCHEM.v7.n8.1999.14_1999.pdf

INTRODUCTION

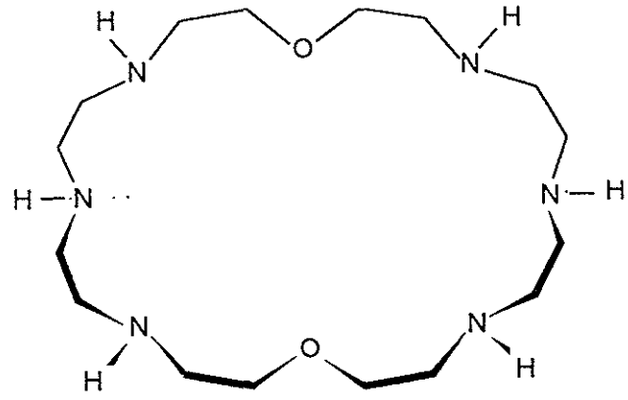
Metal ions are essential in a large variety of biological processes, including those with nucleic acids and their derivatives[1]. For example, both Mg^{2+} and Zn^{2+} are directly involved in the 3′- to 5′- exonuclease activity of the Klenow fragment of DNA polymerase I from *Escherichia Coli* [2,3]. DNA polymerase contains tightly bound Zn^{2+}, and there is evidence that this metal ion binds the enzyme to DNA[4,5].

DNA strand scission reactions are of considerable interest, both in understanding the ubiquitous phosphate ester hydrolysis reactions carried out in nature and in designing new artificial restriction enzymes[6]. Redox properties of a variety of metals have been exploited as DNA cleaving agents[7,8]. The full therapeutic potential of these compounds cannot be realized because these oxidative cleavage agents require activation by either light or an oxidant and tend to produce diffusible free radicals and products that are not amenable to further enzymatic manipulation. Hydrolytic cleavage agents do not require coreactants and therefore could be more useful in drug design[9].

Recently, Cu(II)[9-11], Zn(II), Ni(II)[11,12], Cd(II)[11] and Au(III)[13] complexes shown to hydrolyze DNA, and polyamines also possess potential as catalytic agents, in view of the propensity of polyammonium species for complexing phosphates and nucleotides[14,15]. Large ring macrocyclic exhibits a range of catalytic ability even in the absence of metal ion[16-18].

In order to identify possible mechanisms by which metal ions promote phosphodiester hydrolysis, model compounds, like bis(2,4-dinitrophenyl) phosphate and *p*-nitrophenyl phosphate, have been used to study the reactivity with metal complexes[19-22].

The 24-membered macrocycle 1, 4, 7, 13, 16, 19-hexaaza-10,22-dioxacyclotetracosane (OBISDIEN) has been discovered to be an exceptional mimic for enzymes that hydrolyze adenosine triphosphate (ATP), i.e., the ATPases[16,23]. The protonated forms of the macrocyclic OBISDIEN catalyze ATP hydrolysis through a nucleophilic pathway wich involves a phosphorylated intermediate[24,25].



1. OBISDIEN

We have previously demonstrated that OBISDIEN-Cu(II) complex interacts with dipeptides[26] and that OBISDIEN-Zn (II) complexes hydrolyze glycylglycine[27]. In this paper we report the discovery that binuclear OBISDIEN-Zn(II) complexes also cleaves DNA and bis(2,4-dinitrophenyl)phosphate. The formation constant of Zn(II)-OBISDIEN complexes with ATP were investigated and the results were used to interpret the cleavage ability of these complexes towards DNA.

EXPERIMENTAL

The OBISDIEN. 6 HBr in the form of colorless hexahydrobromide was synthesized by the method described in literature[28,29]. The adenosine 5′-triphosphate disodium salt hydrate and pBR322 plasmid DNA from *Escherichia Coli* were obtained from Aldrich Chem. Co. and used without further purification. The stock solution of $ZnCl_2$ was standardized by titration with EDTA (ethylenediaminetetraacetic acid)[30]. Carbonate-free solution of 0.100 M KOH were prepared from Dilut-it (Baker) ampoules and were standardized by titration with potassium acid phthalate. Potassium chloride, the supporting electrolyte, was obtained as reagent grade quality.

Potentiometric equilibium measurements

Potentiometric studies of OBISDIEN, in the absence and presence of Zn (II), and ATP were carried out with a Micronal-B 375 research pH meter fitted with blue-glass and Ag-AgCl reference electrodes. The potentiometric apparatus was calibrated with standard HCl and KOH solutions to read $-\log[H^+]$ directly and pK_w for water at $\mu = 0.100$ M was 13.78 [31]. The temperature was maintained at 25.00 °C and the experimental solutions, adjusted to 0.100 M in ionic strength by addition of KCl, were titrated with 0.100 M CO_2-free KOH solution. Equilibrium measurements were made on solutions containing 2:1 molar ratio of metal ion to OBISDIEN, 1:1 molar ratio of metal ion to ATP, and the ternary system containing OBISDIEN, Zn (II) and ATP were studied at molar ratio of 1:2:1 with 0.050mmol of OBISDIEN, 0.100 mmol of Zn (II) and 0.050 mmol of ATP. Potentiometric studies were carried out on 50.00 mL of experimental solution in a thermostated cell, purged with argon cleaned by an alkaline solution of KOH. Each system was titrated at least three times. The range of accurate p[H] measurements was considered to be 2.7 – 11. All stability constants were determined using procedures outlined in detail in the literature[31].

Computations

Computations were all carried out with the BEST7 program and species diagrams were obtained with SPE and SPE-PLOT programs[31].

Cleavage of bis(2,4-dinitrophenyl) phosphate

The instrument utilized for absorption measurements was a diode array spectrophotometer (Hewlett-Packard), model 8452A, equipped with a thermostated compartment at constant temperature (50°C). This instrument was attached to a microcomputer HP Wectra 386/33N and a HP Deskjet 500 printer.

Samples containing 1*m*M of OBISDIEN and 1.9 *m*M of Zn(II) with $\mu = 0.100$ M (KCl) have the p[H] adjusted by small increments of 1.0 M KOH or 1.0 M HCl. After that, solution of bis(2,4-dinitrophenyl) phosphate (BDNPP) was added to the experimental solution containing OBISDIEN-Zn. The final concentration of BDNPP was 7.0×10^{-5}M. The absorbance measurements as a function of time were read after addition of BDNPP to the experimental solutions. The hydrolysis was monitored by following the visible absorbance change at 400 nm due to the release of 2,4-dinitrophenolate anion.

Cleavage of DNA and Electrophoresis analysis

DNA degradation assay: Two different sources of DNA, a circular plasmid DNA (pBR322, Sigma D 9893), or a linear double stranded genomic DNA purified from mussels, was treated at a concentration of 10ng/μl in a final volume of 400 μl and pH 8.0 at 25°C or 40°C. The OBISDIEN-Zn(II) concentration varied from 0 to 25 μM, as indicated on figure legends. The reaction was stopped with loading buffer 2X (20mM Tris-Cl pH 8.0, 10mM borate, 2mM EDTA and 20% glycerol). An aliquot of the sample (10 μL) was applied to a 0.8% agarose gel, and an electric field (5V/cm) applied for 1-2h. The DNA was visualized by ethidium bromide staining or silver staining[32].

RESULTS AND DISCUSSION

Equilibrium measurements in the OBISDIEN-Zn(II)-ATP system

The protonation and Zn (II) binding constants of OBISDIEN and its interactions with bromide ions were reported earlier[33-35]. The protonation constant of ATP and its binding constants with zinc(II) ion were redetermined under the present experimental conditions from titration data. Figure 1 shows the titration curves OBISDIEN, OBISDIEN-Zn(II) 1:2 molar ratio, OBISDIEN-ATP 1:1 molar ratio and OBISDIEN-ATP-Zn(II) 1:1:2 molar ratio. The equilibrium constants determined are reported in Table 1 and 2, which includes comparison with the values reported in the literature[24,34].

The equilibrium constants found for the formation of ternary systems are defined by Eqs. (1) – (5), where LZn_2 is the binuclear OBISDIEN-Zn(II) complex, the receptor complex, and A^{4-} is the basic deprotonated form of ATP.

		K	
$H_nL + A^{4-} \rightleftharpoons H_nLA^{n-4}$		$\frac{[H_nLA^{n-4}]}{[H_nL][A^{4-}]}$	(1)
$H_6L + HA^{3-} \rightleftharpoons H_7LA^{3+}$		$\frac{[H_7LA^{3+}]}{[H_6L][HA^{3-}]}$	(2)
$LZn_2^{4+} + A^{4-} \rightleftharpoons LZn_2A$		$\frac{[LZn_2A]}{[LZn_2^{4+}][A^{4-}]}$	(3)
$LZn_2A + nH^+ \rightleftharpoons H_nZn_2A^{+n}$		$\frac{[H_nZn_2A^{+n}]}{[LZn_2A][H^+]^n}$	(4)
$LZn_2A \rightleftharpoons LZn_2(OH)A^- + H^+$		$\frac{[LZn_2(OH)A^-][H^+]}{[LZn_2A]}$	(5)

Equilibrium studies of the ternary system show that the binuclear OBISDIEN-Zn(II) complexes hold on ATP molecule coordinated in their cavities at p[H] values above 5.5.

Table 1. Log values of protonation constants of ATP, their binding constants with Zn(II) and the stability constants for the complexes ATP-OBISDIEN at 25.0 °C (μ = 0.100 (KCl))

Equilibrium quotient	log K	
	This work	Others [24,34]
$[HA^{3-}]/[A^{4-}][H^{+}]$	6.76 (0.01)	6.50
$[H_2A^{2-}]/[AH^{3-}][H^{+}]$	4.05 (0.04)	4.00
$[AZn^{2-}]/[A^{4-}][Zn^{2+}]$	4.34 (0.13)	4.87
$[HAZn^{-}]/[AZn^{2-}][H^{+}]$	4.76 (0.12)	4.57
$[AZn(OH)^{3-}][H^{+}]/[AZn^{2-}]$	-8.36 (0.62)	-8.76
$[H_4LA]/[H_4L][A^{4-}]$	5.75 (0.02)	4.80
$[H_5LA]/[H_5L][A^{4-}]$	7.83 (0.12)	8.15
$[H_6LA]/[H_6L][A^{4-}]$	10.14 (0.11)	11.0
$[H_6LHA]/[H_6L][HA^{3-}]$	7.01 (0.06)	7.85

Table 2. Log values of protonation and stability constants for the ternary species formed by Zn(II) complexes of OBISIDIEN with ATP at 25.0 °C (μ = 0.100 (KCl))

Equilibrium quotient	log K
$[LZn_2A]/[LZn_2^{4+}][A^{4-}]$	5.97 (0.18)
$[HLZn_2A^{+}]/[LZn_2A][H^{+}]$	6.98 (0.09)
$[H_2LZn_2A^{2+}]/[HLZn_2A^{+}][H^{+}]$	6.47 (0.22)
$[LZn_2A(OH)^{-}]\ [H^{+}]/[LZn_2A]$	-9.50 (0.12)

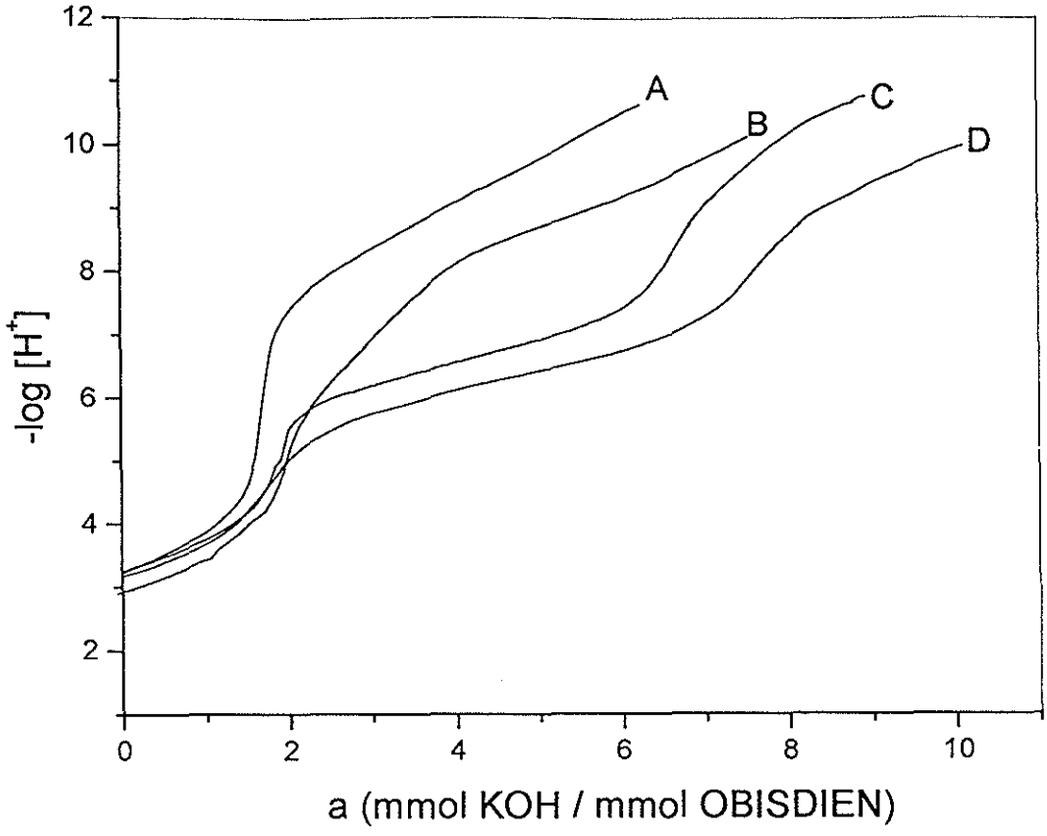


Figure 1. Potentiometric p[H] profiles for solutions containing A) 0.05 mmoles OBISDIEN; B) 0.05 mmoles OBISDIEN and ATP; C) 0.05 mmoles OBISDIEN and 0.1 mmol Zn(II); D) 0.05 mmoles OBISDIEN, ATP and 0.1 mmol Zn(II) at 25.0 °C and μ = 0.100 M (KCl).

This is clearly seen by analysis of the species distribution diagram shown in Figure 2. The diprotonated species, H_2LZn_2A, is 27 % formed at p[H] = 6.3. Above this p[H] it decreases giving place to the monoprotonated species, $HLZn_2A$. This species is 31 % formed at p[H] 6.9 and decreases at higher p[H] values. The normal species, LZn_2A, is 77 % formed at p[H] 8.2. This species has ATP coordinated to the bimetallic center. The hydroxide species appears at p[H] values above 8 and it is 38% formed at p[H] 9.8.

The capacity of OBISDIEN in acting as a receptor of anionic molecules is fundamental on its ability to interage with ATP. The presence of zinc (II) ions in its cavity facilitates the coordination of ATP, favoring the formation of ternary species at neutral and alkaline p[H].

Lehn, et al,[24] and Mertes, et al,[23] relate that OBISDIEN can hydrolyze the phosphoric ester bond of ATP molecule. In p[H] 7.6 and 70°C the $k_{obs} = 25 \times 10^{-3}$ min^{-1}. However, the OBISDIEN-Zn(II) at 1:1 molar ratio diminishes the hydrolysis rate of ATP comparing with OBISDIEN without metal ion ($k_{obs} = 3.2 \times 10^{-3}$ min^{-1})[23]. Potentiometric studies and ^{13}C NMR indicated strong interactions between the metal and macrocycle, the retardation in rates for these ternary macrocycle-ATP-Zn(II) system is probably the result

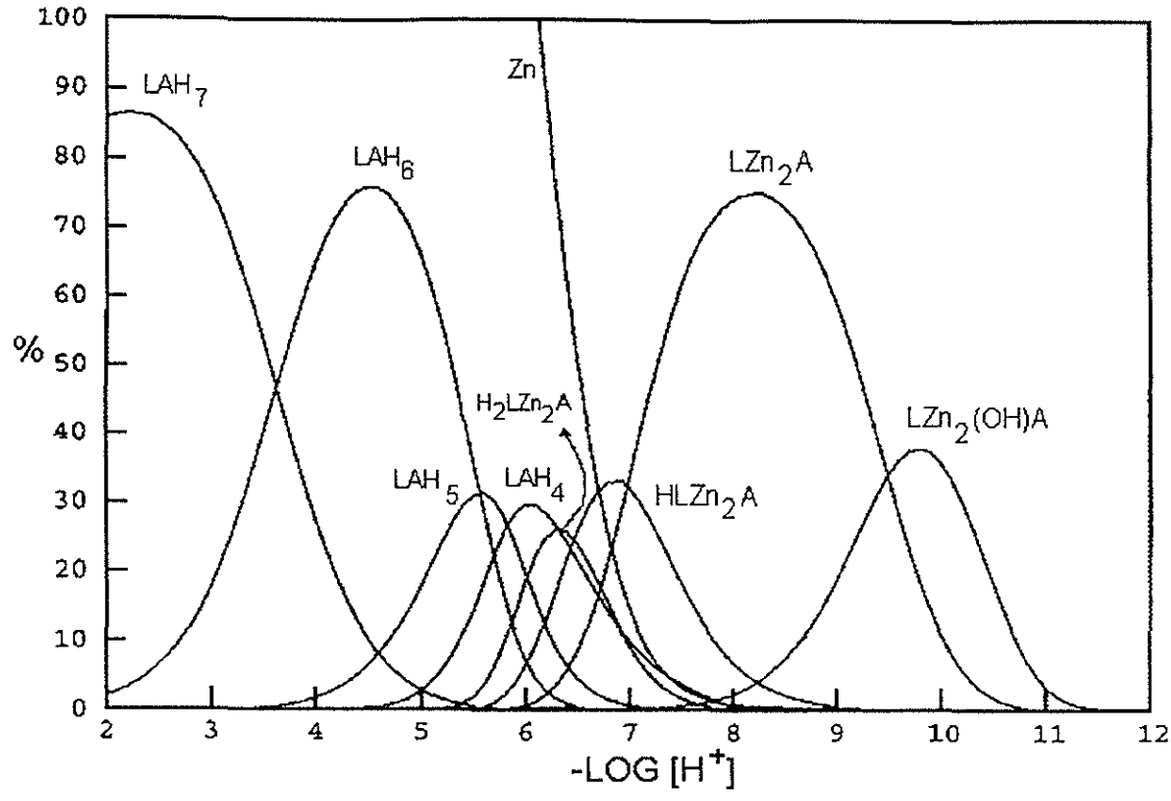


Figure 2. Species distribution curves of the 1:1:2 OBISDIEN-ATP-Zn(II) system in aqueous solution, at 25.o °C and μ = 0.1 M (KCl).

of competitive inhibition. Kinetic studies were done in the 1:1.5:1.5 molar ratio of ATP:OBISDIEN:Zn(II), respectively. However, our results show ATP coordinated to the binuclear OBISDIEN-Zn(II) complexes. The two Zn(II) ions in the cavity of OBISDIEN are in favorable arrangement interacting with the substrate as in a binuclear arrangement, predominating species 1:1 molar rate of OBISDIEN:Zn(II). On the other hand, OBISDIEN-Zn(II) system, studied by Mertes, et al, does not have a complex in an appropriate association with ATP. The binuclear arrangement leaves coordination sites in the metal center occupied by water molecules that can be substituted by an ATP molecule during its association.

The results of equilibrium studies of OBISDIEN-ATP-Zn(II) system show the predomination of two species near physiological p[H] values (Figure 2), LZn_2A and $HLZn_2A$. These results prompted us verify the DNA cleavage by binuclear OBISDIEN-Zn(II) complexes.

Cleavage of bis(2,4-dinitrophenyl) phosphate (BDNPP)

The rate of binuclear OBISDIEN-Zn(II) complex promoted cleavage of BDNPP was monitored by following the increase in the visible absorbance at 400 nm due to the release of 2,4-dinitrophenolate (see UV-vis spectra in Figure 3) at different pH's. Figure 4

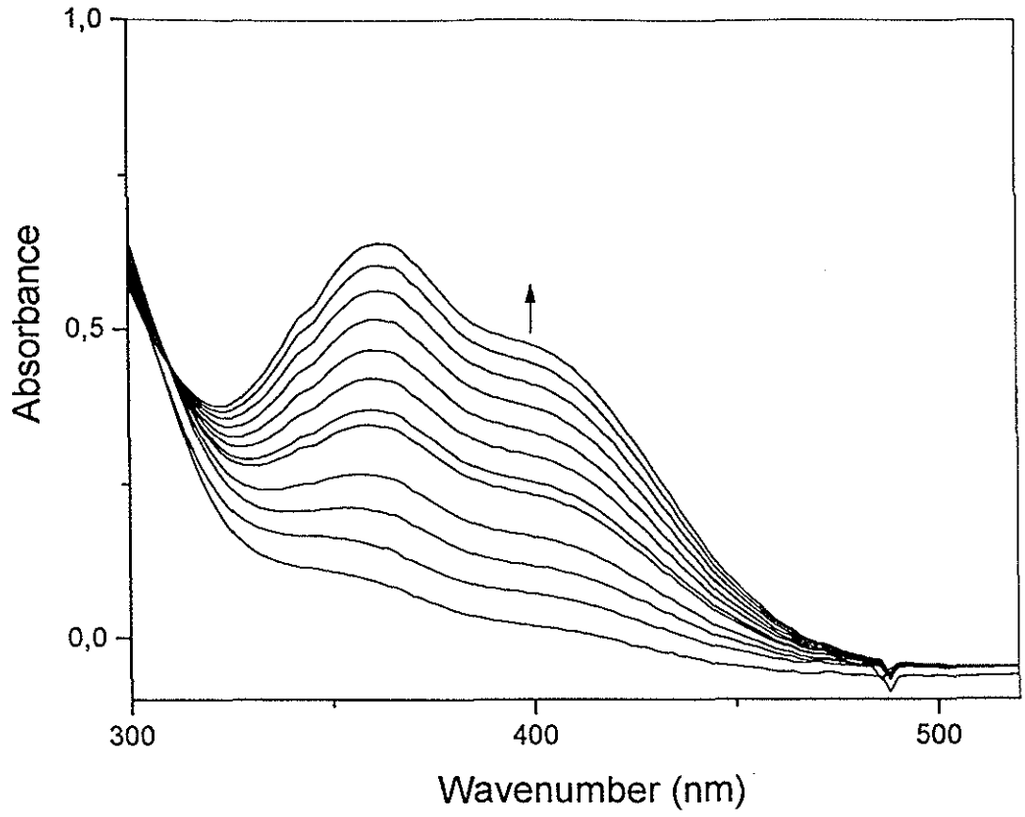


Figure 3. UV-Vis spectra of a solution containing 1 x 10^{-3} M of OBISDIEN, 1.9 x 10^{-3} M of Zn(II) and 7 x 10^{-5} M of BDNPP at pH 8.5, μ = 0.1 M (KCl) and 50 °C. Spectra measured during 7 h.

shows the pseudo-first order plot[36] for the hydrolysis reaction in pH 8.5 with a rate of 2.42 x10^{-5} s^{-1} and a correlation coefficient R = 0.9998. The observed rate constants determined in this way are summarized in Table 3.

It is known from the species distribution curves of OBISDIEN-Zn_2-ATP system that as the p[H] is increased above 8, the hydroxide species appears. The normal complex is maximum near p[H] 8 and below p[H] 7 the protonated species are the major ones. The increase of rate from p[H] 6.5 to 8.2 is mostly due to increase of binuclear OBISDIEN-Zn(II) complexes rather than many differences in the catalytic actives of protonated and normal ternary complex. However, at higher p[H] values the increase in the rate can be attributed to the presence of a ternary hydroxide species. In this species intramolecular catalysis is favored by the proximity of the hydroxide group coordinated to the bimetallic center. This interpretation is in agreement with potentiometric results of OBISDIEN-Zn(II)-ATP (1:2:1 molar ratio) system.

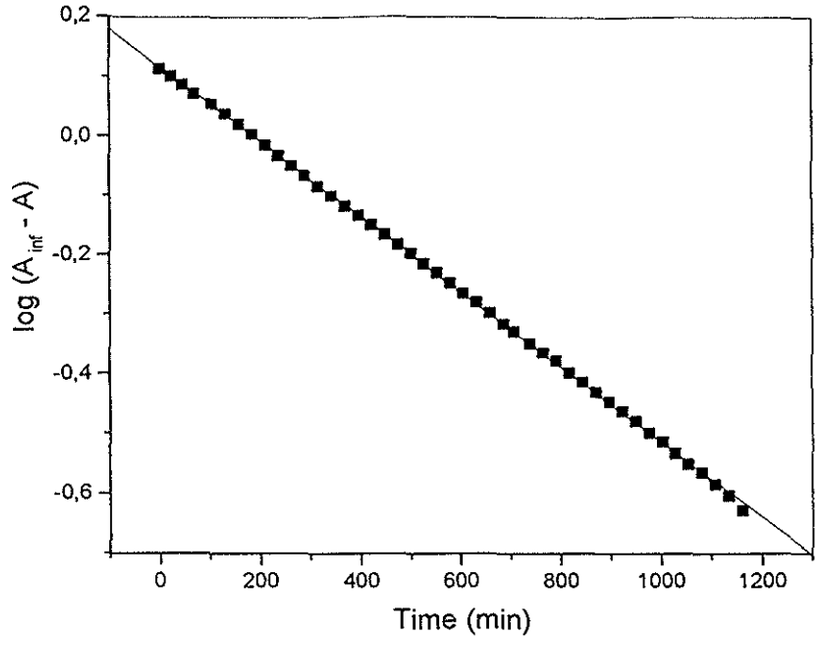


Figure 4. Pseudo-First-order plot for the hydrolysis of bis(2,4-dinitrophenyl)phosphate by OBISDIEN-Zn. The solution containing 1 x 10^{-3} M of OBISDIEN, 1.9 x 10^{-3} M of Zn(II) and 7 x 10^{-5} M of BDNPP at pH 8.5, μ = 0.1 M (KCl) and 50°C. A_{inf} represent the absorbance in infinite time and A the absorbance in each measurement.

Table 3: Observed rate constants for the pseudo-first order reaction of the binuclear OBISDIEN-Zn(II) (1:2 molar ratio) complex with BDNPP, at 50.0°C, μ = 0.100 M (KCl).

p[H]	k_{obs} (10^5 s^{-1})
6.5	0.80
7.4	1.30
8.2	1.80
8.5	2.42
9.5	5.22

DNA cleavage analysis

A typical assay to demonstrate DNA cleavage is the transformation of supercoiled circular double stranded DNA on its relaxed form by a single strand nick[37,38].

In Figure 5 the effect of treatment of pBR322 circular DNA with OBISDIEN-Zn(II) complex (25 μM) at 25°C is observed. The supercoiled DNA was transformed to a relaxed form (single strand nicked DNA) only in the presence of the OBISDIEN-Zn(II) complex in a time dependent manner. The effect is also dependent on temperature, at 40°C the DNA is completely degraded in 1h (not shown). In Figure 6 the source of DNA was a double stranded, linear genomic DNA, and the complex was also capable of degrading this DNA as evidenced from disappearance of the high molecular weight band, the reaction was strictly dependent on OBISDIEN-Zn(II) concentration. The observed smear on the gel is

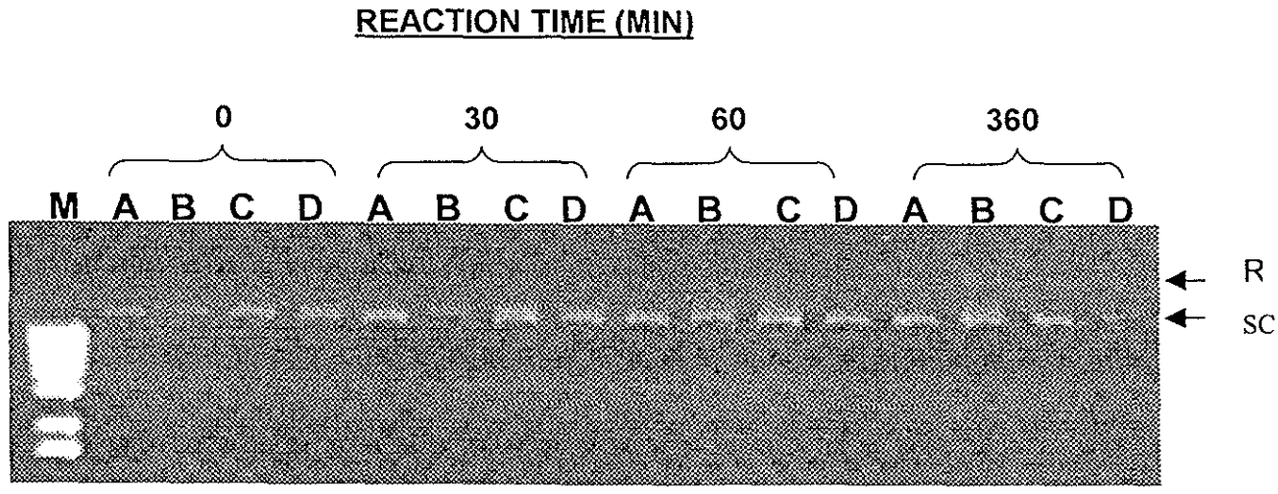


Figure 5. Plasmid DNA (pBR322) incubated at 25°C for 0, 30, 60 and 360 min as shown. R and SC at the right of the figure refer to 'relaxed' and 'supercoiled' forms of pBR322. A) DNA at pH 8.0; B) DNA-OBIS-Zn(II), C) DNA-OBIS; D) DNA-Zn(II), M) molecular weight marker (Gibco BRL 1kb ladder).

due to numerous different fragments appearing as a result of OBISDIEN-Zn(II) cleavage reaction.

As evidenced from these results, OBISDIEN-Zn(II) may be considered a "chemical nuclease" since it is able to cut DNA directly under physiological conditions by apparently all positions regardless of the nucleotide linked to the deoxyribose as for example Fe-EDTA[39], various metalloporphyrins[40] and octahedral complexes of 4,7-diphenyl-1,10-phenanthroline[41].

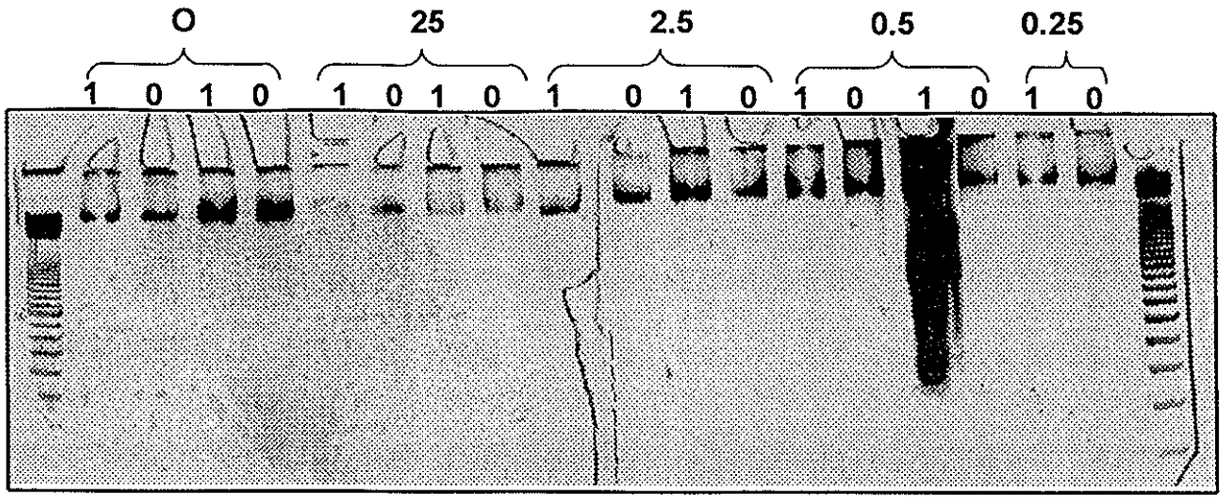


Figure 6. OBIS-Zn(II) complex treatment on genomic DNA. Various concentrations of OBISDIEN-Zn(II) (0 to 25 μM) were added to genomic DNA, at 40 °C and incubated for 1h, 0 and 1, on the top of the figure refers to incubation time (h), 0 is the control. At each side of the figure the molecular weight marker (100bp, Gibco) is observed. On lane 0.5, 1 an artifact of silver staining is observed.

The reaction mechanism of these 'chemical nucleases' distinguish them from other chemical modification reagents widely used in nucleic acid chemistry, for example, dimethyl sulfate, diethyl pyrocarbonate, osmium tetraoxide, and permanganate react preferentially with the various bases and do not cause strand scission without subsequent base treatment (e.g., piperidine as in Maxam-Gilbert sequencing)[42].

The results shown suggest that OBISDIEN-Zn(II) cleave DNA is in a random and nonspecific manner, since no distinguishable low molecular weight bands were observed after treatment, the staining method employed detected only uncut genomic DNA or the supercoiled and relaxed forms of pBR322 plasmid DNA, suggesting again the randomness of the cleavage reaction. Further studies may confirm this hypothesis and should introduce OBISDIEN-Zn(II) as a novel footprinting reagent.

REFERENCES

1. H. Sigel, K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs, *J. Am. Chem. Soc.*, 102: 247-260 (1981).
2. P. S. Freemont, J. M. Friedman, L. S. Beese, M. R. Sanderson, T. A Steitz, *Proc. Natl Acad. Sci.*, 85:8924 (1988).
3. L. S. Beese, T. A Steitz, , *EMBO J.*, 10:25 (1991).
4. G. L. Eichlhorn, *"Metal Ions in Biological Systems"*, vol. 10, pp. 1-21, (1980).
5. J. P. Slater, A. S. Mildvan, L. A. Loeb, *Biochem. Biophys. Res. Commun*, 44:37-43 (1971).
6. J. K. Barton, L. A. Basile, A. L. Raphael, *J. Am. Chem. Soc.*, 109:7550-7551 (1987).
7. L. A. Basile., J. K. Barton, *J. Am. Chem. Soc.*, 109:7548-7550 (1987).
8. W. R. Pogozelski, T. J. Mcneese, T. D. Tullius, *J. Am. Chem. Soc.*, 117:6428-6433 (1995).
9. J. N. Burstyn, E. L. Hegg, *Inorg. Chem.* 35:7474-7481 (1996).
10. K. D. Karlin, N. N. Murthy, H. H. J. Sun, S. T. Frey, *Inorg. Chimica Acta,* 242:329-338 (1996).
11. J. Rammo, H. J. Schneider, *Inorg. Chimica Acta*, 251:(1-2) 125-134 (1996).
12. W. Trogler, D. F. Harvey, C. L. Walton, L.H. Freeman, M. A. DeRosch, E. A. Kesicki, *Inorg. Chem.*, 32:5851-5867 (1993).
13. P. Calamai, A. Guerri, L. Messori, P. Orioli, G. P. Speroni, *Inorg. Chimica Acta*, 285:309-312 (1999).
14. E. Kimura, M. Kodama, T.Yatsunami, *J. Am. Chem. Soc.*, 104:3182-3187 (1982).
15. B. Dietrich, M. W. Hosseini, J-M. Lehn, R. B. Sessions, *J. Am. Chem. Soc.*, 103:1282-1283 (1981).
16. J-M. Lehn, J. W. Hosseini, *J. Chem. Soc., Chem. Commun.*, 107:1155-1157 (1985).
17. P. G. Yohannes, M. P. Mertes, K. B. Mertes, *J. Am .Chem. Soc.*,107:8288-8289 (1985).
18. A. E. Martell, Q. Lu, R. J. Motekaitis, *Inorg. Chimica Acta*, 251:365-370 (1996).
19. J. Chin, R. C. Hynes, D. Wahnon, J. M. Young, *J. Am. Chem. Soc.*, 117:9441-9447 (1995).
20. T. C. Bruice, K. A. Browne, *J. Am. Chem. Soc.*, 114:4951-4958 (1992).
21. E. Kimura, M. Shiro, M. Inoue, T. Koik, *J. Am. Chem. Soc.*, 118:3091-3099 (1996).
22. W. H. Chapman, R. Breslow, *J. Am. Chem. Soc.*, 117:5462-5469 (1995).
23. K.B. Mertes, M. P. Mertes, K. E. Plute, P. G. Yohannes, *Inorg. Chem.*, 26:1751-1755 (1987).
24. J-M. Lehn, M. P. Mertes, M. W. Hosseini, *Helvetica Chimica Acta*, 245:2454-2466 (1983).
25. J-M Lehn, M. P. Mertes, M. W. Hosseini, L. Maggiora, K.B. Mertes, *J. Am. Chem. Soc.*,109:537-544 (1987).
26. M. T. B. Luiz, B. Szpoganicz, M. Rizzoto, A. E. Martell, M. G. Basallote, *Inorg. Chimica Acta,* 254:345-351 (1997).
27. M. T. B. Luiz, B. Szpoganicz, M. Rizzoto, A. E. Martell, M. G. Basallote, *Inorg. Chimica Acta,* 287:134-141 (1999).
28. J-M. Lehn, S. H. Pine, E. Watanabe, A. K. Willard, *J. Am. Chem. Soc.*, 99:6766 (1977).
29. J. Commarmond, P. Plumere, J-M. Lehn Y. Angus, R. Louis, R. Weiss, A. Kahn, I. Morgenstern-Badarau, *J. Am. Chem. Soc.*, 104:6330 (1982).

30. G. Schwarzenbach, H. Flaschka, "*Complexometric Titration*" Methuen, London, pp. 260 (1969).
31. A. E. Martell, R. J. Motekaitis, "*Determination and Use of Stability Constants*", VCH, New York, 2nd ed. (1992).
32. Ausubel, F. *in: "Short Protocols in Molecular Biology"*, Willey, 3rd ed. (1995).
33. A. E. Martell, R. J. Motekaitis, J.P. Lecomte, J-M. Lehn, *Inorg. Chem.*, 22:609-614 (1983).
34. R. M. Smith, A. E. Martell, Y. *Chen. Pure App. Chem.*, 63:1015 (1991).
35. N. D. Rosso, B. Szpoganicz, A. E. Martell, *Inorg. Chimica Acta,* 287:193-198 (1999).
36. J.W. Moore, R. G. Pearson, "*Kinetics and Mechanism*", John Wiley & Sons, USA, pp. 70-74 (1981).
37. Ausubel, F. *in*: Short Protocols in Molecular Biology, 3rd ed. Willey; Hegg, E.L (1995).
38. J. N. Burstyn, *Inorg. Chem.* 35:7474-7481 (1996).
39. T. D. Tullius, B .A. Dombroski, *Proc. Natl. Acad. Sci.*, 83:5469-73 (1986).
40. B. Skorobogaty, J. C. Dabrowak, *Biochemistry*, 25:6875-6883 (1986).
41. J. K. Barton, *Science,* 233:727-734 (1986).
42. D. S. Sigman, *Biochemistry,* 29:9097-9105 (1990).

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

BIOLOGICAL ACTIVE ACYLHYDRAZIDE I. THE O-ACYL-DERIVATIVES NATURE OF MONOACYLATION PRODUCTS OF CYCLIC MALEIC- AND PHTHALIC-HYDRAZIDE.

I. Panea[a], Lucia Bodochi[b], Teodora Panea[b], Daniela Zinveliu[b] and Violeta Paşcalău[b]

a) "Babeş-Bolyai" University, Department of Organic Chemistry, Arany Janos 11, 3400 Cluj-Napoca, Romania

b) Research and Production Center "BIOS", Academy of Agricultural and Forest Sciences, OP 1 CP 2, 3400 Cluj-Napoca, Romania

ABSTRACT

We confirmed, on the basis of chemical and physico-chemical (melting points, IR- and ^{1}H-NMR-spectra) data, that the products isolated by monoacylation of cyclic maleic- and phthalic-hydrazides have only the O-acylderivatives nature although owing to tautomerism these hydrazides may give rise to N- or/and O-acylderivatives in such reactions. Simultaneously, we showed that the claims for the obtainment of N-acylderivatives of cyclic maleic- and phthalic-hydrazides were not valid. Also by reacting the cyclic maleic-, respectively phthalic-hydrazide, with 4-chlorobenzoylchloride two new O-monoacylderivatives [3-(4-chlorobenzoyloxy)-1-H-pyridazin-6-one and 1-(4-chlorobenzoyloxy)-3-H-phthalazin-1-one] were obtained.

RESUMO

Através de estudos químicos e físico-químicos confirmamos que os produtos isolados da monoacilação de hidrazidas maléica e ftálica cíclicas tem somente o caráter de O-acil-derivados. Devido a tautomerismo, estas hidrazidas podem resultar em derivados de N- ou O-acila. Também demonstramos que as pretensões de obtenção de N-acil derivados de hidrazidas cíclicas maléica e ftálica não são válidas. A reação de hidrazida maléica e ftálica cíclicas com cloreto de 4-clorobenzoila leva a dois novos O-monoacil derivados: 3-(4-benzoiloxi)-1-H-piridazina-6-ona e 1-(4-clorobenzoiloxi)-3-H-ftalazina-1-ona, respectivamente.

KEYWORDS : O-acylderivatives of cyclic maleic- and phthalic-hydrazides. Melting points, IR- and ^{1}H-NMR-spectra.

DOI: 10.48141/SBJCHEM.v7.n8.1999.27_1999.pdf

INTRODUCTION

Many acylated acyclic (1)[1-12], semicyclic (2)[1,5,8,9,12b,c,13-16] and cyclic (3, 4)[3,4,12b,13a,14,17-34] hydrazides of 1,4-dicarboxylic acids have been described. Most of these have various useful biological actions like: tuberculostatic[2a], herbicide[6-8], plant growth regulation[9,10,12,22,25a,26,29a,b,30,32,34], of thrombocytes antiagregation[11], cytostatic[24a,28], bactericide[27c], respectively are intermediary or component parts of some useful products, inclusively biological ones[2a,5,8,12d,17c,26,30].

The structure of these acylhydrazides is uncertain, respectively controversial[3,4,12-15,17-34]. Thus, for the products obtained by the monoacylation of acyclic hydrazides (5, R^1 = acyl) with the cyclic anhydrides of 1,4-dicarboxylic acids (6), some authors assigned an acyclic acylhydrazide (1)[2-12] structure, while others considered that under given conditions a semicyclic (2, R=H; R^1=acyl)[12c,13,14b], respectively cyclic (3, R=H; R^1=acyl; Y=H, H) (7)[3a,26] N-acylhydrazides (for acyl see the R^1 significances from Scheme 1) would result. According to other authors, the type 7 derivatives have not been isolated till now[4,12b,c] contrary to other affirmations[3,14a,24,26,28,29c,30-32].

Likewise, the nature of the obtained products by cyclic hydrazides (8) acylation with acyclic anhydrides (9) or acid chlorides (10), namely the O-[3,4,12b,17b,18-20,21-25,27,33,34] or/and N-[4,13a,17a,24,27,28,31,32] -acylderivatives nature (3, 4, 11), by the monoacylation, respectively diacylation [4,13a,17a,18-20,27,28], is uncertain[14d,22,29a] or controversial.

The possibility of N-(3) or/and O-acyl (4, 11) derivatives formation from cyclic hydrazides (8) of dicarboxylic acids is based on their tautomerism[3a,4,17b,18-20,23,25,27,35] (see Scheme 1).

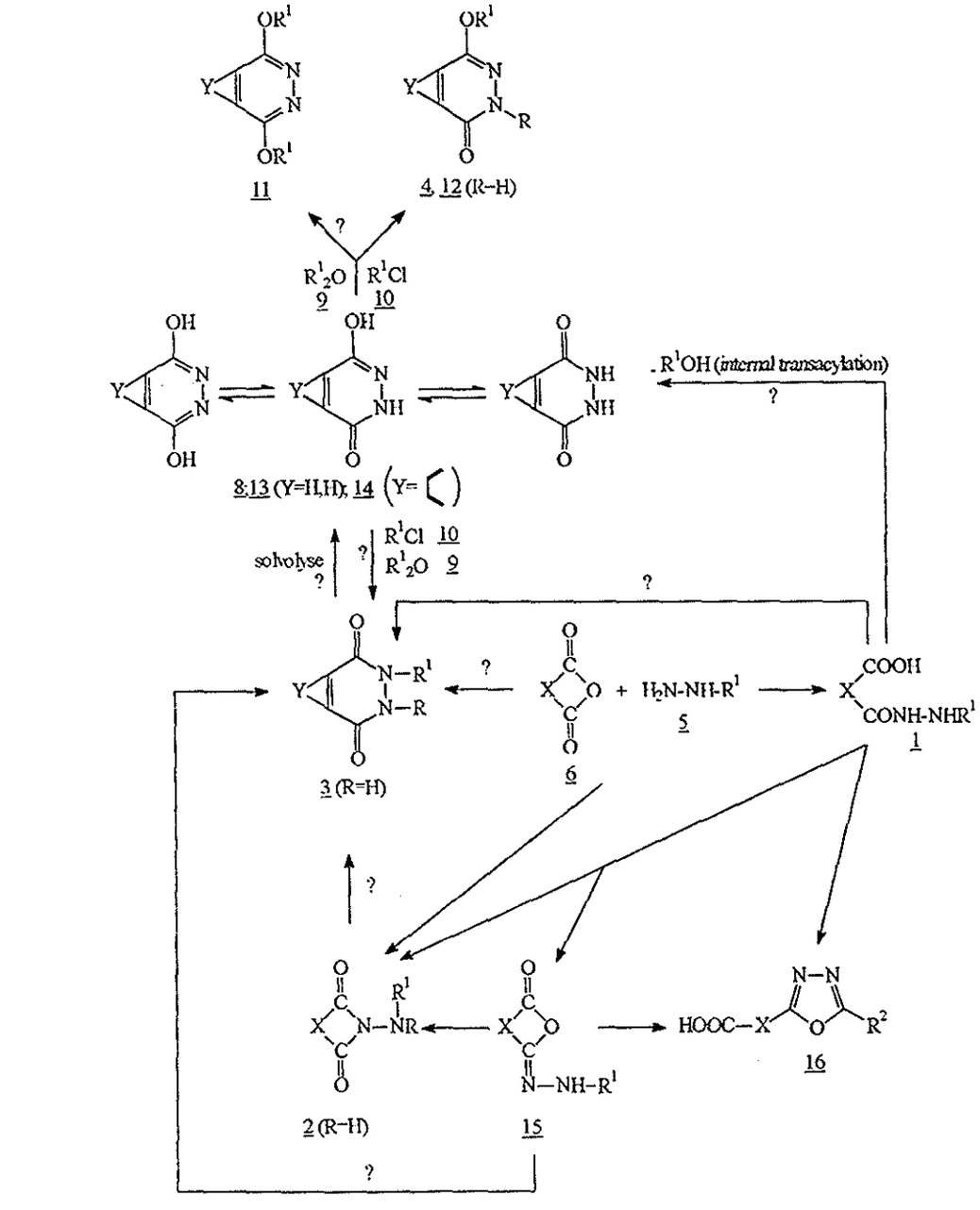
The unclear situation and controversies on acylhydrazides structure (1-4, 11), prompted us to undertake a systematic study on them.

We consider that the study is necessary in as much as it concerns substances of biological interest and a knowledge of the exact structure is an absolute requirement for establishing structure-activity relationship.

In this paper we discuss the O-acyl derivatives nature (4, R=H) (12) of the products obtained by us[34], by monoacylation of cyclic hydrazides 8 (Y=H, H; maleic; Y = ⟨ ; phthalic) and in this context we deal with all of the monoacylation products of cyclic hydrazides (8).

EXPERIMENTAL

The cyclic maleic hydrazide (13)[3b,17a,36], the cyclic phthalic hydrazide (14)[13a,17a] and the acid chlorides (10)[37] have been obtained in accordance with cited references. The cyclic anhydrides (6) were commercial products. The monoacylation products (12) were prepared by treating to reflux cyclic maleic-(13) or phthalic-(14) hydrazide (0,012 moles), suspended in toluene (30 ml), with acid chloride (0,0133 moles) (10)[34]. The mixture was refluxed in addition 2-4 hours, then cooled at room temperature, filtered off, washed with ethanol and dried (yield 83-87%). The purity was checked by thin layer chromatography. The elemental analysis data agreed with that of monoacylation products. Under similar conditions, working instead in the presence of equimolar quantities of pyridine, one new monoacylation product was obtained from each of cyclic



R=H, CH_3-CO ; X= CH_2—CH_2 ; CH=CH ; . Y=H, H; .

R^1=R^2-CO; R^2=CH_3; C_2H_5; $ClCH_2$; C_6H_5; (2-or 4-)ClC_6H_4; 4-O_2N-C_6H_4; 4-H_2N-C_6H_4; 2-HO-C_6H_4; 2-CH_3-$OCOC_6H_4$; H_5C_6-CH_2; α-naphthyl-CH_2; H_5C_6-OCH_2; 4-ClC_6H_4-OCH_2; 2,4-$Cl_2C_6H_3OCH_2$; β-naphthoxy-CH_2; 4-pyridyl; indolyl-CH_2; H_2N; CH_3-O; C_2H_5-O, etc.

Scheme 1.

hydrazide (13, 14), through their reaction with p-chlorobenzoic acid chloride (10, R^1=OC-C_6H_4-4-Cl).

6-(4-chlorobenzoyloxy)-2,3-dihydro-3-oxo-pyridazine (12, Y=H, H; R^1=4-Cl-C_6H_4-CO) formed colorless needles from ethanol. Yield : 77%, m.p. = 227-230°C

Anal. : Calcd. for $C_{11}H_7N_2O_3Cl$ (250.5); MS 250
C-52.60; H-2.8; N-11.9; Cl-14.5;
Found : C-52.80; H-3.15; N-11.94; Cl-14.4.

1-(4-Chlorobenzoyloxy)-3,4-dihydro-4-oxo-phthalazine (12, Y= ; R^1=4-Cl-C_6H_4-CO) formed colorless needles from ethanol. Yield : 65%, m.p. = 193-195°C

Anal. : Calcd. for $C_{15}H_9N_2O_3Cl$ (300.5); MS 300
C-69.3; H-4.4; N-8.09; Cl-10.3;
Found : C-69.2; H-4.5; N-8.20; Cl-10.23.

In order to determine the spectra of compounds claimed to be O-[23,25,26] (12) or N-[3,17b,24a,26,32] (3, R=H) monoacylderivatives of cyclic hydrazides (8), respectively of the monoacylderivatives of hydrazides (8) with no specified O- or N-derivatives nature[22], we prepared a series of them using the same conditions described by previous authors.

With the purpose to have spectra for comparison we synthesized, in accordance with references[2a,3a,b,4,6-8,11-13a,16b], a series of acyclic hydrazides (5)[3b], their acyclic acylation products (1) with dicarboxylic acid anhydrides (6) and also the transformation products (2, 15, 16) of initial acyclic acylderivatives (1) (see Scheme 1).

The ^{1}H-NMR spectra were recorded with a Brucker Fourier Transform NMR spectrometer of 90 MHz, respectively Varian FT-80A NMR of 80 MHz, respectively Varian Gemini 300 (300 MHz), in deuterated solvents at room temperature.

Mass spectra were registered with a Matt 311 mass spectrometer with double focussing and inverse Nier geometry.

IR spectra were registered as KBr pellets with a UR-20 spectrophotometer (C. Zeiss-Jena). The melting points are uncorrected.

RESULTS AND DISCUSSIONS

Some of physico-chemical data of the compunds under consideration and other related ones are presented in Tables 1, 2. Relative to the alkylation products of cyclic hydrazides – maleic-(13) and phthalic-(14) – the exact nature of N-, respectively O-alkyl derivative was succesfully established based on structure proof synthesis[3a,4c,20,28,35, 38].

From literature works[4c,20,28,38] it results that O-alkylderivatives of maleic-(13) and phthalic-(14) hydrazide have much lower melting points (about 150°C) than the cyclic unsubstituted hydrazides and lower (about 50°C) than the N-alkylderivatives (see Table 1). The reason is that O-alkylderivatives are ethers and the initial hydrazides (8) and their N-alkylderivatives are amides. It is well-known that ethers are a class of compounds more volatile than amides, the latter having high melting points.

The monoacylated products of maleic-(13) and phthalic-(14) hydrazides also have much lower melting points (some of them more than 150°C lower) than starting hydrazides (see Table 1). This situation is more consistent with the O-acylderivatives (12) than the N-acylderivatives (3, R=H) structure of cyclic monacylated hydrazides (compare[17b]). The option for O-acylderivatives (12) takes into consideration their relation to esters, which are more volatile compunds (see[25b]) than amides, to which the starting hydrazides and their N-acylderivatives (3, R=H, R^1= acyl) belong.

Table 1. Physical-chemical data of acylhydrazides with controversial structure and of some related compounds. References are given in square brackets.

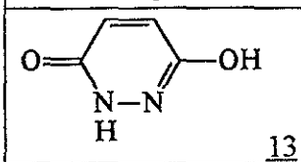
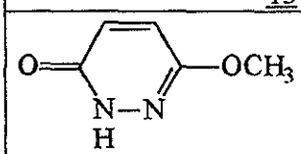
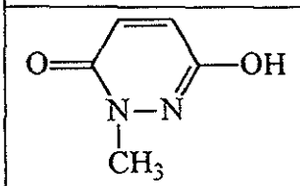
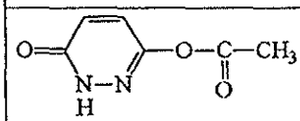
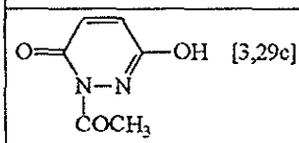
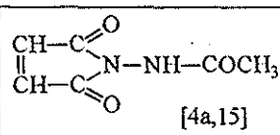
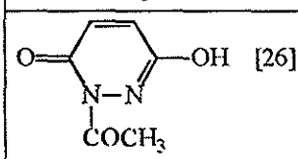
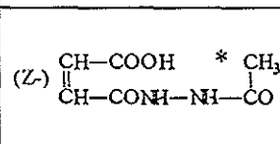
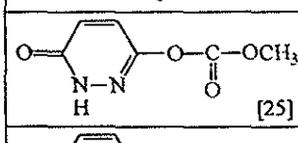
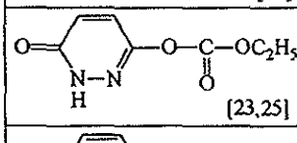
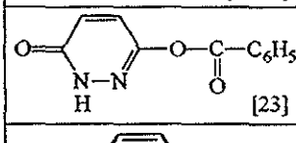
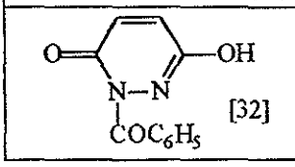
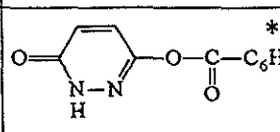
Structure according to reference	The structure confirmed for the compound by present study	Melting point [°C]	I.R. -$\nu_{C=O}$ with highest wave number [cm^{-1}]	Molecular peak from mass spectra
1	2	3	4	5
 13	as in first column [35]	300 [3a,4a]	1660 [15] 1676 [4a]	-
	as in first column [4c,38]	154 [38]	-	-
	as in first column [4c,35,38]	210 [38]	-	-
	as in first column [3a,4]	122-123 [3a,4]	1755 [4a]	-
 [3,29c]	 [4a,15]	164-166 [4a] 160-162 [3]	1725 [4a] 1715 [15]	-
 [26]		176 [26] 173-175*	1718* for COOH	-
 [25]	as in first column*	118-120* 121-122[25b] 105-107[25a]	1790*	170*
 [23,25]	as in first column*	103-105* 106-108 [23] 101-103[25b]	1765*	184*
 [23]	as in first column*	154-156* 162-164 [23]	1745*	-
 [32]		154-156*	1745*	-

Table 1 (continued)

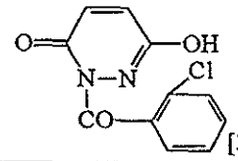
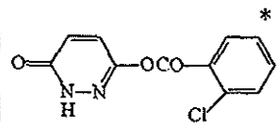
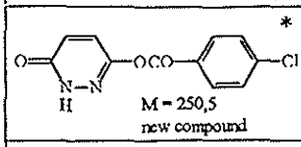
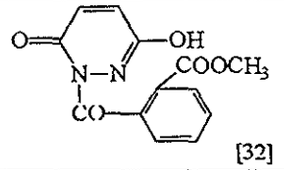
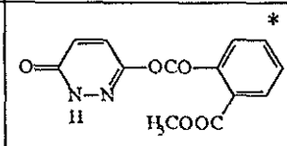
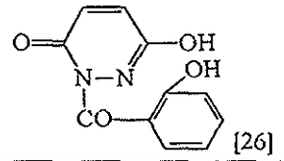
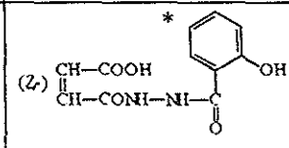
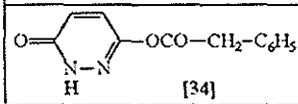
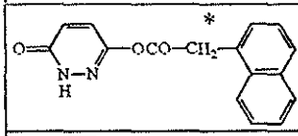
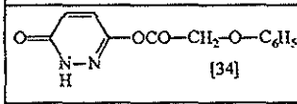
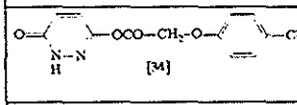
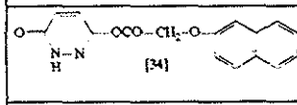
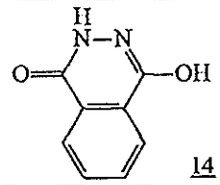
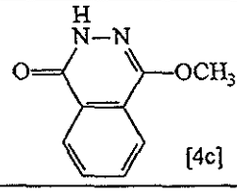
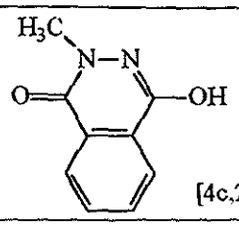
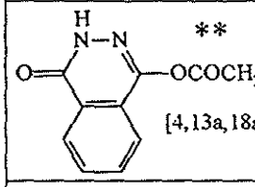
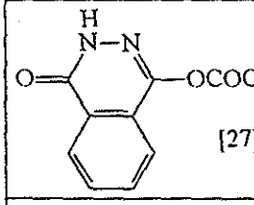
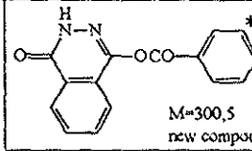
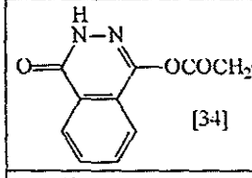
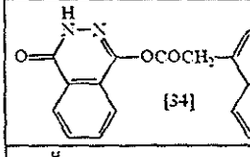
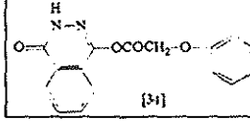
1	2	3	4	5
 [32]	 *	>210 [32]	-	-
 M = 250,5 new compound	as in first column*	226-228*	1735*	250*
 [32]	 *	144-146* 144-145 [32]	1770*	-
 [26]	 *	192 [26] 189-191*	1710* for COOH	-
 [34]	as in first column*	136-138* [34]	1767* [34]	-
 *	as in first column*	177-178*	1775*	280*
 [34]	as in first column*	143-145* [34]	1780* [34]	-
 [34]	as in first column*	146-147* [34]	1787* [34]	-
 [34]	as in first column*	124-126* [34]	1775* [34]	-
 14	as in first column [27]	344 [4] 341-344[13a]	1660 [4,27]	-
 [4c]	as in first column [4c]	188-189 [4c,20]	-	-

Table 1 (continued)

1	2	3	4	5
 [4c,20]	as in first column [4c,20a]	238-240 [4c,20a]	-	-
 ** [4,13a,18a,27]	as in first column [4c,27]	175 [27] 172-173 [13a,20a] 170 [18a]	1770 [4a] 1765 [27]	-
 [27]	as in first column [27]	227-228 [27]	1745 [27]	-
 M=300,5 new compound	as in first column*	193-195*	1765*	300*
 [34]	as in first column*	178-180 [34]	1760*	-
 [34]	as in first column*	199-200 [34]	1762*	330*
 [34]	as in first column*	212-213 [34]	1745*	346*

* Structure assigned (see Discussion and compare also with Table 2), respectively data obtained, by authors of this work.

** Among the cyclic phthalic hydrazide monoacylations, one in which the N-acylderivative formation is claimed, but only beside O-acylderivative, is the acetylation [4a, 4b, 13a]. However we point out that in a subsequent work [4c] of Le Berre and coworkers, concerning this acetylation, there is no reference to the N-acetylderivative. Other authors [27a] also deny the N-acetylated phthalic hydrazide formation on the basis of IR data.

The O-acylderivative nature of monoacylation products of cyclic hydrazides (8) is also supported by the presence of an absorbtion band between 1735-1790 cm^{-1} in their IR spectra (see Table 1). This band is characteristic for carbonyl stretching frequency ($\nu_{C=O}$) within a ester group[26,27a,39], which is present in all O-acylderivatives. The O-acylderivative nature (12) of monoacylation products is sustained by the parameters of their ^{1}H-NMR spectra too (see Table 2).

It was found in published ^{1}H-NMR data[4c,35] of maleic hydrazide alkylderivatives (13) that the chemical shift (δ) for the olefinic hydrogens signals have bigger and more differentiated values for O-alkyl than N-alkylderivatives. These chemical shift values for O-alkylderivatives are also bigger than those of maleic hydrazide. On the other hand, for N-alkylderivatives, only the chemical shift (δ) for olefinic hydrogen of distant position to N-alkylsubstitute is sometimes slightly bigger than in maleic hydrazide.

In ^{1}H-NMR spectra registered by us for maleic hydrazide monoacylderivatives (see Table 2) the signals for olefinic protons have sensible differentiated chemical shifts values (Δδ~ 0,5 ppm) and are allways bigger than those of maleic hydrazide (13). These results correlated with the debates upon maleic hydrazide alkylderivatives support the O-acylderivatives nature (12) for examined monoacylderivatives (see reference 4).

The sensible differentiation of chemical shifts for olefinic protons and their bigger chemical shift values, especially for the hydrogen atom close to O-substitute in O-derivatives towards maleic hydrazide and N-alkylderivatives is due to a deshielding steric effect. In the case of O-derivatives (see 12), the O-substitute is much nearer in space to olefinic protons than N-substitute in N-derivatives (see 3, R=H). Probably the N-substitute has no deshielding steric effect upon olefinic protons since for its manifestation the very close steric proximity is important (see reference 40a).

Also, the O-acylderivative nature of monoacylated cyclic hydrazides-8 is sustained indirectly by a series of other data. One of these arguments is that the authors who claimed[17c,24a,29c,32] the obtainment of N-acylated cyclic maleic hydrazide or suggested[14d,29a] the N-acylderivatives formation besides O-acylderivatives in the monoacylation reaction of cyclic maleic hydrazide do not give any proof for their claim or suggestion. On the contrary, many authors[3a,4,12b,17b,23,26,27,33,34] who claimed only O-acylderivatives (12) formation, in the monoacylation reaction of cyclic maleic hydrazide (13), presented more or less convincing proofs for this. In accordance with the last authors our IR and ^{1}H-NMR spectral data (see Table 1, 2) for cyclic maleic hydrazide monoacylderivatives, claimed to be N-acylderivatives[17c,29c,32] or O-acylderivatives[23,25,34], respectively with unspecified structure[22], are compatible only with O-acylderivatives structures (12). Thus, the monoacylation products of cyclic maleic hydrazide (13) with carbonic acid monochlorides-monoesters (10, R^1=OC-OCH_3; OC-OC_2H_5)[23,25], α-naphthylacetic (10, R^1= OC- α-naphthyl[22,34]), benzoic (10, R^1=CO-C_6H_5[23]) or 2-carbomethoxy-benzoic (10, R^1=CO-C_6H_4-(2)$COOCH_3$)[32] chlorides – reproduced by us under the conditions described by the authors[22,23,25,32,34] – all show an absorbtion band in the IR, specific of esters $\nu_{C=O}$ stretching vibrations, between 1745-1790 cm^{-1} as well as two ^{1}H-NMR signals, as doublets – for the olefinic hydrogens – with well differentiated chemical shifts (Δδ~0,4ppm) that are bigger than in maleic hydrazide (δ> 7ppm).

We point out that regardless the O- or N-acylderivative claimed, respectively unspecified nature, all the monoacylated cyclic maleic hydrazide compunds show similar properties: low melting points, IR band specific to ester carbonyl group and ^{1}H-NMR signals almost identical for each type of olefinic protons (see Table 1, 2).

Table 2. ^{1}H-NMR Data (Chemical shift - δ, in ppm, multiplicity*, number of hydrogens and coupling constant - J, in Hz) of acylhydrazides with controversial structure and of some related compounds.

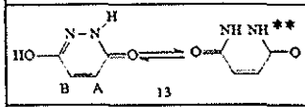
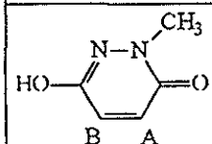
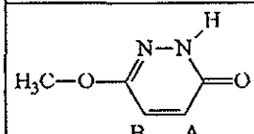
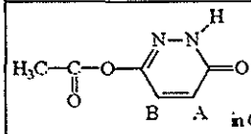
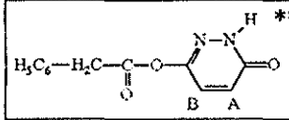
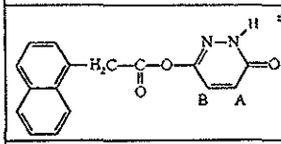
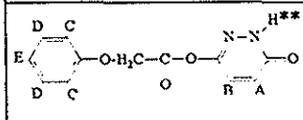
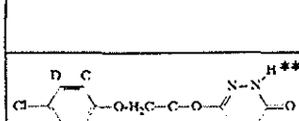
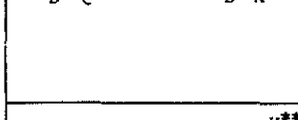
Compound	Methylic and Methylenic hydrogens	Olefinic hydrogens	Aromatic hydrogens	Hydrazidic hydrogens (NH or OH)
1	2	3	4	5
 13	-	$\delta_{HA,B}$ = 6,94 (s)	-	11,55 (b)
 [35a,4c]	δ_{CH3-N} = 3,50 (s) 3H	δ_{HA} = 6,87 (d); 1H δ_{HB} = 7,04 (d); 1H J_{AB} = 9,4	-	11,06 (b)
 [4c]	δ_{CH3-O} = 3,76 (s) 3H	δ_{HA} = 7,06 (d); 1H δ_{HB} = 7,29 (d); 1H J_{AB} = 10	-	-
 [4] in $CDCl_3$	δ_{CH3-CO} = 2,3 (s) 3H	δ_{HA} = 7,07 (d); 1H δ_{HB} = 7,22 (d); 1H J_{AB} = 10	-	-
	δ_{CH2-CO} = 3,97 (s) 2H	δ_{HA} = 6,99 (d); 1H δ_{HB} = 7,4 (d); 1H J_{AB} = 9,8	δ_{C6H5} = 7,33 (s) 5H	-
	δ_{CH2-CO} = 4,45 (s) 2H	δ_{HA} = 7,0 (d); 1H δ_{HB} = 7,4 (d); 1H J_{AB} = 9,7	δ_{C10H7} = 7,35-8,2 (m) 7H	-
	δ_{CH2-O} = 5,06 (s) 2H	δ_{HA} = 7,01 (d); 1H δ_{HB} = 7,44 (d); 1H J_{AB} = 10	$\delta_{HC,E}$ = 6,85-7,1 (m) δ_{HD} = 7,2-7,4 (m) 2H	-
	δ_{CH2-O} = 5,1 (s) 2H	δ_{HA} = 7,0 (d); 1H δ_{HB} = 7,45 (d); 1H J_{AB} = 10	δ_{HC} = 7,01 (d); 2H δ_{HD} = 7,33 (d); 2H J_{CD} = 8,5	-
	δ_{CH2-O} = 5,25 (s) 2H	δ_{HA} = 7,01 (d); 1H δ_{HB} = 7,45 (d); 1H J_{AB} = 10	δ_{OC10H7} = 7,1-7,9 (m) 7H	-

Table 2 (continued)

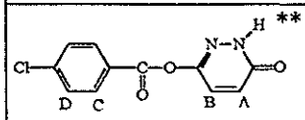
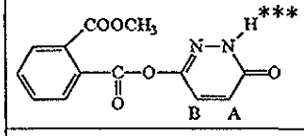
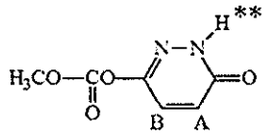
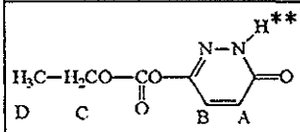
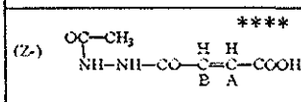
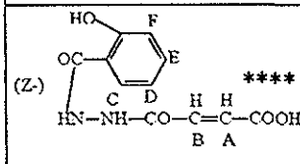
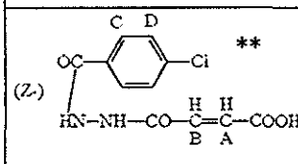
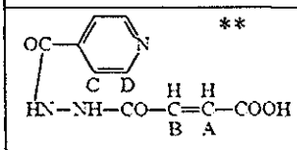
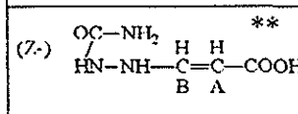
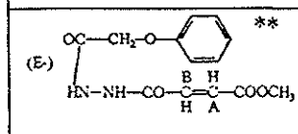
1	2	3	4	5
	-	δ_{HA} = 7,01 (d); 1H δ_{HB} = 7,53 (d); 1H J_{AB} = 10	δ_{HC} = 8,06 (d); 2H δ_{HD} = 7,63 (d); 2H J_{CD} = 8,6	-
	δ_{OCH_3} = 3,81 (s) 3H	δ_{HA} = 7,05 (d); 1H δ_{HB} = 7,47 (d); 1H J_{AB} = 10	$\delta_{C_6H_4}$ = 7,6-8,0 (m) 4H	-
	δ_{OCH_3} = 3,81 (s) 3H	δ_{HA} = 7,0 (d); 1H δ_{HB} = 7,49 (d); 1H J_{AB} = 10	-	-
	δ_{CH_3} = 1,24 (t) 3H δ_{CH_2-O} = 4,23 (q) 2H J_{CD} = 7	δ_{HA} = 7,0 (d); 1H δ_{HB} = 7,49 (d); 1H J_{AB} = 10	-	12,8 (b)
	δ_{CH_3-CO} = 1,9 (s) 3H	$\delta_{HA,B}$ =6,31 (s) 2H	-	10,1 (b)
	-	$\delta_{HA,B}$ =6,35 (s) 2H	δ_{HC} =7,85 (d);1H δ_{HD} = 6,94 (t);1H δ_{HE} = 7,4 (t); 1H δ_{HF} =6,93 (d);1H J_{orto} = 8	10,7 (b)
	-	δ_{HA} = 6,26 (d); 1H δ_{HB} = 6,41 (d); 1H J_{AB} = 11	δ_{HC} = 7,86 (d); 2H δ_{HD} = 7,51 (d); 2H J_{CD} = 8,7	10,7 (b)
	-	δ_{HA} = 6,32 (d); 1H δ_{HB} = 6,43 (d); 1H J_{AB} = 12	δ_{HC} = 7,75-7,85 (m) 2H δ_{HD} = 8,7-8,9 (m) 2H	10,92 (b)
	-	δ_{HA} = 6,22 (d); 1H δ_{HB} = 6,4 (d); 1H J_{AB} = 12,2	-	8,03 (b)
	δ_{CH_3-O} = 3,65 (s) 3H δ_{CH_2-O} = 4,6 (s) 2H	δ_{HA} = 6,6 (d); 1H δ_{HB} = 7,0 (d); 1H J_{AB} = 15,5	$\delta_{OC_6H_5}$ = 6,65-7,25 (m) 5H	10,5 (b)

Table 2 (continued)

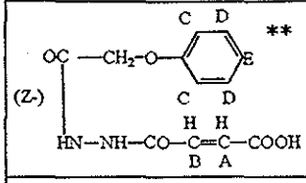
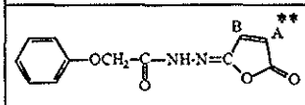
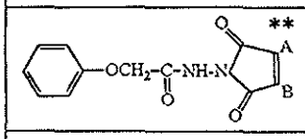
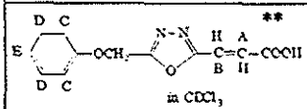
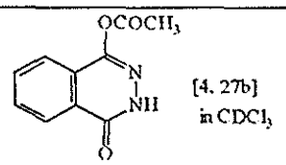
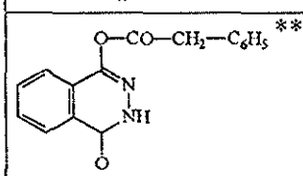
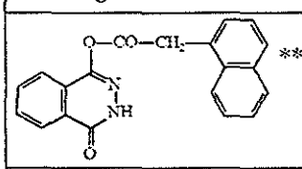
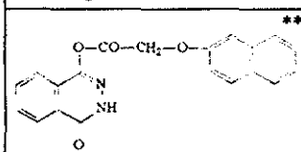
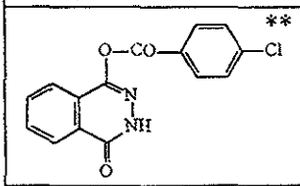
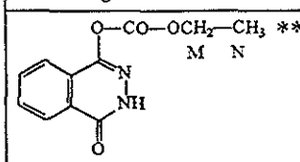
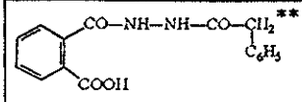
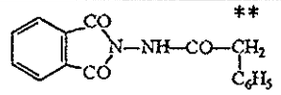
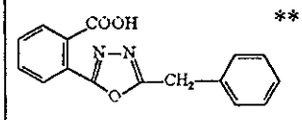
1	2	3	4	5
	δ_{CH_2-O} = 4,58 (s) 2H	δ_{HA} = 6,2 (d); 1H δ_{HB} = 6,37 (d); 1H J_{AB} = 12	$\delta_{HC,E}$ =6,75-7,15 (m) 3H δ_{HD} = 7,2 (t) 2H	10,35 (b)
	δ_{CH_2-O} = 4,74 (s) 2H	δ_{HA} = 6,82 (d); 1H δ_{HB} = 7,8 (d); 1H J_{AB} = 5,6	$\delta_{OC_6H_5}$ = 6,75-7,26 (m) 5H	11,54 (b)
	δ_{CH_2-O} = 4,7 (s) 2H	$\delta_{HA,B}$ = 7,09 (s) 2H	$\delta_{OC_6H_5}$ = 6,9-7,0;7,2-7,35 (m); 5H	10,82 (b)
	δ_{CH_2-O} = 5,32 (s) 2H	δ_{HA} = 6,87 (d); 1H δ_{HB} = 7,63 (d); 1H J_{AB} = 16	δ_{HC} =7,02 (d);2H δ_{HD} =7,34 (t); 2H δ_{HE} = 7,05 (t);1H J_{CD}=8,6; J_{DE}=8	-
	δ_{CH_3-CO} = 2,47 (s) 3H	-	$\delta_{C_6H_4}$ = 7,80 (m); 3H 8,47 (m); 1H	-
	δ_{CH_2-CO} = 4,14 (s) 2H	-	$\delta_{C_6H_5}$ = 7,34 (m); 5H $\delta_{C_6H_4}$ = 7,75-8,5 (m); 4H	-
	δ_{CH_2-CO} = 4,62 (s) 2H	-	$\delta_{C_{10}H_7;\ C_6H_4}$ = 7,35-8,5 (m); 11H	-
	δ_{CH_2-O} = 5,50 (s) 2H	-	$\delta_{C_{10}H_7;\ C_6H_4}$ = 7,1-8,5 (m); 11H	-
	-	-	$\delta_{C_6H_4;\ C_6H_4}$ = 7,4-8,4 (m);	-
	δ_{CH_2-O} = 4,31 (q) 2H δ_{CH_3} = 1,3 (t) 3H J_{MN} = 7,0	-	$\delta_{C_6H_4}$ = 8,4 (m) 1H 7,7-8,2 (m); 3H	-

Table 2 (continued)

1	2	3	4	5
	δ_{CH_2-CO} = 3,56 (s) 2H	-	$\delta_{C_6H_5}$ = 7,3 (s); 5H $\delta_{C_6H_4}$ = 7,45-7,7 (m); 3H 7,75 (m); 1H	10,32 (b)
	δ_{CH_2-CO} = 3,72 (s) 2H	-	$\delta_{C_6H_5}$ =7,3 (s);5H $\delta_{C_6H_4}$ = 7,85 (m); 4H	11,0 (b)
	δ_{CH_2} = 4,32 (s) 2H	-	$\delta_{C_6H_5}$ = 7,25-7,4 (m); 5H $\delta_{C_6H_4}$ = 7,7-7,75 (m); 3H 7,9-7,94 (m); 1H	-

* s=singlet; d=doublet; t=triplet; q=quartet; m=complex multiplet; b=broad
CD_3SOCD_3 was used as solvent with two exceptions that are mentioned beside the respective formula
** data obtained by authors of this article
*** for this compound Hoffmann and Patel [32] have claimed the N-acylated cyclic maleic hydrazide structure but the properties of the reproduced compound are compatible only with isomeric O-acylated structure (see Discussion and also Table 1)
**** for these compounds Kühle [26] has claimed the N-acylated cyclic maleic hydrazide structure but their ^{1}H-NMR spectra show that they are N-acylaminomaleamic acids (see Discussion)
The cited references are given in square brackets.

Evidently, this indicates the same type of structure, namely that of O-acylderivative (12).

The fact that the N-acylderivatives (3, R=H) of the cyclic maleic-(13) and phthalic-(14) hydrazides were not found among the products of reactions that begin by treating the acyclic hydrazides (5, R^1=acyl) with maleic-, respectively phthalic-anhydride[4,12b,c,13a] (see Scheme 1) is another indirect argument for the O-acylderivatives (12) structure of monoacylation products of cyclic maleic-(13) and phthalic-(14) hydrazides.

The acylation with maleic-, respectively phthalic-anhydride of alkyl- and aryl-hydrazines (5, R^1=alkyl, aryl) is considered a structure proof synthesis for N-alkyl, respectively N-aryl- cyclic maleic-[15,28,38,41-43] or phthalic-[20a] hydrazides (3, R=H, R^1=alkyl, aryl). For this reason it was considered that the analogue acylation of acylhydrazines – namely the acyclic hydrazides (5, R^1=acyl) – with dicarboxylic acid anhydrides (6) led to cyclic N-acylhydrazides (3, R=H)[3,26]. Le Berre and co-workers[4] contested the statemants of Feuer and co-workers[3,14a] by invalidation of the N-acetylated cyclic maleic hydrazide structure (3, R=H, R^1=$COCH_3$) for a product formed as a result of transformations involving, as a first step, the reaction between acethydrazide (5, R^1=acetyl) and maleic anhydride (6, X=-CH=CH). This polemic and other dissagrements[14c,43b] concerning the structure of compounds formed in the

reactions that begin by treating substituted hydrazines (5) and maleic anhydride (see Scheme 1) have determined Feuer research group[15,44] to reconsidered previous apreciations. Furthermore, they tried to explain why the formation of some N-substituted cyclic maleic hydrazide with electron acceptor substitutes, including acyl groups (3, R=H, R^1=acyl) failed[15,44].

To check if N-acylated cyclic hydrazides (3, R=H, R^1=acyl) can be formed under Kühle's given conditions[26] we reproduced his acylation of acethyl hydrazide (5, R^1=$COCH_3$) and of salicylic acid hydrazide (5, R^1=OC-C_6H_4-2-OH) with maleic anhydride. Our products had the Kühle's physical constants (melting points, IR spectra) (see Table 1). Yet, the ^{1}H-NMR spectra of these products (see Table 2) prove unequivocally their acyclic structure, namely that of N-acylaminomaleamic acids (1, R^1=OC-CH_3; OC-C_6H_4-2-OH). Thus, the chemical shift for the olefinic hydrogens ($\delta_{CH=CH}$ ~ 6,35 ppm) has a close value to that of maleic acid ($\delta_{CH=CH}$ ~ 6,2 ppm) and of other N-substituted maleamic or 3-aminocarbonyl propenoic acids ($\delta_{CH=CH}$= 6,25-6,7ppm)[4,12a,15,44,45]. On the other hand, for N-acylated cyclic maleic hydrazides (3, R=H; Y=H, H; R^2=acyl) it would be expected that the chemical shift of olefinic hydrogens ($\delta_{CH=CH}$), have values correspondent to maleic hydrazide (13), respectively their N-alkyl and N-aryl derivatives (3, R=H; Y=H,H; R^2= alkyl, aryl) which are about 7 ppm (see[4,15,35,43a,44,47]). Furthermore, extending the acylation with maleic anhydride, under Kühle's[26] conditions to other acyclic hydrazides (5, R^1 = acyl with the meanings from Scheme 1) we obtained N-acyl-aminomaleamic acids (1, R^1=CO-C_6H_4-4-Cl; izonicotinyl; OC-NH_2) that show in their ^{1}H-NMR spectra a pair of doublets, for the olefinic hydrogens, with a vicinal coupling constant $J^{1,3}_{CH-CH}$ = 11-12 Hz (see Table 2). This value is known to be characteristic for acyclic olefinic hydrogens with Z-(cis) configuration[40b,44]. Otherwise, the N-acylated cyclic maleic hydrazide structure (3, R=H) for Kühle's[26] products is contradicted by the position of IR band, due to $\nu_{C=O}$ stretching vibration, recorded by Kühle at 1708 cm^{-1} for the derivative obtained from p-nitrobenzoic acid hydrazide. This value is characteristic for $\nu_{C=O}$ within the carboxylic group of α,β-unsaturated acids (see[3a,4a,15,39b,44]) to which N-acyl aminomaleamic acids (1) also belong.

We could not obtain[12b] N-acylated cyclic hydrazides (3, R=H, R^1=acyl) either by submission the N-acylaminoamic acids (1, R^1=acyl) to some conditions in which the N-alkyl or N-arylaminoamic acids are cyclocondensed[15,41-46], for instance, to imides (2), isoimides (15) or N-alkylated or N-arylated cyclic hydrazides (3, R=H, R^1=alkyl, aryl)* or to conditions under which the N-acylaminomaleamic acids (1, R^1=acyl; X=-CH=CH) transformation – directly[3] or through the N-acetylaminomaleinimide (2, R=H, R^1=acetyl; X=-CH=CH)[14a] or through the N-acetylaminoisomaleinimide (15, R^1=acetyl; X=-CH=CH)[14c] – to N-acetylated maleic hydrazides (3, R=H; R^1= $COCH_3$; X=-CH=CH) is claimed. In these experiments we however obtained, in accordance with Scheme 1 (see Table 2), a series of new N-acylamino-imides (2) and -isoimides (15)[12b,16b], respectively oxadiazole derivatives (16)[12b,d].

Very probably, the N-acylated cyclic maleic- and phthalic-hydrazides (3, R=H, R^1=acyl) were not found neither in cyclic maleic-(13) and phthalic-(14) hydrazides acylation experiments, or in acylations of acyclic hydrazides (5, R^1=acyl) with

dicarboxylic acid anhydrides (6).This is because of their (3) instability, knowing that they would correspond to triacylhydrazines (N-acylated cyclic diacylhydrazines[31,47]) which are very sensible to solvolysis[3b,4a,b,13a,20a].

In conclussion, we have shown that the claims of those who affirmed the obtainment of N-acylated cyclic hydrazides (3, R=H; R^1=acyl) were not valid.

Simultaneously we have confirmed the O-acyl-derivatives nature (12) of the products isolated by monoacylation of cyclic maleic-(13) and phtalic-(14) hydrazide. This conclussion is entirely compatible with the melting points, IR and ^{1}H-NMR spectra of cyclic maleic-(13) and phthalic-(14) hydrazide monoacylation products.

---

* We must also point out here that there are wrong assignments concerning the ^{1}H-NMR published data on the products formed in the transformations that begin by treating maleic anhydride and aryl hydrazine (5, R^1=aryl, Scheme 1). Thus, the characteristic parameters for N-arylaminoisomaleinimides (15, R^1=aryl; X=-CH=CH), respectively for N-arylaminomaleinimide (2, R=H, R^1=aryl; X=-CH=CH) (see[15, 44]), are wrongly assigned[43a, 45] for N-arylated cyclic maleic hydrazides (3, R=H, R^1=aryl; X=-CH=CH).

REFERENCES

1. Th. Curtius, *J. Prakt. Chem.*, **92**, 74 (1915).
2. a) H. Fox, J.T. Gibas, *J. Org. Chem.*, **18**, 1375 (1953).
   b) R. Huisgen, J. Sauer, H. Sturm, J. Markgraf, *Chem. Ber.*, **93**, 2124 (1960).
3. a) H. Feuer, H. Rubinstein, *J. Amer. Chem. Soc.*, **80**, 5873 (1958).
   b) Houben-Weyl, Methoden der Organischen Chemie, Stickstoff Verbindungen, I, Teil 2, Vol. 10/2, 1967, p. 122-168; Vol. 8, 1967, p. 676; Chem. Abstr. **48**, 7580c (1954).
   c) B. Lippert, H.P. Fritz, P. Barkert, *Chem. Ber.*, **108**, 478 (1975).
4. a) J. Godin, A. Le Berre, *Bull. Soc. Chim. Fr.*, 1968, 4210.
   b) A. Le Berre, J. Godin, R. Gorreau, *C.R. Acad. Sci.*, Paris, Ser. C, **265**(10), 570 (1967).
   c) A. Le Berre, B. Dumaitre, J. Petit, *Bull.Soc.Chim.Fr.*, 1970, 4376.
5. F.B. Short, L.M. Long, *J. Heteroc. Chem.*, **6**, 707 (1969).
6. O. Wakabayashi, M. Tsunoda, M. Tsuda, Japan 72.08.292 (1972); Chem. Abstr. **76**, P 153383w (1972).
7. M. Okutsu, O. Wakabayashi, Japan 73.07.766 (1973); Chem. Abstr. **81**, P 10454n (1974).
8. W. Brouwer, J.E. Mac Pherson, Can. Pat. 966.490 (1975); Chem. Abstr. **83**, P 114415h (1975).
9. A.M. Reinbold, G.S. Pasechinic, D.P. Popa, *Izv. Akad. Nauk Mold. SSR*, Ser. Biol. Khim. Nauk., 1989 (4), 55.
10. S.R. Sandler, S.B. Advani, U.S. Pat. 4.906.280 (1990).

11. Yu. S. Andreichikov, V.O. Koz'mymykh, USSR SU 1.121.932 (1992); Chem. Abstr. **117**, 205205b (1992).
12. a) M.L. Bodochi, I. Panea, T. Panea, A.R. Pop, E.D. Zinveliu, E. Bucur, Rom. Pat. 113.144 (1998); A. Pop, T. Panea, L. Bodochi, D. Zinveliu, I. Panea, 35th IUPAC Congress, Instanbul, Turkey, 1995, p. 612; L. Bodochi, I. Panea, A. Pop, A. Căprar, D. Zinveliu, T. Panea, 12th International Conference on Organic Synthesis, Venezia, Italy, 1998, Book of Abstracts, p. 46.
b) T. Panea, A. Pop, I. Panea, L. Bodochi, D. Zinveliu, M. Ţerbea, Proceedings of 34th IUPAC Congress, 1993, Beijing, p. 167.
c) L. Bodochi, I. Panea, A. Pop, T. Panea, D. Zinveliu, 1th International Conference of Chemical Society of South-East European Countries, Chemical Sciences and Industry, Halkidiki, Greece, 1998, Book of Abstracts, Vol. II, p. 681.
d) D. Zinveliu, T. Panea, L. Bodochi, I. Panea, A. Pop, *Chimia (Schweiz)*, **51**, (7), 469, (1997).
13. a) H.D.K. Drew, H.H. Hatt, *J. Chem. Soc.*, 1937, 16.
b) Brit. Pat. 746.047 (1956); Chem. Abstr. **51**, 9707e (1957).
14. a) H. Feuer, J.P. Asunskis, *J. Org. Chem.*, **27**, 4684 (1962).
b) E. Hedaya, R.L. Hinman, S. Theodoropulos, *J. Amer. Chem. Soc.*, **85**, 3052 (1963).
c) E. Hedaya, R.L. Hinman, S. Theodoropulos, *J. Org. Chem.*, **31**, 1311 (1966).
d) Ibidem, 1317 (1966).
15. H. Rubinstein, J.E. Skarabek, H. Feuer, *J. Org. Chem.*, **36**, 3372 (1971).
16. a) M.J. Hearn, S.B. Prisch, *Org. Prep. Proced. Int.*, **13**(6), 421 (1981); Chem. Abstr. **96**, 6318p (1982).
b) I. Panea, L. Bodochi, D. Zinveliu, A. Pop, T. Panea, 35th IUPAC Congress, Instanbul, Turkey, 1995, p. 584.
17. a) Th. Curtius, H.A. Foesterling, *J. Prakt. Chem. (2)*, **51**, 371 (1895).
b) P. Ruggli, C. Hartmann, *Helv. Chim. Acta*, **3**, 493 (1920).
c) Belg. Pat. 634.526 (1963); Chem. Abstr. **61**, 4541b (1964); K.M. Milton, U.S. Pat. 3.295.981 (1967); Chem. Abstr. **66**, P 120802x (1967).
18. a) D. Rădulescu, V. Georgescu, *Bull. Soc. Chim. Fr. (4)*, **37**, 881 (1925).
b) H. Feuer, R. Harmetz, *J. Org. Chem.*, **24**, 1501 (1959).
19. G. Gheorghiu, *Bull. Soc. Chim. Fr. (4)*, **47**, 630 (1930).
20. a) F.M. Rowe, A.T. Peters, *J. Chem. Soc.*, 1933, 1331 .
b) G. Heller, *J. Prakt. Chem.*, **111**, 4 (1925).
21. H. Feuer, G.B. Bachman, E.H. White, *J. Amer. Chem. Soc.*, **73**, 4716 (1951).
22. J.W. Zukel, W.D. Harris, U.S. Pat. 2.614.917 (1952); Chem. Abstr. **47**, 1889 (1953); Ger. Pat. 886.147 (1953); *Chem. Zentr.*, 1954, 4243.
23. D. Stefanye, W.L. Howard, *J. Org. Chem.*, **19**, 115 (1954).
24. a) E. Kühle, R. Wengler, G. Domagk, Ger. Pat. 1.008.739 (1957); Chem. Abstr. **53**, P 18071b (1959).
b) G. Zinner, R. Moll, B. Böhlke, *Arch. Pharm.*, **299**, 441 (1966).
25. a) E. Kühle, L. Eue, Ger. Pat. 1.014.999 (1957); Chem. Abstr. **53**, P 18073g (1959).
b) L. Fischbein, W.L. Zielinski, *J. Chromatogr.*, **18**, 581 (1965).
26. E. Kühle, Ger. Pat. 1.022.592 (1958); Chem. Abstr. **54**, 4502c (1960).

27. a) K. Kormendy, P. Sohár, *Acta Chim. Acad. Sci. Hung.*, **39**, 93; 109 (1963); P. Sohár, ibidem, **40**, 317 (1964).
b) P.G. Parsons, H.J. Rodda, *Austr. J. Chem.*, **17**(4), 491 (1964); Chem. Abstr. **60**, 15711e (1964).
28. Z. Prezezdziecki, A. Chrzaszewska, *Lodz. Towarz Nauk. Wydziol III Acta Chim.*, **10**, 109 (1965); Chem. Abstr. **65**, 13697e (1966); Z. Prezezdziecki, *Roczniki Panstwowego Zokladu Hig.*, **17**(2), 229 (1966); Chem. Abstr. **65**, 7859g (1966).
29. a) R. Geary,U.S. Pat. 3.244.714 (1966).
b) V.A. Greulach, D.B. Plyler, J. Elisha Mitchell, *Sci. Soc.*, **82**, 18 (1966); Chem. Abstr. **66**, 94105a (1967); C.F. Delong, G.L. Erion, III, Braz. Pedido. PI. 77.07.845 (1979); Chem. Abstr. **93**, 63612s (1980).
c) K. Disch, D. Kuehling, P. Krings, H. Bellinger, J. Perner, Ger. Pat. 1.949.561 (1971); Chem. Abstr. **74**, P 143619x (1971).
30. M. Okutsu, O. Wakabayashi, Japan 73.32.337 (1973); Chem. Abstr. **81**, P 59329q (1974).
31. J.P. Anselme, *Tetrahedron Lett.*, 1977 (41), 3615; Chem. Abstr. **88**, 136547h (1978).
32. a) O.L. Hoffmann, N.R. Patel, U.S. Pat. 4.256.884 (1981); Chem. Abstr. **95**, 19717x (1981).
b) E. Parups, I. Hoffman, H.V. Morley, *Can. J. Biochem. Physiol*, **40**, 1159 (1962); Chem. Abstr. **57**, 15549c (1962).
33. M. Tonegawa, Y. Nishimma, C. Fukasawa, K. Kitahara, J. Yamashita, H. Satoh, *Tokyo Ika Daigaku Kiyo*, **15**, 1 (1989); Chem. Abstr. **112**, 77083t (1990).
34. T. Panea, V. Paşcalău, I. Panea, E.T. Marcu, Rom. Pat. 103.399 (1991); Chem. Abstr. **121**, 255815d (1994).
35. a) A.R. Katritzky, A.J. Waring, *J. Chem. Soc.*, 1964, 1523; O. Ohashi, M. Mashima, M. Kube, *Can. J. Chem.*, **42**, 970 (1964).
b) M. Augustin, H.D. Schädler, P. Reinemann, *Z. Chem.*, **12**, 230 (1972).; H.P. Fritz, F.H. Köhler, B. Lippert, *Chem. Ber.*, **106**, 2918 (1973).
36. T. Panea, M. Morar, E. Bucur, E.T. Marcu, V. Paşcalău-Văidean, Rom. Pat. 84.822 (1984).
37. Organicum, Chimie organică practică, translated from German, Ed. Şt. şi Encicloped., Bucureşti, 1982, p. 463-464.
38. K. Eichenberger, A. Stachelin, J. Druey, *Helv. Chim. Acta*, **37**, 837 (1954).
39. L.J. Bellamy "The Infrared Spectra of Complex Molecules", 2nd ed. a) p. 179-182; b) 162, 168; Methuen, London, 1964.
40. L.M. Jackman, S. Sternhell "Applications of NMR Spectroscopy in Organic Chemistry, 2nd ed.; a) p. 71, 72; b) 301-305, Pergamon Press, London, 1969.
41. J. Druey, Kd. Meier, A. Staehelin, *Helv. Chim. Acta*, **45**, 1485 (1962).
42. M. Augustin, P. Reinemann, *Z. für Chemie*, **12**, 101 (1972); ibidem, **13**, 214 (1973).
43. a) S. Baloniak, A. Mroczkiewicz, *Rocz. Chem.*, **48**(3), 399 (1974).
b) S. Baloniak, *Rocz. Chem.*, **41**, 1143 (1967); **42**, 1231; 1867 (1968); **43**, 315, 1187 (1969).
44. H. Rubinstein, M. Parnarouskis, H. Feuer, *J. Org. Chem.*, **38**, 2166 (1973).
45. A. Krutošiková, M. Dandárova, V. Konečny, *Collect. Czech. Chem. Commun.*, **55**, 2707 (1990).
46. H. A. Hageman, W.L. Hubbard, U.S. Pat. 3.257.414 (1966).
47. W.H. Pirkle, P.L. Gravel, *J.Org. Chem.*, **42**, 296 (1977).

The SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY (ISSN: 2674-6891; 0104-5431) is an open-access journal since 1993. Journal DOI: 10.48141/SBJCHEM.
http://www.sbjchem.com.
This text was introduced in this file in 2021 for compliance reasons.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

Luzia Otília Bortotti Favero[1], Ervim Lenzi[1(*)], Eduardo Bernardi Luchese[2], e Luciano Márcio de Moraes[1]

[1] - Chemistry Department of State University of Maringá (Departamento de Química da Universidade Estadual de Maringá). Av. Colombo, 5790; Caixa Postal, 331; CEP 87.020-900; Maringá; Paraná - Brasil. [2] - Agronomy Department of State University of Paraná West (Departamento de Agronomia da Universidade Estadual do Oeste do Paraná). Rua Pernambuco, 1777; CEP-85.960-000; Marechal Candido Rondon; Paraná - Brasil. (*) – Author for correspondence.

ABSTRACT: *Samples of six types of soil were tested in a greenhouse. Three doses of boric acid: 0.0 g (control); 0.10 g and 0.20 g were applied to the prepared soils. Seeds of cabbage were sown and only two plants remained after cutting. Mehlich 1, HCl 0.05 mol.L^{-1} and the saturation extract were used to determine the boron in the soil. After determining the dry matter and calcined the plants ashes were dissolved in HCl 0.6 mol.L^{-1}. The boron concentration was determined by the Azomethine-H method. The correlation between boron concentration in the plant (μg.g^{-1}) and total boron in the plant (μg.plant^{-1}) versus boron concentration in the soil showed that among the three extractors the HCl 0.05 mol.L^{-1} and saturation extract presented the best correlation coefficients (r): 0.857 and 0.832, 0.858 and 0.841 respectively. The Mehlich 1 extractor presented the lowest correlation coefficients for all conditions studied.*

Key words: boron extractors, micronutrient, boron, element extraction, boron in soil.

RESUMO: *Amostras de 6 tipos de solo foram testadas em casa de vegetação. Nos solos preparados foram aplicadas três doses de ácido bórico: 0,0 (testemunha); 0,10 g e 0,20 g. Sementes de couve foram semeadas. No desbaste foram deixadas 2 plantas. Os extratores: Mehlich 1; HCl 0,05 mol.L^{-1} e extrator de saturação foram usados para extrair o boro do solo. Após determinar a massa seca, e respectiva calcinação, as cinzas das plantas foram dissolvidas em solução de HCl 0,6 mol.L^{-1}. A concentração do boro foi determinada pelo método da Azometina-H. As correlações entre a concentração de boro na planta (em μg.g^{-1}) e o total de boro na planta (em μg.plant^{-1}) verso a concentração de boro nos solos mostraram que entre os três extratores o HCl 0,05 mol.L^{-1} e o extrator de saturação apresentaram os melhores coeficientes de correlação (r): 0,857 e 0,832; 0,858 e 0,841 respectivamente. O extrator Mehlich 1 apresentou os coeficientes de correlação mais baixos para todas as condições analisadas.*

INTRODUCTION

Extraction by hot[1] water is the most widely used method to detect boron critical content in the plants. Though it is feasible to find in limed soils a better

DOI: 10.48141/SBJCHEM.v7.n8.1999.43_1999.pdf

correlation between boron extracted by $CaCl_2$ 0.01 $mol.L^{-1}$ and Mehlich 1 extractors and that absorbed by the plant, this is not a normal condition in the soils routinely[2] analyzed in the laboratory.

Boron deficiencies in plants are found when the concentration values interval of this element in soil ranges from 0.1 to 0.7 ppm [3-4-5].

Using coffee shrub as reference and saturation extract as extractor, it was verified that for the DRL (dystrophic red latosol) the boron concentrations in the extract can be considered low when below 0.3 ppm, suitable when close to 0.6-0.8 ppm and high when over 1.0 ppm[6].

Lopes and Carvalho[7] summarized the most frequent methodologies used to determine the boron available for the plants and consider: $Ca(H_2PO_4)_2.H_2O$ solution, hot water, saturation extract, H_2SO_4, HF and HCl diluted solutions as extractors. The soil/extractor relation varies from 1:1 to 1:2 during a five-minutes to sixteen-hours shaking period. Up to now the amplitude of critical levels varies from 0.2 to 2 ppm for the different extractors proposed.

Undoubtedly, hot water is the most appropriate extractor for the determination of boron available, but it does not allow the use of pyrex-type borosilicated glasses. Otherwise, it is a laborious technique which makes it difficult to determine this element in routine laboratory analysis.

With the purpose of dynamizing the routine analysis either as a function of the time spent or the handling of the materials, new extractors such as Mehlich 1, diluted acids, 0.5 $mol.L^{-1}$ mannitol + $CaCl_2$, $CaCl_2$, and 0.005 $mol.L^{-1}$ [8-6-9] are being tested and used. The solution of $BaCl_2$. $2H_2O$ 1.25 $g.L^{-1}$ heated in a microwave stove was also recommended for the extraction of boron in routine laboratory analyses, as well as, for the determination in ICP-AES (Inductively Coupled Plasma – Atomic Emission Spectroscopy)[10]. The characterization of these extractors for several types of soil and plants, and the correlation of the critical levels between them constitutes a vast field of study.

Our purpose is to study three extractors: Mehlich 1, HCl 0.05 $mol.L^{-1}$ and the saturation extract using cabbage as a test plant.

MATERIALS AND METHODS

List of soils

Six classes of soils (DRL-dystrophic red latosol, ERL-eutrophic red latosol, DRAL – dark red allic latosol, AC-allic cambisol, DSRS-dystrophic structured red soil and DDRL-dystrophic dark red latosol), already used in a previous experiment to evaluate cotton plant response to boron[11] application, were used in this study.

Preliminary determination in the soil samples

Some conditions of soil fertility which influence boron concentration in soil was assessed through the routine analysis in the *Laboratório de Agroquímica do Departamento de Química da UEM (Universidade Estadual de Maringá* - Laboratory of Agrochemistry-Department of Chemistry of the *Universidade Estadual de Maringá*), see Table 1.

The pipet[12] method was used for the granulometric analysis in the Laboratory of Pedology, Sedimentology and Palynology of the Department of Geography of the Universidade Estadual de Maringá, (see Table 1).

Boron extraction and determination in the soil

The extractors used to classify boron available for the plants in the soils listed were as follow, with their respective procedures:

HCl 0.05 mol.L^{-1} [6]: to each 10 g of ADFE (air-dried fine earth), add 20 mL of HCl 0.05 mol.L^{-1}, shake for 5 minutes and filter in a Whatman-42 paper filter.

Mehlich 1[8]: transfer 5 mL of ADFE to a erlenmeyer-container, add 50 mL of Mehlich 1 solution, (H_2SO_4 0.0125 mol.L^{-1} + HCl 0.05 mol.L^{-1}), shake for 5 minutes and decant during one night.

Saturation extract[6]: transfer 500 g of soil to plastic recipients, moisten with a determined quantity of distilled water to complete saturation. Then rest it for 12 hours and vacuum-extract the solution.

The Azomethina-H[13] colorimetric method was used to analyze the boron in the respective extracts.

Procedures of the experiment.

The experiment was set up in a greenhouse, using 2.5-liter vases. The triplicate treatments were 0.0 g, 0.10 g and 0.20 g of boric acid-H_3BO_3 respectively. For 15 days the vases were filled with water up to 70% retention capacity. Six cabbage seeds were sown in each vase and after ten days of germination they were pared down, remaining only two plants in each vase.

The vases were kept in a greenhouse according to a totally randomized design and regularly watered to maintain humidity.

Collection of vegetal material and soil samples from the vases.

Eight weeks and half after seeding the aerial portion of the plants was close-cut, washed with distilled and deionized water, dried in a 65 °C hothouse till reaching their constant weight, ground in a Wiley grinder with a 1mm-mesh sieve and packed in plastic bags ready for the analysis[14].

After the plant collecting the vases were emptied and the soils air-dried, ground, in a 2-mm-mesh sieved, homogenized and kept in plastic bags for future analysis[15].

Boron analysis in the plants and soils

For four hours[16] 0.40 g vegetal material, contained in porcelain vases, was incinerated in muffle at 550 °C temperature. After cooling 20 mL of HCl 0.6 mol.L^{-1} were added. Using the complexant Azomethina H[13], the boron concentration was determined in the extract obtained by means of UV-VIS spectrophotometry.

Boron determination in the soils followed the above-mentioned extraction and complexations.

RESULTS AND DISCUSSION

Contents of organic matter and clay and pH which characterize the soils used in the experiment are shown in Table 1. These factors may influence boron micronutrient availability in the soils.

According to Table 2, the extractors behaved differently in the boron-extraction procedure for all types of soil. The Mehlich 1 extractor provided the greatest quantity of the micronutrient while the saturation extract, the smallest. The HCl 0.05 $mol.L^{-1}$ and the Mehlich 1 extractor removed the same quantity of boron content only from the DDRL soil, which is a very sandy soil with low content of organic matter as it can be seen in Table 1.

Table 1 – Characteristics of the soils used in the experiment

Type of soil*	pH (H_2O)	Organic matter $g.dm^{-3}$	Clay %	Silt %	Sand %
DDRL	7.2	5.35	9.65	3.35	87.0
DRL	6.2	6.69	65.47	31.53	3.0
ERL	6.3	12.06	59.45	34.56	6.0
DRAL	6.2	5.35	38.77	47.73	13.5
AC_1	5.7	28.14	39.57	55.43	5.0
AC_2	5.7	30.75	52.62	45.38	2.0
DSRS	6.6	18.75	65.40	33.60	1.0

* **DDRL** - dystrophic dark red latosol; **DRL** – dystrophic red latosol; **ERL** – eutrophic red latosol; **DRAL** – dark red allic latosol; **AC_1** – allic cambisol; **AC_2** - allic cambisol and **DSRS** – dystrophic structured red soil.

The boron extractors HCl 0.05 $mol.L^{-1}$ and the saturation extract showed to be more efficients, as boron extractors, than Mehlich 1 when their respective boron concentrations extracted from the soils were correlated with the boron concentrations absorbed by the plants cultivated. The variance analysis was significant on the level of 1% for these extractors once all data were considered in relation to the boron concentration in the aerial portion in $\mu g.g^{-1}$ as well as in relation to the boron absorbed by the plant in $\mu g.plant^{-1}$ with the correlation coefficient of 0.857 and 0.832 respectively for hydrochloric acid and 0.858 and 0.841 for the saturation extract in Table 3.

Taking into account only the vases that were fertilized with borate (Table 4) the variance analysis was 1% of significance for the HCl 0.05 $mol.L^{-1}$ and saturation extractors. For both extractors, the correlation coefficients remained close to 0.8 in relation to the boron content in the vegetal tissue as well as for the boron absorbed by the plant. Bartz and Magalhães[17] also found better correlation between the boron extracted by acid extractors and the boron absorbed by the plants, as compared to boron fertilized soils. However, Renan and Gupta[18] found a positive correlation between the boron extracted from the soil with HCl 0.05 $mol.L^{-1}$ and that absorbed by the plant in any class of soil.

Table 2 - Boron concentration in soils determined by different extractor solutions

Soil*	Extractors	$\mu g.g^{-1}$ (values intervals)	$\mu g.g^{-1}$ (average)
DRL	Mehlich 1	1.00 - 10.00	5.06
	HCl 0.05 $mol.L^{-1}$	0.03 - 4.57	2.01
	Saturation extract	0.06 - 1.42	0.54
ERL	Mehlich 1	1.16 - 7.35	4.41
	HCl 0.05 $mol.L^{-1}$	0.23 - 2.34	1.37
	Saturation extract	0.03 - 0.52	0.26
DRAL	Mehlich 1	2.67 - 6.11	4.16
	HCl 0.05 $mol.L^{-1}$	1.00 - 3.76	2.69
	Saturation extract	0.05 - 0.70	0.32
AC_1	Mehlich 1	2.15 - 9.14	6.29
	HCl 0.05 $mol.L^{-1}$	0.16 - 4.25	1.98
	Saturation extract	0.19 - 1.80	1.06
AC_2	Mehlich 1	3.32 - 13.34	8.27
	HCl 0.05 $mol.L^{-1}$	0.84 - 3.00	1.76
	Saturation extract	0.27 - 2.73	1.28
DSRS	Mehlich 1	1.45 - 10.94	5.28
	HCl 0.05 $mol.L^{-1}$	0.03 - 3.50	1.61
	Saturation extract	0.03 - 1.01	0.37
DDRL	Mehlich 1	0.47 - 8.70	4.53
	HCl 0.05 $mol.L^{-1}$	0.38 - 8.89	4.21
	Saturation extract	0.13 - 4.49	1.91

* **DDRL** - dystrophic dark red latosol; **DRL** – dystrophic red latosol; **ERL** – eutrophic red latosol; **DRAL** – dark red allic latosol; **AC_1** – allic cambisol; **AC_2** - allic cambisol and **DSRS** – dystrophic structured red soil.

For the soils of the control vases the correlation between boron content extracted by HCl 0.05 $mol.L^{-1}$ and boron concentration in the plant or boron absorbed by the plant was significant at the level of 5% and the correlation coefficients were 0.627 and 0.523 respectively (Table 4).

The Mehlich 1 extractor showed low correlation coefficients (Tables 3 and 4), verifying the literature on the subject. For the hydromorphic and alluvial soils under flooded rice cultivation, Paula *et al.*[19] found correlation coefficients close to Mehlich 1 and hot water extractors, thus indicating the feasibility of replacing hot water by Mehlich 1.

Table 3 – Linear correlation coefficients of boron concentration absorbed by the plant in $\mu g.g^{-1}$ and $\mu g.plant^{-1}$ respectively with the boron content available in the soil in the extractors used for all data obtained in the experiment.

Extractor	Boron concentration in the plant ($\mu g.g^{-1}$)	Boron absorbed by the plant ($\mu g.plant^{-1}$)
Mehlich 1	0.342*	0.411**
HCl 0.05 $mol.L^{-1}$	0.857**	0.832**
Saturation extract	0.858**	0.841**

Levels of significance (* - 5%) (** - 1%)

Table 4 - Linear correlation coefficients of boron concentration absorbed by the plant in $\mu g.g^{-1}$ and $\mu g.plant^{-1}$ respectively with the boron content available in the soil in the extractors used before and after fertilization.

Extrator	Boron concentration in the plant ($g.g^{-1}$)		Absorbed boron by the plant. ($\mu g.plant^{-1}$)	
	Without fertilization (control)	With fertilization	Without fertilization (control)	With fertilization
Melich 1	0.389	0.109	0.326	0.177
HCl 0,05 $mol.L^{-1}$	0.627*	0.874**	0.553*	0.828**
Saturation extract	0.226	0.808**	0.283	0.799**

Levels of significance (* - 5%) (** - 1%)

Table 5 - Linear correlation coefficients between contents of soil boron micronutrients determined by different extractors

Extractor	HCl 0.05 $mol.L^{-1}$	Saturation extract
Mehlich 1	0.527**	0.604**
HCl 0.05 $mol.L^{-1}$	-	0.800**

Level of significance (** - 1%)

The variance analysis of the linear correlation between the extractors was significant at the level of 1% for the Mehlich 1 x HCl 0.05 $mol.L^{-1}$, Mehlich 1 x saturation extract and HCl 0.05 $mol.L^{-1}$ x saturation extract correlations of which the last one showed the best correlation coefficient (0.800) (Table 5).

Figure 1 shows a linear correlation between the boron content absorbed by the plants and that extracted by the three extractors and their respective equations.

CONCLUSIONS

The Mehlich 1 extractor extracted the greatest amount of boron from the soils, while the saturation extract, the least.

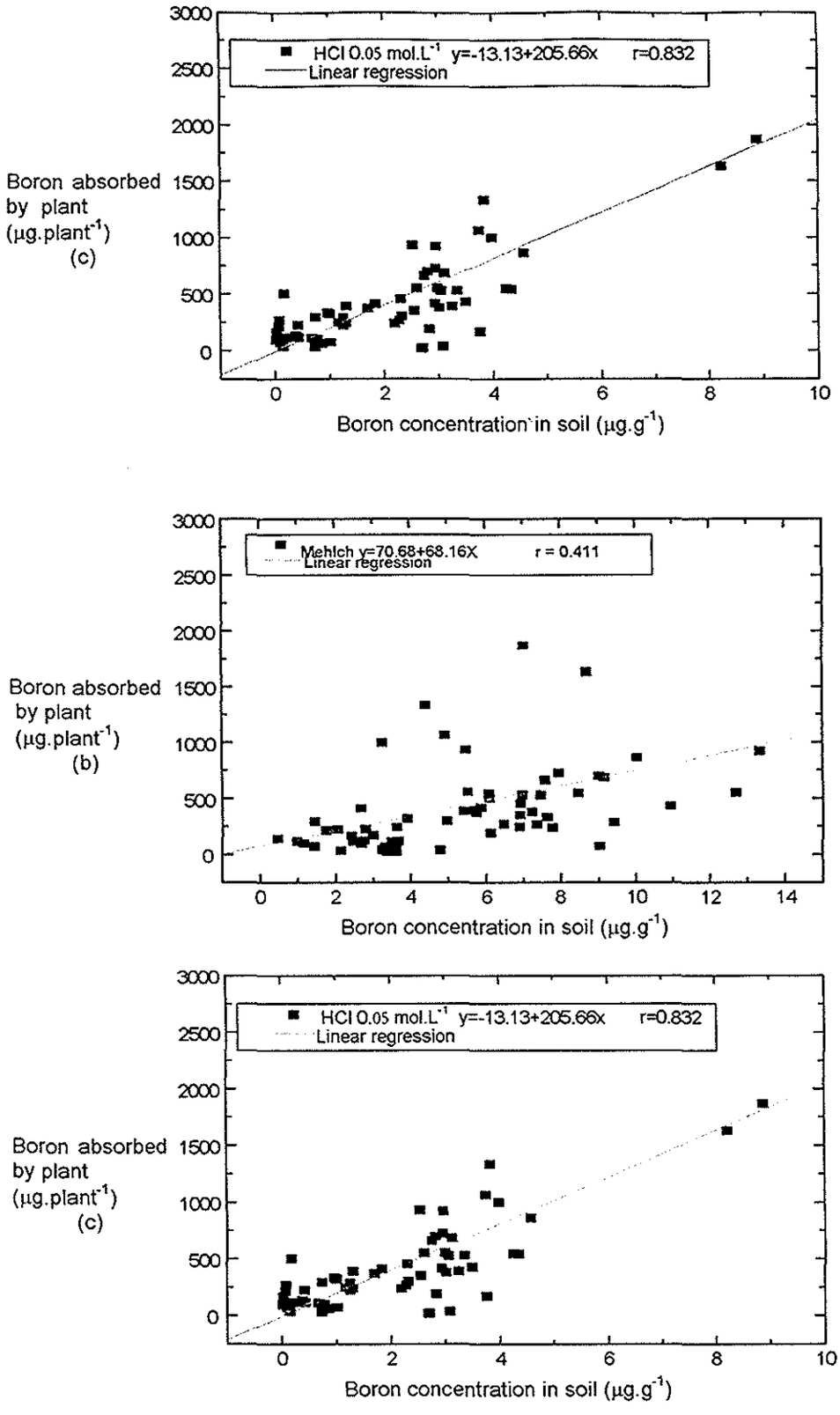


Figure 1 – Linear correlation graphs for boron absorbed by the plant and boron concentration in the soil extracted by (a) saturation extract, (b) Mehlich 1 (c) HCl 0.05 mol.L^{-1}.

Saturation extract and HCl 0.05 mol.L^{-1} extractors showed the best correlation coefficients between boron concentration in the plant in $\mu g.g^{-1}$, and the total boron per plant, in g/plant x the boron extracted from the soil by these extractors.

The correlation coefficient between the HCl 0.05 mol.L^{-1} and the saturation extract extractors was the best (0.800).

Taking into consideration the correlation coefficients and execution facility, the HCl 0.05 mol.L^{-1} is the best among all extractors studied.

AKNOWLEDGMENTS

We would like to thank *Departamento de Química – DQI* (Department of Chemistry = DC); *GEMA (Grupo de Estudos Multidisciplinares do Ambiente* = The Multidisciplinary Environment Study Group); *UEM (Universidade Estadual de Maringá)* and *PBIC (Programa de Bolsa de Iniciação Científica/UEM* = Scientific Research Initiation Scholarship Program = SRISP) and *Laboratório de Tradução e Versão, e Revisão de Textos em Língua Portuguesa (LTR/STR-DLE)* for their support.

BIBLIOGRAPHY

01. B.Van Raij, *Fertilidade do Solo e Adubação.* Editora Agronômica Ceres, Piracicaba, (1991), 343 p.
02. C. Bataglia & B.Van Raij, *Rev. Bras. Ciênc. Solo, 13*:205-212 (1989).
03. F. R. Cox & E. J. Kamprath, Micronutrient Soil Tests. *In*: J. J. Mortvedt, P. M. Giordano and W. L. Lindsay, *Micronutrients in Agriculture,* Soil Science Society of America, Madison, (1972), p. 289-313.
04. R. L. Mitchell, *In*: F. E. Bear, *Chemistry of the soil.* Reinhold Publishing Corporation, New York, (1965).
05. S. Buzetti, T. Muroka & M. E. Sá, *Rev. Bras. Ciênc. Solo, 14*, 157-166 (1990).
06. A . E. Correa, M. Miyazawa & A . Pavan, *Pesq. Agropec., 20(2)*, 177-181 (1985).
07. A . S. Lopes & J. G. Carvalho, *Anais XVII Reunião Brasileira de Fertilidade do Solo,* 133-174, IAPAR, Londrina, Paraná, 1988.
08. A. C. Ribeiro & T. Sarabia, *Rev. Bras. Ciênc. Solo, 8*, 85-90 (1984).
09. C. Bataglia & B. Van Raij, *Rev. Bras. Ciênc. Solo, 14*, 25-31 (1990).
10. C. A. Abreu, M. F. Abreu, B. Van Raij, O . C. Bataglia and J. C. Andrade, *Commun. Soil Sci. Plant Anal. 25(19, 20)*, 3321-3333 (1994).
11. E. B. Luchese, E. Lenzi, L.O .B. Favero e A . Lanziani, *Arq. Biol. Tecnol., 37(4)*: 775-785 (1994).
12. EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), *Manual e Métodos de Análise de Solo*, Serviço Nacional de Levantamento e Conservação de Solos, Rio de Janeiro (RJ) (1979), não paginado.
13. N. D. Basson, R. G. Bohner & D. A . Staton, *Analyst. 94*: 1135-1141 (1969).
14. C. Bataglia, A. M. C. Furlani, J. P. F. Teixeira, P. R. Furlani e J. R. Gallo, *Métodos de Análises Química de Plantas,* Boletim Técnico nº. 78. Instituto Agronômico, Campinas (SP) (1983), p. 5-6.

15. M. A. Pavan, M. F. Bloch, H. C. Zempulski, M. Miyazava, D. C. Zocoler, *Manual de análise química de Solo e controle de qualidade,* Circular nº. 76, Instituto Agronômico do Paraná, Londrina (PR) (1992), p. 7.
16. J. R. Sarruge, *Análise Química em Plantas,* Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba (SP) (1974), p. 25-29.
17. H. R. Bartz & A .F. Magalhães, *Agron. Sulriograndense, 11,* 89-96 (1975).
18. L. Renan & V. C. Gupta, *Commun. Soil Sci. Plant Anal., 22(9,10),* 1003–1012 (1974).
19. M. B. Paula, J. G. Carvalho & A . F. Souza, *Anais XXV Congresso Brasileiro de Ciência do Solo,* 1172 – 1174, Viçosa, Minas Gerais (1995).

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

QUANTITATIVE DETERMINATION OF FURANOCOUMARINS AND IDENTIFICATION OTHER CHEMICAL CONSTITUENTS OF RHIZOMES AND LEAVES FROM *DORSTENIA TUBICINA* AND COMMERCIAL SAMPLES

CLAUDIA A. L. CARDOSO[1,*], WAGNER VILEGAS[2] AND NELI K. HONDA[3]

[1]Universidade Estadual de Mato Grosso do Sul, Rua: Pereira Gomes, 355, 79400-000, Coxim, MS, Brazil.
[2]Instituto de Química - UNESP, Departamento de Química Orgânica, Caixa Postal 355, 14800-900, Araraquara, SP, Brazil.
[3]Universidade Federal de Mato Grosso do Sul, Departamento de Química, Caixa Postal 549, 79070-900, Campo Grande, MS, Brazil.

ABSTRACT

The chemical composition of the hexane extracts from the rhizomes and leaves of Dorstenia tubicina Ruiz et Pavon (Moraceae) and four commercial samples of Dorstenia was investigated by high resolution gas chromatography-flame ionization detector (HRGC-FID) and high resolution gas chromatographic-mass spectrometry (HRGC-MS). This is a rapid and sensitive method for the quantification of furanocoumarins and identification isoprene derivatives (triterpenes, steroids and vitamins) from apolar crude extracts of Dorstenia species.

RESUMO

A composição química dos extratos hexânicos das rizomas e folhas de Dorstenia tubicina Ruiz et Pavon (Moraceae) e quatro amostras comerciais de Dorstenia foram investigadas por cromatografia gasosa de alta resolução com detector de ionização de chama (HRGC-FID) e cromatografia gasosa de alta resolução com detector de massas (HRGC-MS). Este é um método rápido e sensível para a quantificação de furanocumarinas e identificação derivados isoprênicos (triterpenos, esteróides e vitaminas) de extratos apolares de espécies de Dorstenia.

KEYWORDS: *Dorstenia tubicina*, furanocoumarins, gas chromatography-mass spectrometry, steroids, triterpenes, vitamins

DOI: 10.48141/SBJCHEM.v7.n8.1999.52_1999.pdf

INTRODUCTION

Preparations based on plants used in traditional medicine have been widely employed in Brazil as an alternative to pharmaceuticals. The cost of modern pharmaceuticals is probitive for the majority of the population[1].

The chemical investigation of medicinal plants often requires the examination of several related species of one genus. Traditional phytochemical procedures of isolation and identification can be very time-consuming and generaly require considerable amounts of plant material. In the case of closely related chemical species, however, classical phytochemical methods can be exchanged for an analytical approach, based on chromatographic analyses and identification of known compounds using standards and/or spectrometric data. The process of isolation and identification of minor compounds is often a very complex matter, because plants usually contain only one major class of compounds. To detect such minor compounds, there is a need for a rapid method for the detection of the substances in a complex mixture of other extractives. The genus *Dorstenia* (Moraceae) has 37 Brazilian species[2]. Most of them are known as "carapiá" or "figueirilha". Some of them are used in folk medicine, mainly for skin diseases and as antiophidics[3].

Furanocoumarins are the most abundant compounds in these plants. They have been used empirically for centuries in the treatment of depigmentations, specially in Egypt[4]. Derivatives of psoralen, such as bergapten, are used in therapeutic treatment of psoriasis (buvatherapia) and mycosis fungoides[5]. Some of these compounds can be related to the topical utilization of preparations containing *Dorstenia* rhizomes in the treatment of skin diseases such as psoriasis and vitiligo[6]. Furthermore, these compounds are responsible for the pleasant odour of these plants, that are mixed to pipe tobacco[7]. A number of methods has been described for the analysis of furanocoumarins, among them high performance liquid chromatography-ultraviolet (HPLC-UV) and gas chromatography coupled to a flame ionization detector (GC-FID)[6,8-10].

Besides furanocoumarins, terpenoids also occur in the *Dorstenia* genus[3,6]. It has also been suggested that the terpenoidal constituents might be related to the antiophidical activity of some *Dorstenia* species[11-12]. However, as most of terpenoids lack a chromophore group, their analysis by spectrophotometry poses a problem to the analysis of crude *Dorstenia* extracts.

GC-FID and high resolution gas chromatography - mass spectrometry (HRGC-MS) have proved to be a valuable tool for separation and identification of mixtures of apolar furanocoumarins and terpenoids[6]. Consequently, they are an useful alternative for the investigation either of new species of this genus or for the analysis of samples found in local commerce.

Dorstenia tubicina is an herbaceous plant used in the city of Campo Grande, Mato Grosso do Sul State, Brazil for the treatment of antiophidics and vitiligo. There are no phytochemical or pharmacological studies reported on *D. tubicina* in the literature.

In this work we report the identification of the components present in the hexane extracts of rhizomes and leaves of *D. tubicina* and in four commercial samples obtained in the local commerce of Campo Grande and quantification of furanocoumarins using GC-FID and HRGC-MS.

EXPERIMENTAL

Plant material. The plant *Dorstenia tubicina* Ruiz et Pavon was collected in Aquidauana, Mato Grosso do Sul, Brazil in 1996 and identified by Dr. Jose Pedro Carauta from Jardim Botânico do Rio de Janeiro, Brazil. A voucher specimen is kept in the Herbarium of the University.

The commercial samples were purchased in the local commerce in Campo Grande, MS. Standard substances were obtained from a collection of our laboratory.

Sample extraction. D. *tubicina* (rhizomes and leaves) and the four commercial samples were separatedly dried at 37°C for 1 day. A quantity of 1g of each sample was powdered and extracted with 30 mL hexane (maceration in sonic bath for 30 min). The solutions were filtrared with a filter paper. The solvents were evaporated under vacuum. One milligramas of each extract was redissolved with 1 mL of hexane, filtered with a Millex filter of 0.45 μm and directly analysed by HRGC-FID with standards and also by HRGC-MS. Samples were extracted in triplicate.

Extract characterization. GC analyses were performed with a VARIAN 3400 gas chromatograph equipped with a capillary fused sílica LM-5 (15m x 0.2 mm i.d., film thickness 0.5 μm) and with a flame ionization detector (FID). H_2 was used as carrier gas at a flow rate 0.8 mL/min and the injection split ratio was 1:20. The injection temperature was 280°C. Column temperature was programmed 150-240 °C, linear increase 10 °C/min, 240°-280 °C , linear increase 5 °C/min, held for 20 min. The detector temperature was 300 °C . Samples of 1 μL were injected with a 10 μL Hamilton syringe.

The HRGC-MS analysis were performed on an SHIMADZU QP 5000, with electron impact ionization (70eV), coupled to an SHIMADZU GC-17B gas chromatograph in the same column and temperature program described above. Helium was used as carrier gas at a flow rate 0.8 mL/min. The MS scan range was 45-550 u. Samples of 1 μL were injected with a 10 μL Hamilton syringe.

Determination of the GC-FID detection limit. The GC-FID detection limit was determined by injecting in triplicate 6 solutions of known concentrations (1.0 μL each injection), and lowering the concentration of the sample until the detection of a peak with twice the height of base noise line; the corresponding

concentration was considered being the minimal amount detectable by GC-FID for those substances.

Calibration curves. Estimation of the content of psoralen and bergapten in plant material was performed by external calibration. The compounds were dissolved separately in analytical grade chloroform in order to obtain the stock solutions which were appropriately diluted to concentrations ranging from 1-100 μg/mL of compounds. Aliquots (1.0 μL) for seven dilutions of each standard were analysed by GC-FID. Each determination was carried out in triplicate.

RESULTS AND DISCUSSION

In order to optimize the analyses for furanocoumarins and isoprene derivatives, several chromatographic runs were made by GC-FID under different conditions. At the conditions presented, furanocoumarins are eluted first (t_R among 4.0-7.0 min), the monoterpenic furanocoumarin DT is eluted with t_R 16-17 min and isoprene derivatives (triterpenes, steroids, vitamins) are eluted between 13.0-22.0 min (Fig.1-3,Table I).

The co-injection of authentic standards were also used to confirm the presence of psoralen, bergapten, α-sitosterol, stigmasterol, α- amyrin, β-amyrin, α- amyrin acetate, β-amyrin acetate, α-tocopherol, vitamin E. The presence of DT and isoprenoids (not fully identified) were deduced on the basis of matching with the NIST 62500 data bank (with 62235 compounds) and also by their MS-fragmentation pattern compared to the literature[6,13-18].

The calibration curve was linear in the range 10-100 (R=0,9999) μg/mL for psoralen and 5-90 μg/mL (R=0,9997)for bergapten. The limits of detection for psoralen 0.1 μg/mL and for bergapten 0.3 μg/mL.

Table II shows the contents of furanocoumarins in the *Dorstenia* species.

The HRGC-FID and HRGC-MS profile of the hexane extracts of *Dorstenia tubicina* showed some differences between the chemical composition of the rhizomes and leaves. The major compounds in the rhizomes are the furanocoumarins, while isoprene derivatives predominate in the leaves. Furanocoumarin DT is present only in trace amounts in the rhizomes. A common characteristic observed in the rhizomes and leaves was the presence of almost equal amounts of α- amyrin acetate and β-amyrin acetate. Sitosterol is present in significative amounts only in the leaves, where the major constituent is vitamin E. Some minor compounds were lost because of their noisy mass reports.

The chemical composition of *D. tubicina* herein examined showed to be similar to other *Dorstenia* species, in which the major compounds are the furanocoumarins[3, 6-7, 13-14].

Table I. Retention Times of the Components

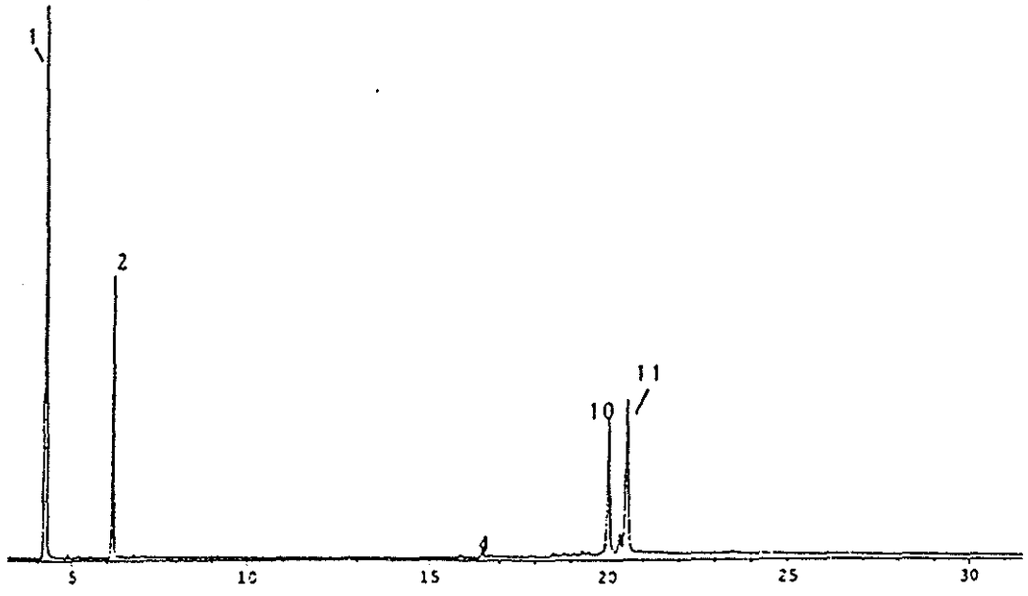
Peak	*Compound*	*MW*	t_r*(min)*
1	Psoralen	186	4.15
2	Bergapten	216	6.25
3	α-tocopherol	416	15.52
4	DT	368	16.55
5	Vitamin E	430	16.67
6	Stigmasterol	412	17.99
7	α-sitosterol	414	18.51
8	β-amyrin	426	18.75
9	α-amyrin	426	19.41
10	β-amyrin acetate	468	20.62
11	α-amyrin acetate	468	21.21

DT=5-[3-(4,5-Dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7H-furo[3-2-g][1] benzopyran-7-one.

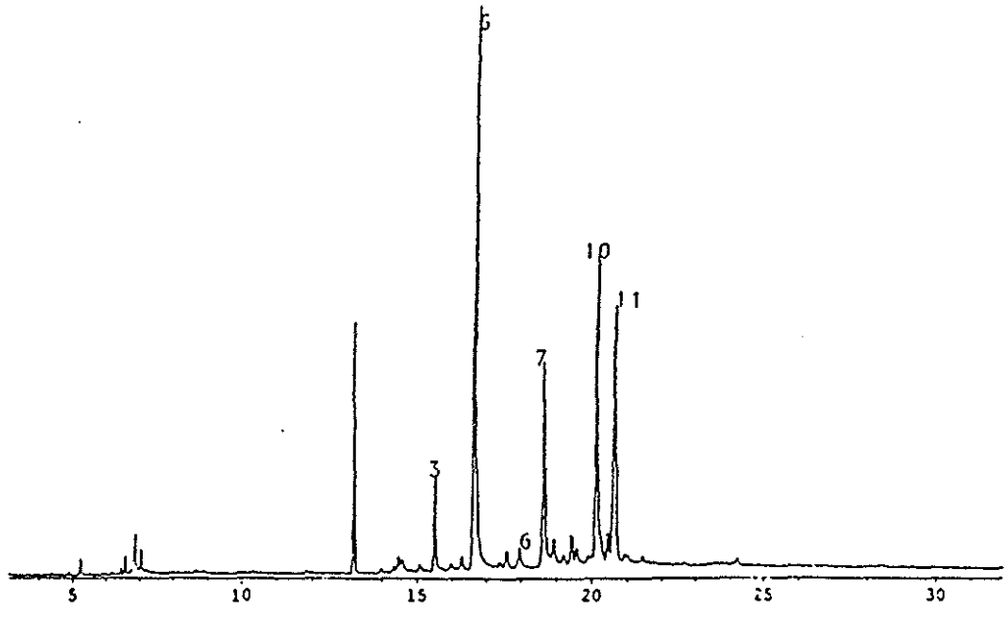
Table II. Contents (μg/g) of Furanocoumarins in *Dorstenia* Species (dry weight) and in Commercial Samples.

Peak	*D.tubicina Rhizomes*	*D.tubicina Leaves*	*Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*
1	3650	-	3355	3590	1790	1335
2	1770	-	1205	1870	3495	-

Average of three determinations; standard deviation < 5%
(-) = Not detected

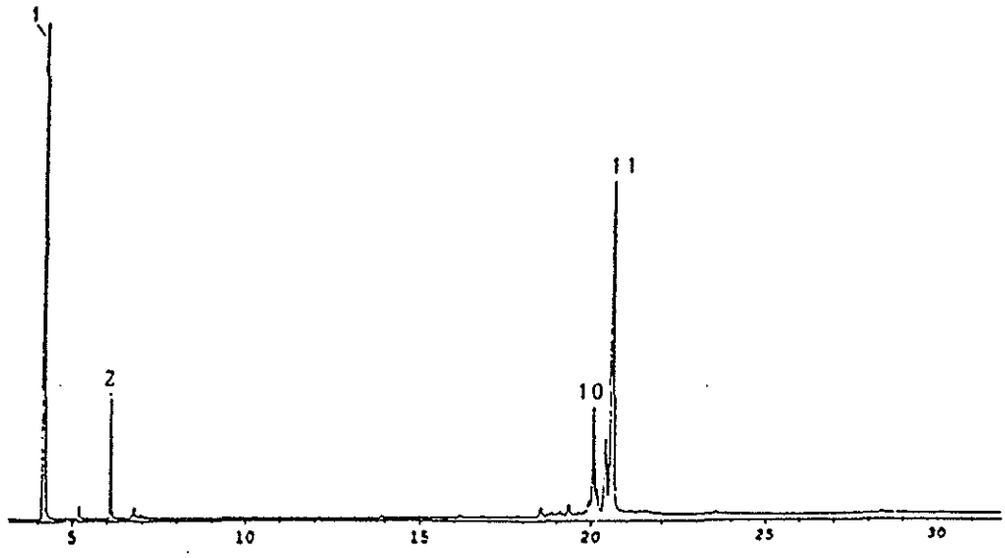


(a)
Time (min)

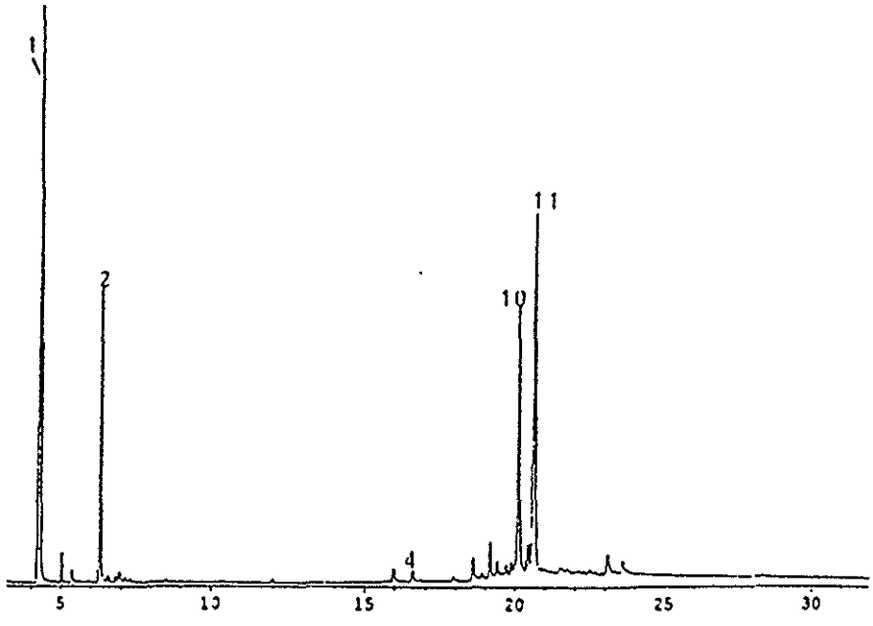


(b)
Time (min)

Figure 1. Gas Chromatographic (GC) analysis of hexane extract from *Dorstenia tubicina*. (a)rhizomes and (b) leaves.



(1)
Time (min)



(2)
Time (min)

Figure 2. Gas Chromatographic (GC) analysis of hexane extract from commercial samples (1 and 2).

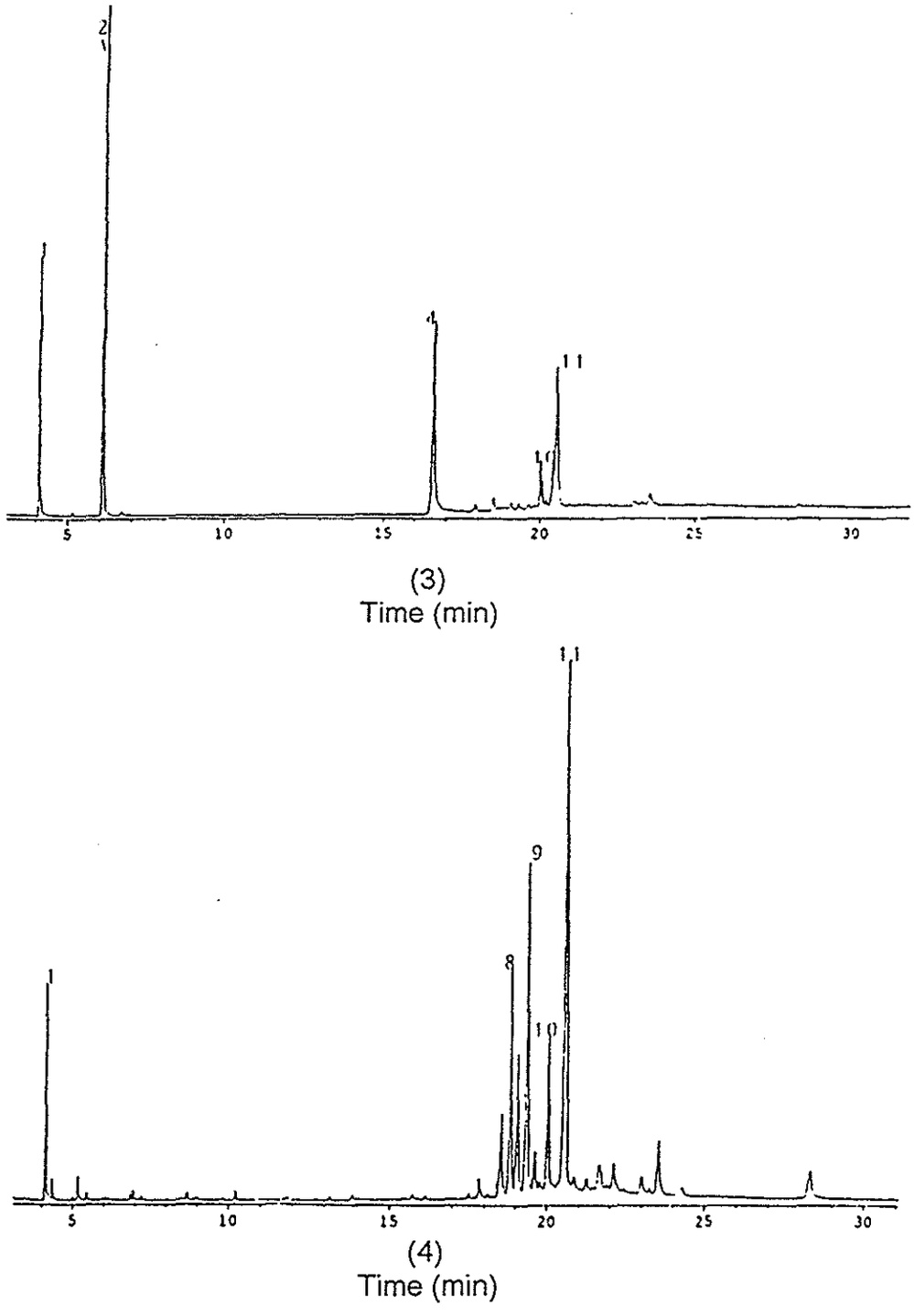


Figure 3. Gas chromatographic (GC) analysis of hexane extract from commercial samples (3 and 4).

The chromatographic pattern of the commercial samples 1 and 2 are similar to that of *D. tubicina*, with psoralen as the major compound. Sample 3 also presents these furanocoumarins, but bergapten predominates over psoralen. The monoterpenic furanocoumarin 4 was found only in samples 2 and 3. On the other hand, sample nr. 4 presented small amounts of psoralen and no bergapten, besides other isoprene derivatives in the range of 13.0-22.0 min, among which we could identify α- amyrin and β-amyrin. It is worth noting that only samples 2 and 3 were sold as the intact rhizome, while samples 1 and 4 were sold as a powder. Indeed, in sample 4 we could verify the presence of small parts of the rhizomes as well as of the leaves. Such type of addulteration is frequent and is done in order to increase the weight of the herb.

The sensitivity of the GC-FID and HRGC-MS for both furanocoumarins and isoprene derivatives allowed the fast chemical characterization of the constituents present in this yet uninvestigated *Dorstenia* specie without the necessity of isolating the individual known substances.

The chromatographic fingerprints of the authentic *Dorstenia tubicina* have permited the identification of the constituents from commercial samples and also the detection of possible adulterations.

ACKNOWLEDGMENTS

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a fellowship to C.A.L.C. and the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) for a grant to W.V.

REFERENCES

1. L.C. Di Stasi, C. A. Hiruma, E. M. Guimarães and C. M. Santos, *Fitoterapia 6*, 529 (1994).
2. M. Pio Correa, "Dicionário de plantas úteis do Brasil a das exóticas cultivadas", Vol.2, Ministério da Agricultura, Rio de Janeiro, 1926, p.161.
3. W. Vilegas, J.H.Y Vilegas and G.L. Pozetti, *Rev. Cienc. Farm.14*, 133 (1994).
4. D. Chouchi and D. Barth, *J. Chromatogr. 672* , 177 (1994).
5. D. A. Averbeck and E. Moustacchi, *Phytochem. Phytobiol. 31*, 475 (1980).
6. J.H.Y. Vilegas, F.M. Lanças, W. Vilegas and G.L Pozetti, *Phytochemical Analysis 4* 230 (1993).
7. W. Vilegas, J.H.Y. Vilegas and G.L. Pozetti, *Rev. Latinoamer. Quim. 23,* 133 (1994).

8. F.R. Stermitz and R.D. Thomas, *J. Chromatogr. 77* , 431 (1973).
9. H.J. Thompson and S.A. Brown, *J. Chromatogr. 314* , 323 (1984).
10. T. Wawrzynowicz and M. Waksmundzka-Hajnos, *J. Liq. Chomatogr. 13*, 3925 (1990).
11. W. B. Mors, M.C. Nascimento, J.P. Parente, G. da Silva Melo, P.A. Suarez- Kurtz, *Toxicon.27*, 1003 (1989).
12. N.A. Pereira, B.M.R. Pereira, M.C. Nascimento, J.P. Parente and W.B. Mors, *Planta. Med.60*, 99 (1994).
13. R.M. Kuster, R.R. Bernardo, A.J.R. Silva, J.P. Parente and W.B. Mors *Phytochemistry 36* , 221 (1994).
14. J.H.Y. Vilegas, W. Vilegas, C.F. Camilot, and G.L. Pozetti, *Rev. Cienc. Farm. 12* 89 (1990).
15. S. Matsunaga, R. Tanaka and M. Akagi, *Phytochemistry 27*, 535 (1988).
16. R.C. Heupel, *Phytochemistry 24* ,2929 (1985).
17. M. Hirohara, Y. Yasuoka, Y. Arai, K. Shiojima, H. Ageta and H.C. Chang *Phytochemistry 45* , 1023 (1997).
18. H. Budzikiewicz, J.M. Wilson and C. Djerassi, *J. Am. Chem. Soc. 85*, 3688(1963).

The SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY (ISSN: 2674-6891; 0104-5431) is an open-access journal since 1993. Journal DOI: 10.48141/SBJCHEM.
http://www.sbjchem.com.
This text was introduced in this file in 2021 for compliance reasons.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, Nº 8, 1999

FUNGITOXIC ACTIVITY OF COMPOUNDS ISOLATED FROM LICHENS

Neli K. Honda* Rosenei L. Brum and Maria Rita Marques[1]
Departamento de Química, [1] Departamento de Morfofisiologia, Universidade Federal de Mato Grosso do Sul, Caixa Postal 549; 79070-900 Campo Grande-MS, Brasil.

Marcelo P. Marcelli
Instituto de Botânica de São Paulo, Seção de Micologia e Liquenologia, Caixa Postal 4005; 01061-970 São Paulo-SP, Brasil.

Mariana Fleig
Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Av. Paulo Gama 110, 90040-370 Porto Alegre-RS, Brasil.

Wagner Vilegas
Departamento de Química Orgânica - Instituto de Química - UNESP, Rua Franscisco Degni s/n, Araraquara -SP, Brasil.

ABSTRACT

Lichens collected in Mato Grosso do Sul, Brazil, were analysed to their secondary metabolites. The compounds isolated were tested against the phytopathogenic fungus Cladosporium sphaerospermum, using a bioautography test. Diffractaic acid, atranorin/chloroatranorin, usnic acid and the technical artifact product ethyl orselinate inhibited the fungus growth.

RESUMO

Liquens coletados em Mato Grosso do Sul, Brasil, foram analisados quanto aos seus metabólitos secundários. Os compostos isolados foram testados quanto a atividade de inibição de crescimento do fungo fitopatogênico Cladosporium sphaerospermum usando a técnica de bioautografia. Atranorina/Cloroatranorina, ácido difractáico, ácido úsnico e o produto de artefato de técnica orselinato de etila inibiram o crescimento do fungo.

KEYWORDS : lichens, fungitoxic activity, *Cladosporium sphaerospermum*, secondary metabolites.

*Author to whom correspondence should adressed (nkhonda@nin.ufms.br)

DOI: 10.48141/SBJCHEM.v7.n8.1999.62_1999.pdf

INTRODUCTION

The lichens like many plants, were used since antiquity as drugs or sources of them[1]. These organisms produce secondary metabolites and many of them are known for presenting biological and/or phaarmacological activities, such as, antimicrobial[2-11], anti-tumor[12-15], antioxidant[16], analgesic and antipyretic[17] and others.

In spite of these properties, the search of new biological and pharmacological activities of these compounds remains important. The search of new antifungal substances has received special attention due to the few drugs existent and which, besides, are of limited efficacy in the treatment of systemic mycoses[18].

During our studies of lichen specimens we have isolated several compounds belonging to the classes of depsides, depsidones, dibenzofurans, and bis-xanthones which have been tested for their biological activities. This paper, describes the results of fungitoxic activities shown by some of these compounds. Evaluation of this activity has been determined by a bioautography method, using spores from the phytopathogenic fungus *Cladosporium sphaerospermum* Penzig.

EXPERIMENTAL

General Experimental Procedures

All melting points are uncorrected. The IR spectra were recorded in KBr with a Perkin Elmer 783 spectrometer. The ^{1}H-nmr spectra were measured at 300 MHz and ^{13}C-nmr at 75 MHz using a Brucker DPX-300 spectrometer. Spectra were measured in $CDCl_3$ and CD_3COCD_3, with chemical shifts reported in δ values (ppm) (using TMS as the internal standard) and coupling constants in Hz. Mass spectra (MS) were obtained from the Analytical Center of the Federal University of Paraíba, João Pessoa, PB, Brazil. Thin-layer chromatography (TLC) was performed on silica gel 60 using the solvent systems a) benzene : dioxane : acetic acid, 90: 25: 4 v/v/v; b) toluene : ethyl acetate : acetic acid, 6 : 4 : 1 v/v/v; and c) toluene : chloroform, 1 : 1 v/v. Spots were visuallized with $MeOH/H_2SO_4$ 10% and *p*-anisaldehyde/H_2SO_4.

Plant Material

The lichens *Parmotrema tinctorum* (Nyl.)Hale, *Ramalina continentalis* Malme, and *Usnea subcavata* Motika were collected in the vilage of Palmeiras and Piraputanga in Mato Grosso do Sul. A voucher specimen of each lichen is deposited in the herbarium of the Chemistry Department of the Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil.

Extraction and Isolation

The powder of each lichen (10.0 to 80.0 grams) was extracted in a Soxhlet apparatus with benzene and then with acetone or with chloroform only. The extracts were concentrated under reduced pressure on a rotaevaporator. Concentrated benzene and chloroform extracts obtained from *P. tinctorum* and *R. continentalis* were treated with ethanol several times to remove pigments. After this treatment, the residues were recrystallized from $CHCl_3$. Fractionation of the benzene extract from *U. subcavata* was by chromatography on a silica gel column with pure solvents and mixtures of hexane, dichloromethane, chloroform, acetone and methanol. The structures of compounds isolated and purified were elucidated by spectral analysis (IR, ^{1}H-nmr, ^{13}C-nmr, and MS).

Biossays

Stock solutions of pure compounds were prepared at 500 μg/mL in an appropriate solvent and were diluted to 250, 50 and 5 μg/mL. 20 μL of each solution corresponding to 10, 5, 1 and 0.1 μg were applied on a plate of silica gel (Merck). The plates were sprayed with a spore suspension of *Cladosporium sphaerospermum* in glucose and saline solution and incubated for 48 hours in the dark in a moist chamber at 30° C[19]. The fungitoxic activity was evaluated by comparison of the size (in mm) of the inhibition zones of fungus growth.

Fungus culture

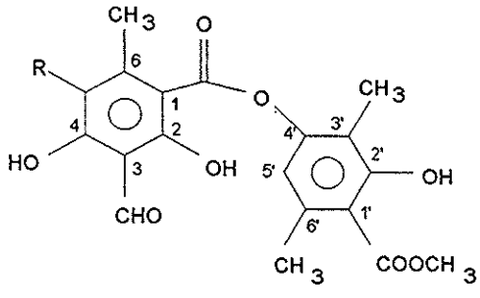
The fungus *Cladosporium sphaerospermum* Penzig was cultured in PDA (potato, dextrose, agar) medium at 28°C in the dark to obtain adequate sporulation. The maintenance of the innoculum was done according to the procedure described by Figueiredo and Pimentel[20].

RESULTS AND DISCUSSION

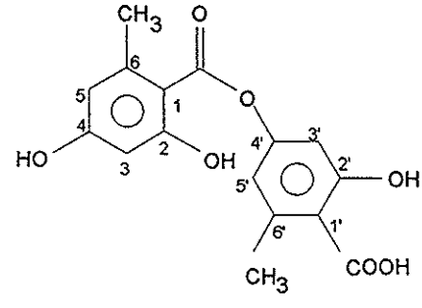
The melting points, IR, ^{1}H- and ^{13}C-nmr of the crystalline residue obtained from the benzene extract of *P. tinctorum* were identical to literature data for atranorin [21,22]. However the TLC of this residue in toluene-chloroform 1:1 v/v showed two components; possibly this fraction is a mixture of depsides atranorin (I) *[methyl (3-formyl-2,4-dihydroxy-6-methylbenzoyloxy-4' 2'-hydroxy-3',6'-dimethylbenzoate)]* and 5-chloroatranorin (II) *[methyl (3-formyl-2,4-dihydroxy-5-chloro-6-methylbenzoyloxy-4' 2'-hydroxy-3',6'-dimethylbenzoate)]*. The residue obtained from the acetone extract of this lichen was dissolved in diethyl ether and treated with $NaHCO_3/H_2SO_4$ [23]. By this procedure were isolated two compounds (III) and (IV). The IR, ^{1}H- and ^{13}C- nmr and mass spectra of these compounds correspond to those reported for the depside lecanoric acid (III)[23,24] *(2,4-dihydroxy-6-methylbenzoyloxy-4' 2'-hydroxy-6'-methylbenzoic acid)* and ethyl orsellinate (IV)[25] *(ethyl 2,4-dihydroxy-6-methylbenzoate)*. The last compound has not been cited for *P. tinctorum* and may have resulted from a technical artifact. The chloroform extract obtained from *Ramalina continentalis* after removal of pigments by treatment with ethanol, was recrystallized from $CHCl_3$ (mp 202-

203°C). It gave the same chromatographic behaviour as usnic acid (V) *[1,3(2H,9bH)-dibenzofurandione-2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl]* , and the IR and ^{1}H- nmr spectra were identical to literature values[21].

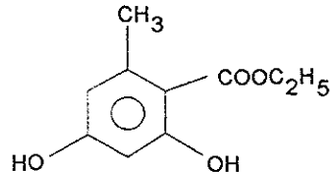
From *Usnea subcavata* we isolated usnic acid and the depside diffractaic acid (VI) *(2,4-dimethoxy-3,6-dimethylbenzoyloxy-4' 2'-hydroxy-3',6'-dimethylbenzoic acid).* These compounds were obtained by fractionation of the benzene extract by chromatography on silica gel column. The structural elucidation of the diffractaic acid was carried out by comparison of spectral data (IR, ^{1}H- and ^{13}C- nmr) with literature values[26, 27] and with the spectral data of an authentic sample of diffractaic acid.



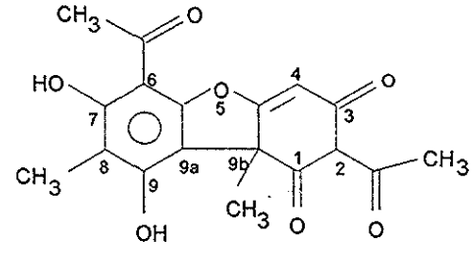
R = H Atranorin (I)
R = Cl 5-Chloroatranorin(II)



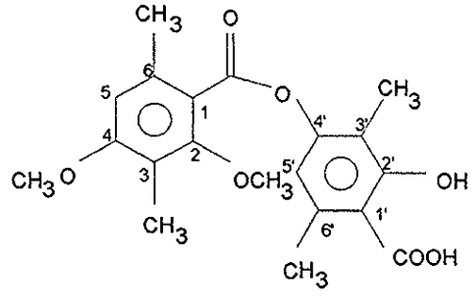
Lecanoric acid (III)



Ethyl orsellinate (IV)



Usnic acid (V)



Diffractaic acid (VI)

The results of the bioautography test using the fungus *Cladosporium sphaerospermum* (Table I), showed that atranorin/chloroatranorin and usnic acid are active in concentrations up to 1 μg. Lecanoric acid was not active at the concentrations tested. Ethyl orsellinate presented an inhibition zone larger that the other compounds at a concentration of 10 μg, however it was inactive in concentration of 1.0 μg . Although

Table 1. Activities of Compounds Isolated from Lichens Against *Cladosporium sphaerospermum*.

Compounds concentration (μg)	Atranorin (I) / Chloroatranorin (II)	Lecanoric acid (III)	Ethyl orsellinate (IV)	Usnic acid (V)	Diffractaic acid (VI)
	inhibition halo diameter (mm)				
10	11.0	0.0	15.0	8.0	4.0
5	9.0	0.0	8.0	8.0	3.0
1	8.0	0.0	0.0	7.0	0.0
0.1	0.0	0.0	0.0	0.0	0.0

the lichen compounds and phenolic derivatives are known to have activities against several microorganisms, the results here presented about the fungitoxic activities of lichenic compounds, and by the technical artifact product, ethyl orsellinate, suggest the need of additional investigations in order to evaluate the use of these compounds, or their derivatives, in the treatment of infections caused by these microorganisms.

ACKNOWLEDGMENTS

We thank to the Coordenadoria de Pesquisa/UFMS for the financial support.

REFERENCES

1. K. O. Vartia, Antibiotics in Lichens. In: V. Ahmadjian and M. E. Hale *The Lichens.* New York, Academic Press, (1973) p. 547-560.
2. P. R. Burkholder, A. W. Evans, I. McVeigh and H. K. Thornton, *Proc. Nat. Acad. Sci. Wash. 30,* 250- 255 (1944).
3. P. R. Burkholder and A.W. Evans, *Bull. Torrey. Bot. Club. 72,* 157-164 (1945).
4. S. Shibata and Y. Miura, *Jap. Med. J. 1,* 512-521 (1948).
5. A. Marshak, G.T. Barry and I. C. Craig, *Science 106,* 2756 (1947).
6. F. Bustinza, *Antibiotics and Chemotherapy I,* 443-446 (1951).

7. L. H. S. M. Cavalcanti, R.F. Maia, E. O. Lima and L. Xavier Filho, *Rev. Microb. São Paulo 14,* 168-171 (1983).
8. J. O. Silva, J. E. M. Leite, M. Q. Paulo and L. Xavier Filho, *Bol. Soc. Brot. 59,* 87-96 (1986).
9. L. Xavier Filho, J. E. M. Leite, M.Q. Paulo and J. O. Silva, *Bol. Soc. Brot. 60,* 79-86 (1987) .
10. E. C. Pereira, G.M. Campos-Takaki, L. Xavier Filho, N.H. Silva, C. Vicente and M. E. Legaz, *Bol. Soc. Brot. 64,* 173-186 (1991).
11. E.C. Pereira, N.H. Silva, E.S.B.A. Britto, J. Cruz and M.I..L. Silva, *Rev. UA 1,* 65-77 (1996).
12. S. M. Kupchan and H. L. Kopperman, *Experientia 31,* 625 (1975).
13. T. Hirayama, F. Fujikawa, T. Kasahara, M. Otsuka, N. Nishida and D. Mizuno, *Yakugaku Zasshi 100,* 755-759 (1980).
14. R.C. Lima, S.C. Nascimento, E.C. Pereira and G.M. Campos-Takaki, *Bol. Soc. Brot. 63,* 339-348 (1990).
15. E.C. Pereira, S.C. Nascimento, R.C. Lima, N.H. Silva, F.M. Oliveira, E. Bandeira, M. Boiatard, H. Beriel, C. Vicente and M. E. Legaz, *Tokai. J. exp. Clin. Med., 19,* 45-52 (1994) .
16. M. E. Hidalgo, E. Fernandez, W. Quilhot and E. Lissi, *Phytochemistry 37,* 1585-1587 (1994) .
17. E. Okuyama, K. Umeyama, M. Yamazaki, Y. Kinoshita and Y. Yamamoto, *Planta Med. 61,* 113-115 (1995).
18. C.D. Hufford and A.M. Clark, In : *"Studies in Natural Products Chemistry"* Atta-ur-Rahman Editor. Elsevier, Amsterdam, 1988, v.2, p. 421-452.
19. A.L. Homans and A. Fuchs, *J. Chromatogr . 51,* 327-329 (1970).
20. M. B. Figueiredo and C.P.V. Pimentel, *Summa Phytopath. 1,* 299-302 (1975).
21. S. Huneck, *"Lichen Substances"*. In : Reinhold L. & Liwschitz, Y. (orgs.) *Progress in Phytochemistry 1*, London, 1968, p. 223-346.
22. P. J. Hylands and K. Ingolfsdottir, *Phytochemistry 24,* 127-129 (1985).
23. G.B. Ahmann and A. Mathey, *Bryologist 70,* 93-97 (1967).
24. A. Sakurai and Y. Goto, *Bull. Chem. Soc. Jpn. 60,* 1917-1918 (1987).
25. C.J. Pouchert and J. Behnke, *"The Aldrich Library of* ^{13}C *and* ^{1}H *FTNMR Spectra"* 1st. ed. Aldrich Company, Milwaukee Wisconsin 1993, v.II p. 1258.
26. Y. Nishitoba, H. Nishimura, T. Nishiyama and J. Mizutani, *Phytochemistry 26,* 3181-3185 (1987) .
27. E.G. Sudholm and S. Huneck, *Chemica Scripta 18*, 233-236 (1981).

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

LIQUID MEMBRANE ION-SELECTIVE ELECTRODES FOR POTENTIOMETRIC DOSAGE OF SOME METAL IONS

Maria Pleniceanu*, Luminiţa Simoiu, Marian Isvoranu, Mihaela Băniceru

Department of Chemistry, Faculty of Sciences, University of Craiova, A.I. Cuza 13,1100 Craiova, Romania

ABSTRACT

Four electrodes with liquid membrane, Cu^{2+} selective and Ni^{2+} selective, not described previously in the literature, were prepared and characterized. From these electrodes, 1 and 2 are based on simple complexes of Cu(II) and Ni(II) with 2,3-dithio-6-nitro-benzoquinoxaline-5,10-dione (dtnbqd), as ligand belonging to the dithiol class. Electrodes 3 and 4 are based on the corresponding mixed complexes in which the ammonia molecule acts as the second ligand and 2,3-dithio-6-nitro-benzoquinoxaline-5,10-dione as the first ligand.

KEYWORDS *copper, nickel, liquid-membrane electrodes, potentiometry, selectivity.*

RESUMO

Foram preparados e caracterizados quatro eletrodos novos com membrana líquida seletivos para Cu^{++} e Ni^{++}. Os eletrodos 1 e 2 foram baseados em complexos simples de Cu(II) e Ni(II) com 2,3-ditio-6-nitro-benzoquinoxalina-5,10-diona (dtnbqd), um ligante da classe do ditiol. Os eletrodos 3 e 4 foram baseados nos complexos mistos correspondentes, onde uma molécula de amonia age como o segundo ligante e 2,3-ditio-6-nitro-benzoquinoxalina-5,10-diona age como o primeiro ligante.

DOI: 10.48141/SBJCHEM.v7.n8.1999.68_1999.pdf

INTRODUCTION

During the past years, ion-selective electrodes for a large series of cations and anions and their use in solving various analytical problems, in industrial processes, the automated control of environmental pollution, biochemistry etc., have been described in the literature[1-6].

This study is a continuation of our previous work described in earlier papers[7-12] and presents the possibility of obtaining Cu^{2+} and Ni^{2+} selective electrodes with a liquid membrane. These electrodes are based on complex combinations of Cu(II) and Ni(II), extractable in organic solvents, non-miscible in water.

The response of these electrodes to the concentration of Cu^{2+} (Ni^{2+}) in solution has been formally attributed to a process of exchange of Cu^{2+} (Ni^{2+}) ions between the analyzed aqueous solution and the solution of the membrane in nitrobenzene.

Considering this thermodynamic hypothesis, the expression of the membrane potential was derived:

$$E = E^{o} + RT/2F \ln a_{M}^{2+} \qquad (1)$$

where a_M^{2+} is the activity of the Cu^{2+} or Ni^{2+} ions in aqueous solution. The possibility of using simple and mixed complexes of Cu(II) and Ni(II) for obtaining selective membranes for the copper or nickel ions has thus been proved.

EXPERIMENTAL

Materials. Analytical grade $CuSO_4$, $Ni(NO_3)_2$ and nitrobenzene with a purity of better than 99% were supplied by Merck. Water used was doubly distilled and deionized. The simple and mixed complexes used as ion selective membranes were synthesized according to a procedure previously described[13-15] and characterized by elemental analysis, UV/VIS spectroscopy, IR spectroscopy and ESR.

Equipment. The electrode used for the determinations has been made according to a procedure previously described by Pleniceanu et al[12].

Construction of the electrode. The body of the electrode is made of a 75 mm long teflon tube (1) (Fig. 1) with an inside diameter of 6 mm at the lower end, where it is closed with a 15 mm long graphite rod (2). This is impregnated with the solution of the active substance (10^{-3} M) in nitrobenzene. The internal reference solution (3) is made up of nitrobenzene in which the complex combination is dissolved. The graphite rod plays both the role of mechanical support for the liquid membrane and electrical support, taking over the electrical potential of the membrane when this one is in contact with the aqueous phase.

The internal reference electrode has been eliminated by introducing a 115 mm long stainless-steel wire (4) having a diameter of 1.5 mm. The wire was introduced inside the graphite rod and makes contact with the connection terminal (6) of the measuring instrument.

In order to prevent evaporation of the internal solution and, as a consequence, any change of concentration in the membrane, the electrode is equipped with a screwed teflon stopper (5) at the top through which the stainless-steel wire

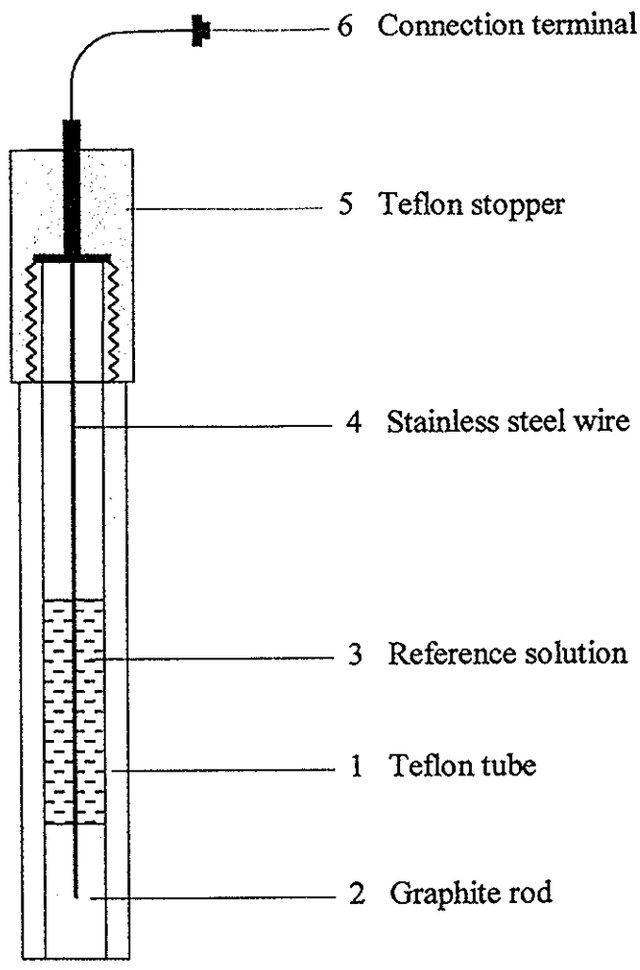
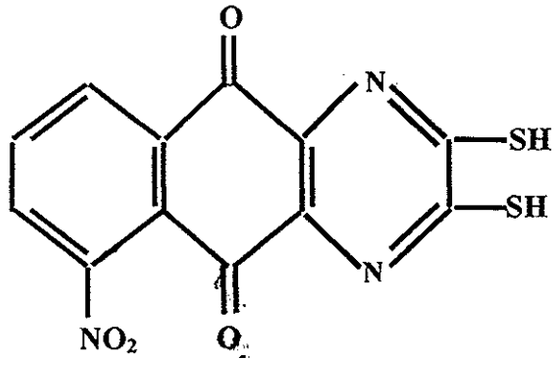


Fig.1 Schematic representation of the electrode.

penetrates, the system being perfectly air tight. The teflon stopper is detachable and allows the filling of the electrode with the organic solution (3).

Electrodes employed. Four new Cu^{2+} and Ni^{2+} selective electrodes with a liquid membrane have been obtained and characterised. Among these, electrodes 1 and 2 are based on simple complexes of Cu(II) and Ni(II) with a ligand of the dithiol class, 2,3-dithio-6-nitro-benzoquinoxaline-5,10-dione, which has the formula shown below:



Electrodes 3 and 4 are based on the corresponding mixed complex combinations with an ammonia molecule, which is present in the structure as a second ligand, along with the ligand belonging to the dithiol class. The formula of the simple and mixed complex combinations of Cu(II) and Ni(II) whose solutions in nitrobenzene constitute the membrane on a graphite rod for the electrodes under consideration are as follows:

Electrode 1: $[(n\text{-}C_4H_9)_4N]_2[Cu(dtnbqd)_2]$;
Electrode 2: $[(n\text{-}C_4H_9)_4N]_2[Ni(dtnbqd)_2]$
Electrode 3: $[Cu(NH_3)_2(dtnbqd)]$;
Electrode 4: $[Ni(NH_3)_2(dtnbqd)]$.

RESULTS

Response of the electrodes to the concentration of Cu^{2+} (Ni^{2+}) ions. Table 1 illustrates the variation of electromotive force obtained with the four ion-selective electrodes at 25 °C and an ionic strength $\mu = 0.4$ (obtained with KNO_3 which does not influence the electrode potential), depending on the concentration of Cu^{2+} and Ni^{2+} ions.

Table 1

The values of the electromotive force of the Cu^{2+} and Ni^{2+} selective electrodes vs. the saturated calomel electrode (SCE) at

25°C, $\mu = 0.4$ (KNO_3) and pH = 4 – 4.2

Electrode 1		Electrode 2		Electrode 3		Electrode 4	
Conc. of Cu^{2+} ions (M)	E (mV)	Conc. of Ni^{2+} ions (M)	E (mV)	Conc. of Cu^{2+} ions (M)	E (mV)	Conc. of Ni^{2+} ions (M)	E (mV)
10^{-1}	330.5	10^{-1}	302	10^{-1}	420	10^{-1}	410
10^{-2}	301.5	10^{-2}	273	10^{-2}	391	10^{-2}	381
10^{-3}	272.5	10^{-3}	244	10^{-3}	362	10^{-3}	352
10^{-4}	243.5	10^{-4}	215	10^{-4}	333	10^{-4}	323
10^{-5}	214.5	10^{-5}	186	10^{-5}	304	10^{-5}	294
10^{-6}	206.5	10^{-6}	171	10^{-6}	275	10^{-6}	265
10^{-7}	-	10^{-7}	-	10^{-7}	263	10^{-7}	251

The influence of pH on the response of the Cu^{2+} (Ni^{2+})-selective electrodes was also studied. For very acidic solutions a part of the 2,3-dithio-6-nitro-benzoquinoxaline-5,10-dione in the mixed complexes is transformed into free dithiol and the electrode potential measured in acid solution are greater. The electrode potential measurements in alkaline solutions have smaller values, because some of the Cu^{2+} (Ni^{2+}) cations are precipitated as hydroxide in alkaline medium.

The pH of the aqueous solution of Cu^{2+} (Ni^{2+}) was set at the desired value by means of Kolthoff and Vleeschhouwer buffer solutions. The pH measurements were made with a MV 85 pH-meter, using a glass electrode and a saturated calomel electrode. Variation of pH between 2.5 and 6.35 for Cu^{2+} and 2 and 8.6 for Ni^{2+} does not affect

the membrane potential. As a consequence the linear portion of the E-pH curves is a function of the Cu^{2+} (Ni^{2+}) concentration in the aqueous phase (Fig. 2, Fig. 3). All the direct measurements of the potential were carried out in solutions of $CuSO_4$, and $Ni(NO_3)_2$ respectively at pH 4 and 4.2, obtained with a CH_3COOH 0.2 M + CH_3COONa 0.2 M buffer solution (82 ml CH_3COOH 0.2 M + 18 ml CH_3COONa 0.2 M for pH = 4; 73.5 ml CH_3COOH 0.2 M + 26.5 ml CH_3COONa 0.2 M for pH = 4.2).

Selectivity of the electrodes. The selectivity constants for the cations indicated in Table 2 were estimated by Srinivasan and Rechnitz's method[16] and checked by Eisenman's procedure[17].

From Table 2 it can be observed that the newly developed electrodes have a cationic response $\Delta E/\Delta \log c$ of 29 mV at 25 °C. This value corresponds to a Nernstian slope of RT/2F. The Nernstian response is obeyed in the concentration range 10^{-1}-10^{-5} M for electrodes 1 and 2 based on the simple complexes, respectively 10^{-1}-10^{-6} M for electrodes 3 and 4, based on the corresponding mixed complexes. Consequently the Cu^{2+} (Ni^{2+}) selective electrodes can be used within these concentration ranges for potentiometric determination of copper and nickel.

According to the experimental data given in Table 2, by comparison with the electrodes based on simple complexes, the Cu^{2+} (Ni^{2+}) selective electrodes based on mixed complexes have a wider range of linear response and a better selectivity for the interfering ions.

Dynamic response and reproducibility of electrodes. The response characteristics of the Cu^{2+} (Ni^{2+}) selective electrodes were estimated using solutions of $CuSO_4$ and $Ni(NO_3)_2$ having different concentrations, (usually 10 times higher) and by recording the values of the potentials as a function of time. The response times of the electrodes in dilute solutions (10^{-4}-10^{-6} M) were of about 2 minutes, whereas for more concentrated solutions (10^{-1}-10^{-3} M), the electrode potential reached an equilibrium value in about 20 seconds.

The reproducibility of the potential measurements was checked during a period of 3-5 weeks for the concentration range 10^{-1}-10^{-5} M $CuSO_4$ ($Ni(NO_3)_2$) in case of electrodes 1 and 2, respectively, 10^{-1}-10^{-6} M for electrodes 3 and 4 (μ = 0.4).

Table 2

The characteristics of Cu^{2+} (Ni^{2+}) selective electrodes (based on simple and mixed complexes) at 25 °C, a constant ionic strength μ = 0.4 (KNO_3) and pH = 4–4.2

Electrode M^{2+} -selective	$\Delta E/\Delta \log c$ (mV)	Range of linear response (M)	Constants of selectivity for the cations:			
			Cu^{2+}	Ni^{2+}	Fe^{2+}	Co^{2+}
1	29	10^{-1}-10^{-5}	-	$8 \cdot 10^{-4}$	$5.55 \cdot 10^{-4}$	$3.12 \cdot 10^{-3}$
2	29	10^{-1}-10^{-5}	$1.07 \cdot 10^{-3}$	-	$6.03 \cdot 10^{-4}$	$3.02 \cdot 10^{-3}$
3	29	10^{-1}-10^{-6}	-	$2.89 \cdot 10^{-4}$	$1.89 \cdot 10^{-4}$	$6.5 \cdot 10^{-4}$
4	29	10^{-1}-10^{-6}	$4.48 \cdot 10^{-4}$	-	$1.19 \cdot 10^{-4}$	$9.05 \cdot 10^{-4}$

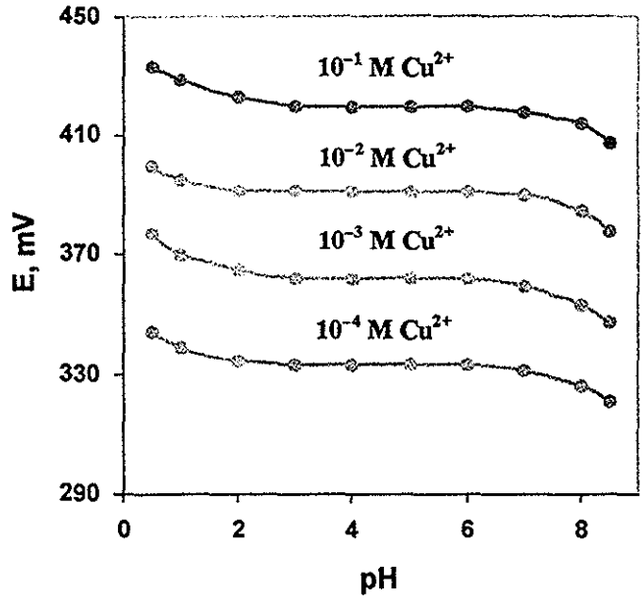


Fig.2 The effect of pH on the response of the Cu^{2+} selective electrode with $[Cu(NH_3)_2(dtnbqd)]$.

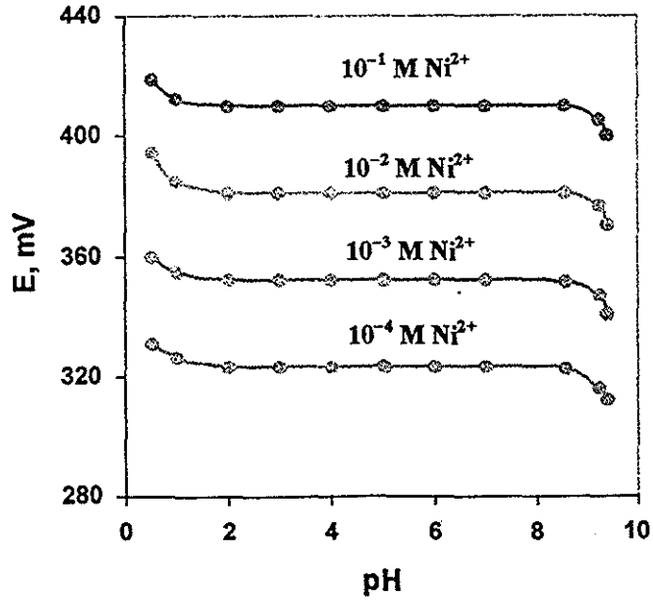


Fig.3 The effect of pH on the response of the Ni^{2+} selective electrode with $[Ni(NH_3)_2(dtnbqd)]$.

Fig. 4.1

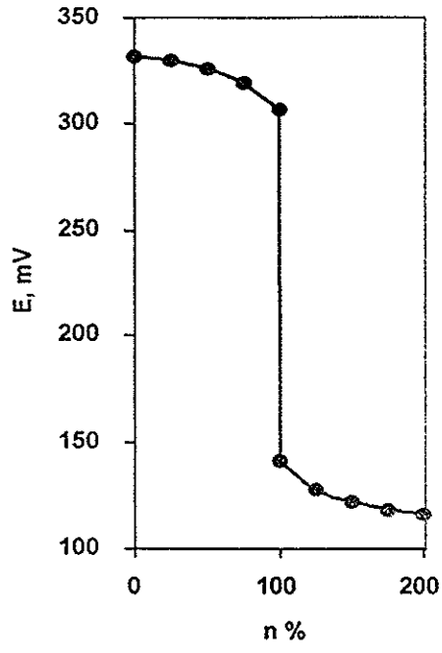


Fig. 4.2

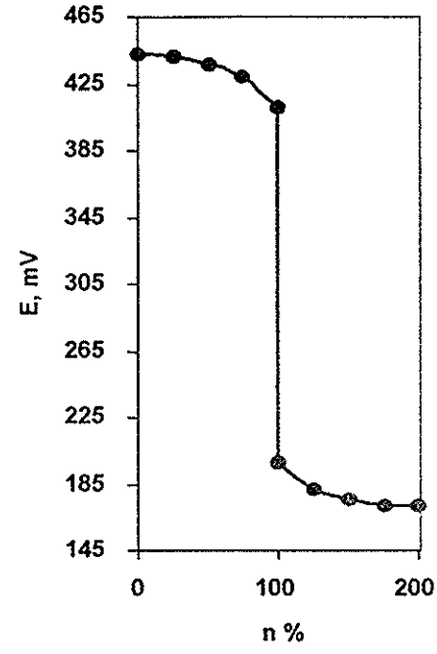


Fig.4 The potentiometric titration curves of Cu^{2+} ions with EDTA, obtained by using the Cu^{2+} selective electrodes based on simple complex, with a membrane of $[(n\text{-}C_4H_9)_4N]_2[Cu(dtnbqd)_2]$ (Fig. 4.1), and mixed complex, with a membrane of $[Cu(NH_3)_2(dtnbqd)]$, (Fig. 4.2).

ANALYTICAL APPLICATIONS

The Cu^{2+} (Ni^{2+}) selective electrodes have been used to determine the Cu^{2+} (Ni^{2+}) ions in aqueous solution both by direct potentiometry and by potentiometric titration with EDTA.

For the direct potentiometric determination, a calibration curve was used. This has been obtained by the variation of the electrode potential of the Cu^{2+} (Ni^{2+}) selective electrodes 1, 2, 3, 4, as a function of $-\log[M^{2+}]$, versus the saturated calomel electrode (SCE) as external reference electrode. The experimental data are shown in Table 1, and are obtained at 25 °C, $\mu = 0.4$ (KNO_3) and pH 4 (4.2). The lower M^{2+}

concentration limit, that can be determined by direct potentiometry is of 10^{-5} M with electrodes 1 and 2 and of 10^{-6} M with electrodes 3 and 4.

The electrodes were also tested for potentiometric titration with EDTA by using 10^{-3} M titrated solutions of $CuSO_4$ ($Ni(NO_3)_2$). These titrations were based on well-defined titration curves and a potential change of 162.5 and 160 mV for electrodes 1 and 2 was observed (Fig. 4). In the case of electrodes 3 and 4 much larger changes of 213.5 and 202 mV were obtained (Fig. 5).

Determination of copper and nickel by direct potentiometry in industrial waters. Samples of water originating from a water treatment station were analyzed; the concentration of copper and nickel has been determined for every sample by atomic absorption spectrometry (AAS). The content of copper was determined by direct potentiometry using the Cu^{2+} selective electrode No. 3, based on $[Cu(NH_3)_2(dtnbqd)]$.

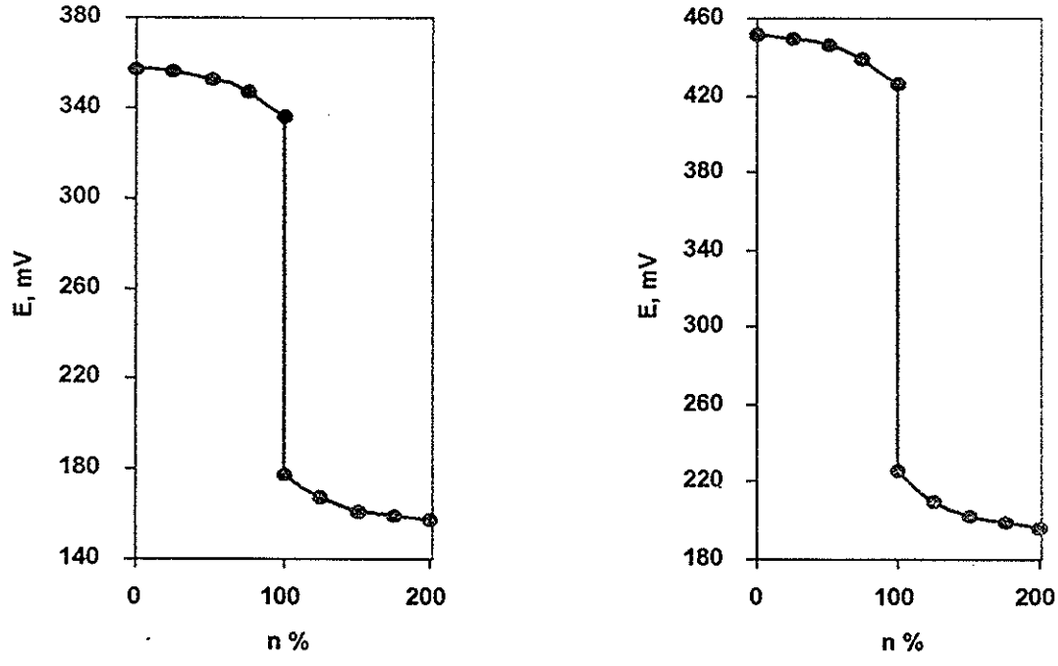


Fig.5 The potentiometric titration curves of Ni^{2+} ions with EDTA, obtained by using the Ni^{2+} selective electrodes based on simple complex, with a membrane of $[(n\text{-}C_4H_9)_4N]_2[Ni(dtnbqd)_2]$ (Fig. 5.1), and mixed complex, with a membrane of $[Ni(NH_3)_2(dtnbqd)]$, (Fig. 5.2).

Table 3

Results of Cu^{2+} (Ni^{2+}) ion determinations in industrial waters

Sample No.	Cu^{2+} (mg L^{-1})		Ni^{2+} (mg L^{-1})	
	Potentiometrically, with the Cu^{2+}-selective electrode No.3	By AAS	Potentiometrically, with the Ni^{2+}-selective electrode No.4	By AAS
1	1.01	1.06	0.15	0.20
2	1.79	1.80	0.21	0.25
3	0.80	0.79	0.09	0.13
4	1.01	1.10	0.18	0.20
5	1.59	1.65	0.23	0.28
6	3.57	3.70	0.11	0.16
7	3.18	3.27	0.29	0.35
8	2.84	2.91	0.33	0.35
9	2.01	2.10	0.42	0.46
10	1.27	1.35	0.19	0.20

Table 4

Results of Cu^{2+} ions determination in industrial waters using the method of standard additions

Sample No.	Cu^{2+} (mg L^{-1})			
	Initial Cu^{2+} (AAS method)	Cu^{2+} added	Theoretical total of Cu^{2+}	Experimental Cu^{2+}, with electrode No.3
1	0.79	1.00	1.79	1.75
2	1.06	1.00	2.06	2.00
3	1.65	1.00	2.65	2.60
4	2.91	0.50	3.41	3.36
5	3.70	0.50	4.20	4.16

Table 5

Results of Ni^{2+} ions determination in industrial waters using the method of standard additions

Sample No.	Ni^{2+}(mg L^{-1})			
	Initial Ni^{2+} (AAS method)	Ni^{2+} added	Theoretical total of Ni^{2+}	Experimental Ni^{2+}, with electrode No.4
1	0.20	2.00	2.20	2.17
2	0.25	1.50	1.75	1.70
3	0.28	1.50	1.78	1.73
4	0.35	1.75	2.10	2.06
5	0.46	1.50	1.96	1.92

In the same way, the content of nickel was determined by using the Ni^{2+} selective electrode No. 4, based on $[Ni(NH_3)_2(dtnbqd)]$.

The results of the experimental determinations are given in Table 3, representing the average values of the 6 measurements. It can be observed that the results obtained with the Cu^{2+} selective electrode No. 3 and the Ni^{2+} selective electrode No. 4 are in agreement with those obtained by AAS. To certify the advantages of using the potentiometric method with the mentioned Cu^{2+} and Ni^{2+} selective electrodes, the experimental data were checked by using the method of standard additions. The results given in Table 4 and Table 5 indicate a good agreement of the AAS and direct potentiometric methods. Also, they suggest that the use of the Cu^{2+} (Ni^{2+}) selective electrodes based on mixed complexes for the determination of cooper (nickel) ions in industrial waters is very appropriate

CONCLUSIONS

Four electrodes with liquid membrane, Cu^{2+} and Ni^{2+} ion-selective electrodes were prepared and characterized. The following characteristics were studied:

- the electrodes response to Cu^{2+} (Ni^{2+}) ion concentration;
- the influence of pH on the response of the Cu^{2+} (Ni^{2+}) selective electrodes;
- the selectivity of the electrodes;
- the dynamic response and reproducibility of the electrodes;

The analytical applications of these electrodes have also been studied by using the direct potentiometric and potentiometric titration methods.

The ion selective electrodes based on mixed complexes have been used for the determination of copper and nickel ions in industrial waters.

The electrodes with mixed complex membranes have a better selectivity for interfering ions and a much larger potential rise on the titration curve. For this reason, they are of practical interest for copper and nickel ion determinations in solutions that are more dilute and in industrial waters.

REFERENCES

1 R. P. Buck, *Analyt. Chem.*, **45**, 28 (1974).
2 E. Pungor and K. Toth, *Kem. Kozlem.*, **39**, 1 (1973).
3 K. Cammaner, *Das Arbeiten mit Ione-Selecktiven Electroden*, Ed.Springer, Berlin (1973).
4 C. Luca and G. Semenescu, *Rev. Chim.*, **26**, 946 (1975).
5 E. Hopîrtean, E. Veress and C. Liteanu, *Rev. Chim.*, **26**, 167 (1975).
6 G. Baiulescu, V. Coşofreţ and C. Cristescu, *Rev. Chim.*, **26**, 429 (1975).
7 C. Luca, M. Pleniceanu and N. Mureşan, *Rev. Chim.*, **43** , 64 (1992).
8 C. Luca, M. Pleniceanu and N. Mureşan, *Rev. Roum. Chim.*, **37**, 555 (1992).

9 M. Pleniceanu, *Rev. Chim.*, **45**, 596 (1994).
10 M. Pleniceanu, Al. Popescu, M. Preda and M. Băniceru, *Rev. Chim.*, **46**, 929 (1995).
11 M. Pleniceanu, M. Preda, N. Mureşan and L. Simoiu, *Anal. Lett.*, **29**, 1485 (1996).
12 M. Pleniceanu, M. Preda, N. Mureşan and C. Spînu, *Bull. Soc. Chim. Fr.*, **134**, 177 (1997).
13 N. Mureşan, *Doctoral Thesis*, Inst. Politehnic Bucureşti, (1975).
14 N. Mureşan, L. S. Mureşan and V. Mureşan, *Anal. Univ. Craiova, Chim.*, **21**, 22 (1993).
15 M. Pleniceanu, *Doctoral Thesis*, Inst. Politehnic Bucureşti, (1986).
16 K. Srinivasan and G. A. Rechnitz, *Anal. Chem.*, **41**, 1203 (1969).
17 G. Eisenman, *Anal. Chem.*, **44**, 1545 (1972).

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

RECOVERY OF MERCURY FROM DENTAL AMALGAMS COLLECTED IN THE NORTHWEST REGION OF PARANA STATE, BRAZIL

Rogerio B. Brasil[1]; Claudenice Rodrigues[1], and Jorge Nozaki[2]
(1) CNPq-PIBIC/UEM-Fellowships (Brazil)
([2]) Departamento de Química, Universidade Estadual de Maringá-UEM.
CEP: 87020-900 Maringá-PR, Brasil.
Fax:(044) 263-5116
E-mail: jnozaki@cybertelecom.com.br

ABSTRACT

Wastes amalgams were collected from dental cabinets of northwest region of the Parana State, Brazil. Amalgams collected were washed with 0.1M nitric acid and distilled water, dried at 80^{0}C, and crushed to 100 mesh. Digestion of the brown powder were performed using two types of reactors. The first was made of pyrex glass and the second reactor of stainless steel, both connected to a PVC tube with MnO_2 and a vacuum pump. The efficiency observed was higher for the second reactor, with 96-98% of mercury recovered, and the minimum time necessary for digestion was 180 minutes. After the digestion, the mercury was transferred to a conventional apparatus for mercury distillation and purification. The distillation was performed two times and the final mercury recovered was 99% pure. Silver and tin were also recovered by chemical and electrochemical methods after the mercury separation

RESUMO

Resíduos de amálgamas dentárias foram coletados em gabinetes odontológicos na região noroeste do Estado do Paraná, Brasil. As amálgamas coletadas foram lavadas com ácido nítrico 0,1 M e água destilada, secadas a 80^{0}C e trituradas a 100 mesh. As digestões dos sólidos escuros foram realizadas em dois tipos de reatores. O primeiro reator foi construído em vidro pyrex e o segundo em aço inoxidável e, ambos foram conectados a tubos de PVC, recheados com dióxido de manganês, e então conectados a uma bomba de vácuo. O reator de aço inox mostrou maior eficiência com a recuperação de 96 a 98% do mercúrio, com o tempo mínimo necessário de 180 minutos para a digestão. Após a digestão, o mercúrio foi transferido para um aparelho convencional de destilação e purificação do mercúrio. Após a bi-destilação recuperou-se o mercúrio com 99% de pureza. A prata e o estanho também foram recuperados por métodos químicos e eletroquímicos.

(2) To whom correspondence should be addressed

Key words: dental amalgams; mercury; environment; pollution; metal recovery

DOI: 10.48141/SBJCHEM.v7.n8.1999.79_1999.pdf

INTRODUCTION

Amalgams are mixtures of metals and mercury used to fill the teeth ,and wastes of dental amalgams consist of old amalgam, and pieces of fresh amalgam from procedures to replace deterioted fillings. Mercury (48-60%), silver (15-37%), tin (12-13%), copper (0-20%), zinc (0-1%) , and palladium (0-2%) are the average composition of dental amalgams used in dentistry. Studies performed with dental amalgams using a radioactive mercury isotope (Hg^{203}) mixed with dental amalgams fillings, and placed in the teeth of adult sheep, showed that the isotope appeared in various organs and tissues within 29 days. Evidence of mercury uptake, as determined by whole-body scanning and measurement of isotope in specific tissues, revealed three specific absorption sites: lungs, gastrointestinal tract and mandibular tissue[1]. Once absorbed, high concentrations of the isotope were found localized in the kidneys and liver.

The effect of mercury on the nervous system is a selective inhibition of protein and amino acid absorption into brain tissue. It inhibits the synaptic uptake of neurotransmitters in the brain and can produce subsequent development of Parkinson's disease. Mercury is also nephrotoxic and causes serious pathological damage[2]. Chronic exposure to mercury may cause an excess of serum proteins in the urine which may progress to nephrotic syndrome and peculiar susceptibility to infections that break into and modify the course of any pre-existing disease[3,4]. Mercury fillings can contribute to a higher level of mercury in the blood, and can affect the functioning of the heart, change the vascular response to norepinepherine and potassium chloride, and block the entry of calcium ions into cytoplasm[5]. It was also studied that mercury released from dental fillings by the practice of using chewing-gun were four times higher compared to the other patients[6]. Mercury in human body can contribute to intelligence disturbances, speech difficulties, limb deformity, and hyperkinesia (hyperactivity resulting from brain damage). Backgrounds levels of mercury in mothers correlate with incidence of fetal birth defects and still births[7].

Wastes of dense, apparently harmless, dental amalgams are usually discarded as refuse in the environment. Mercury is a heavy metal, liquid at room temperature, and with a boiling point of 358^{0}C. However, even at room temperature, the equilibrium $Hg_{(l)} = Hg_{(v)}$ exist and it slowly evaporates, emitting mercury vapor. Mercury can be converted to methyl mercury [$(CH_3)_2Hg$] by the action of anaerobic bacteria such as **Methanobacterium omelanskii**, and methyl mercury is by far the most toxic of mercury compounds. On the other hand, dental amalgams are dense solids, with high consistency and stability, and their boiling points are higher than pure inorganic mercury. Up to the present there is no clear indication about dissolution and contamination of water streams by mercury compounds released from dental amalgams. However, serious environmental problems concerned to dental amalgams are still not clear, because the temperature of furnace for cremating and solid wastes incineration could be high enough for dissolution and release of mercury vapor.

The imported mercury used in dental fillings, gold prospection, caustic soda, and chlorine production in Brazil was approximately 340 ton/year, up to 1993. Besides the amount deducted from importation, the recovery of mercury is also very important for the protection of our environment.

MATERIALS AND METHODS

Dental amalgams were collected by Dr. Jales A. Cardoso (15ª Regional de Saude de Maringa) from dental cabinets of 29 municipal districts, including Maringa County, from the northwest region of Parana State, Brazil. The amounts collected were: 30 kg (1994-1995), 40 kg (1996), and 70 kg (1997).

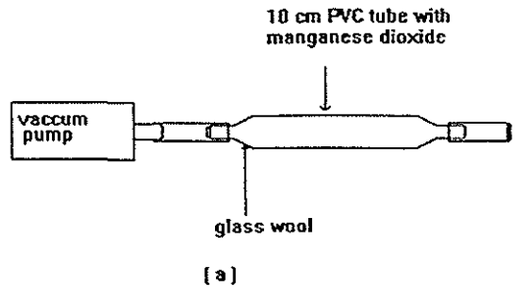
After washing with diluted nitric acid and distilled water, and drying at 80°C, the solids were crushed to a fine powder (about 100 mesh). The digestion of the brown powder was performed in two types of reactors. The first, a 500 mL pirex round-bottom flask was conected to the botton of a 250 mL pyrex erlenmeyer, and the erlenmeyer was connected to a condenser. The condenser was connected to a 1000 mL pyrex heavy-wall filtering flask, and it was connected to a 100 x 10 mm PVC tube with manganese dioxide (MnO_2 - TRONA CHEMICALS-USA), and then to a vacuum pump. As shown in a previous paper[8], manganese dioxide is an excellent material for absorption of gaseous substances including mercury, and was used to protect the vacuum pump and the environment from mercury contamination (Figure 1). The second reactor was made of stainless steel as shown in Figure 2, and a 100 x 10 mm tube of PVC with MnO_2 was also connected between vacuum pump and the filtering flask.

Powder of dental amalgams, approximately 100 g for the first and about 200 g for the second reactor, were used for each digestion. The temperature was slowly and progressively changed, and the dissolution and volatilization of Hg began at ~290-300°C. The distilled mercury collected in the filtering flask was transferred to a conventional mercury distillation apparatus for purification (Figure 6). To the collecting flask of this apparatus a 100 x 10 mm PVC tube with MnO_2 was also connected to protect the vacuum pump and the environment from contamination by mercury vapor. After the second distillation the mercury was transferred to a narrow-mouth bottle with screw cap and stored.

RESULTS AND DISCUSSION

The main disadvantage of the glass reactor was the amount used (~100 g) for digestion. Mercury liquefaction in the corner of the erlenmeyer and before the condenser was another problem observed, and solved by changing of reactor design, as shown in Figure 1. The residue left after digestion and mercury separation was constituted by the oxides of tin, silver,copper, etc. Mercury determination in this residue by cold vapor atomic absorption spectrometry was an important step to calculate the efficiency of mercury separation and recovery. The average efficiency observed was 95% for the glass reactor and 96-98% using the stainless steel reactor. The mercury released as vapor and absorbed on MnO_2 was also determined by cold vapor atomic absorption spectrometry.

Figure 2 shows the stainless steel reactor. The main disadvantage was the superficial corrosion observed after several hours of heating. With 200 g of brown powder inside the reactor, the temperature was slowly increased and the dissolution and volatilization of mercury began at 290-300°C using the PVC tube with MnO_2 and a vacuum pump. Without the vacuum pump and the PVC tube with MnO_2, the minimum temperature for digestion and volatilization of mercury was > 500°C. Figure 3 shows the efficiency of mercury recovered as function of heating time using stainless steel reactor. The amount of manganese dioxide used inside the PVC tube is very important. Higher amounts of manganese dioxide required higher temperature for mercury volatilization as shown in Figure 4, and 25-30g of manganese dioxide should be used and



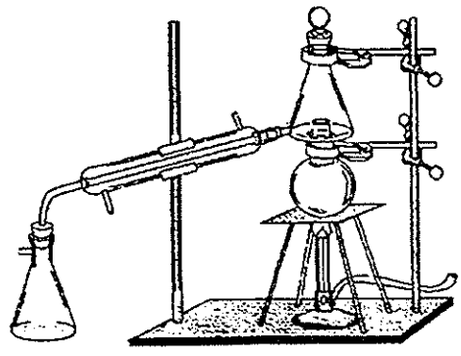


Figure 1. Glass reactor used for amalgams digestion. A 500 mL pirex round-bottom flask, with ~100g of amalgam powder (100 mesh), was used as reactor.[a] 10cm PVC tube with manganese dioxide and glass wool was connected between the vacuum pump and an erlenmeyer collecting flask.

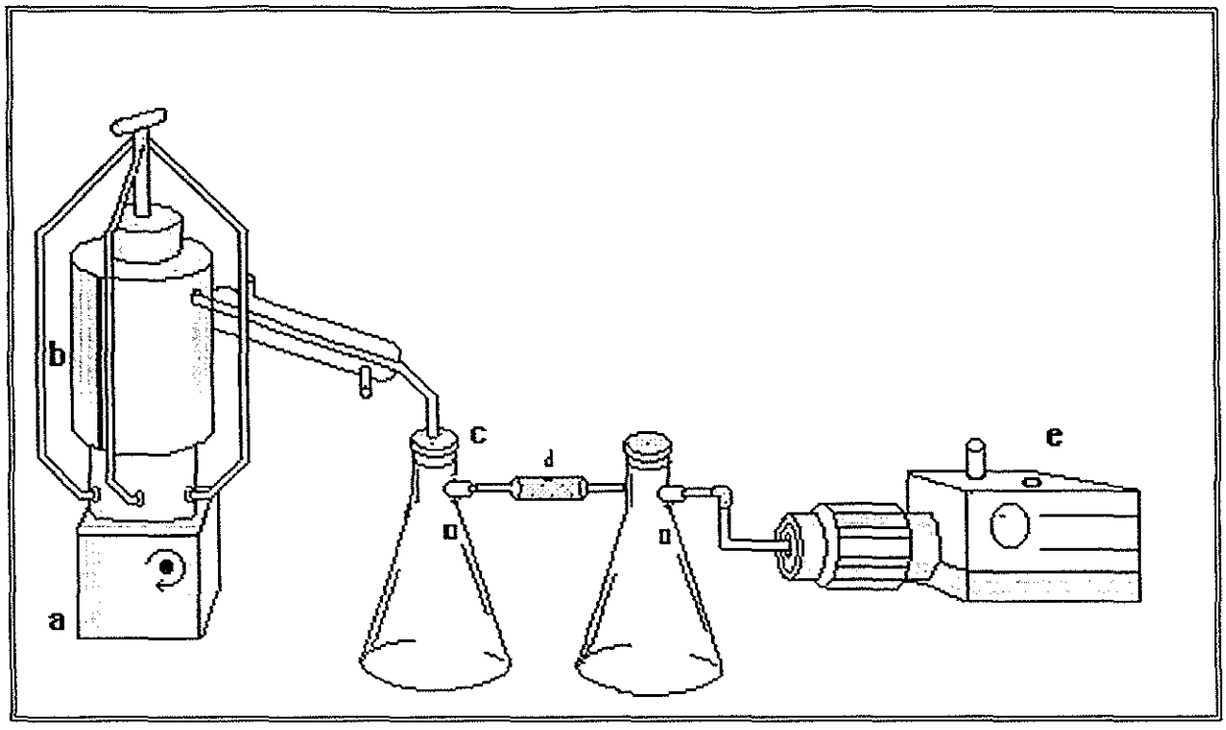


Figure 2. Stainless steel reactor used for amalgams digestion. A 10cm PVC tube with manganese dioxide and glass wool was also connected between the collecting flask and vacuum pump.
a) Rheostat. b) Stainless steel reactor. c) Collecting flask. d) PVC tube (10 x 100 mm) with MnO_2 . e) Vacuum pump.

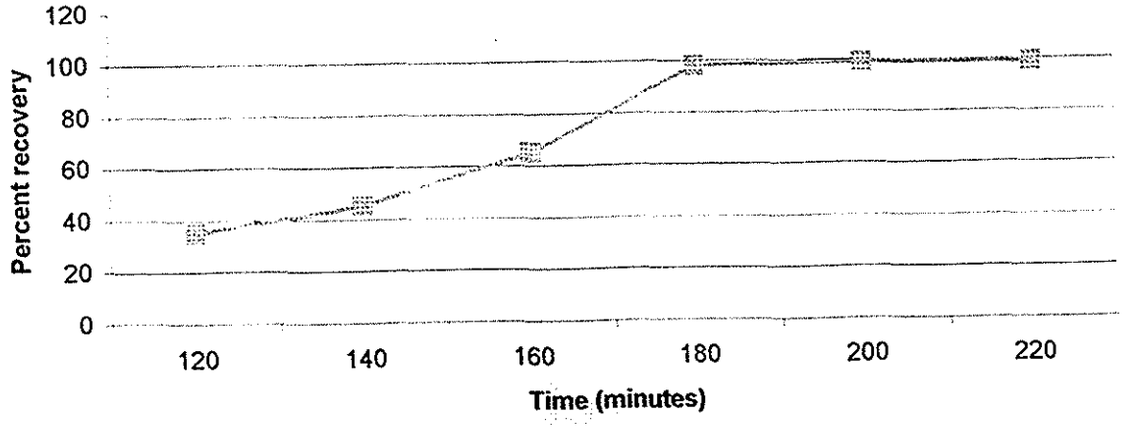


Figure 3. Percent recovery of mercury as function of heating time using a stainless steel reactor. The digestion temperature using the vacuum pump was 295 ± 10^{0}C, and the amount of amalgam powder used was 200g. The digestion temperature using the vacuum pump was 295 ± 10^{0}C, and the amount of amalgam powder used were 200 g.

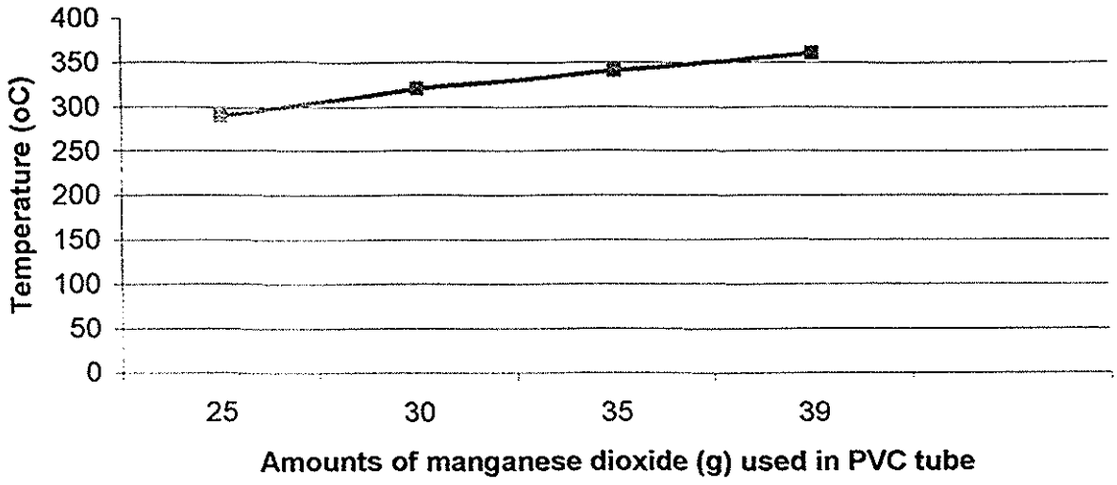


Figure 4. Dependence of Hg volatilization from dental amalgams as function of amount(g) of manganese dioxide used inside the PVC tube. The experiment was performed using a stainless steel reactor with ~200g of dental amalgam powder.

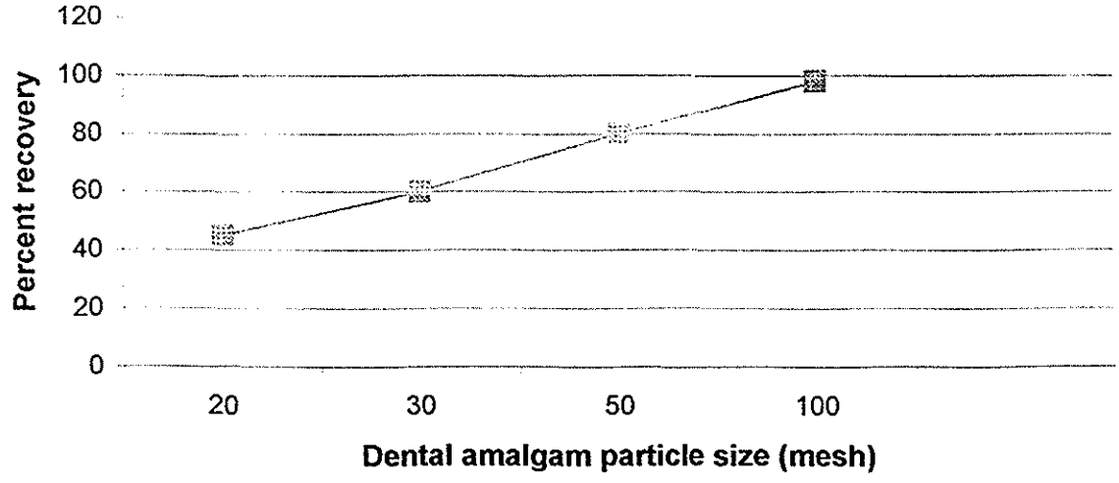


Figure 5. Recovery efficiency (%) of mercury as function of dental amalgam particle size (mesh). The experiment was carried out using a stainless steel reactor with ~200g of dental amalgams, and 25g of manganese dioxide inside the PVC tube.

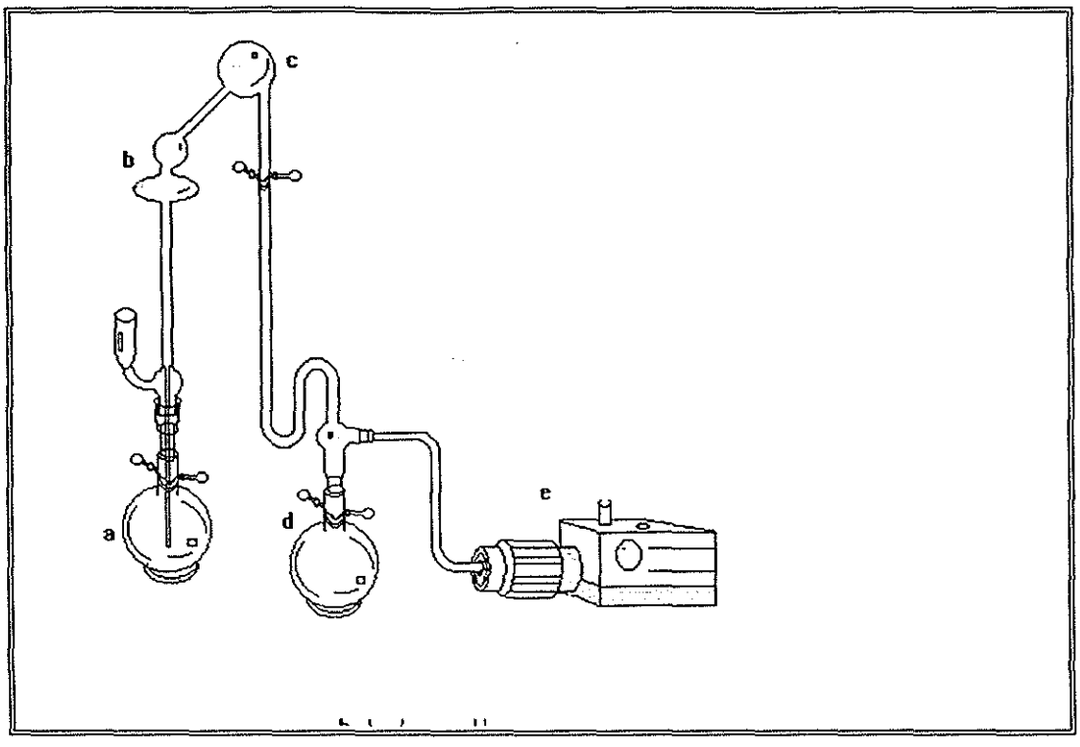


Figure 6. Apparatus used for final mercury purification after separation of silver, tin, copper, zinc, etc., from dental amalgams.[a] Flask with mercury to be purified. [b] Heating device. [c] Condenser. [d] Collecting flask.[e] Vacuum pump. A 10cm PVC tube with manganse dioxide and glass wool, not shown in the figure, was also connected between the collecting flask and vacuum pump.

changed after 24 hours of continuous operation. Amalgam particles size are also important, and higher efficiency was observed with smaller particles size of dental amalgams, as shown in Figure 5.

The environmental problem by mercury released from wastes of dental amalgams could be solved using one of the reactors investigated. Recovery efficiency was very high and the main advantage were low cost, simple operation, and high purity of the mercury recovered. The PVC

tube with MnO_2 connected between the collecting flask and vacuum pump was very important for the absorption of mercury vapor, that otherwise could cause problems to the vacuum pump and the environment. However, the amount of MnO_2 should be carefully investigated, because, with high amounts of MnO_2 the efficiency of the vacuum pump will be lower. The digestion temperature of amalgams was 290-300^{0}C using PVC tube with MnO_2 and vacuum pump, and at least 500^{0}C without the vacuum pump.

ACKNOWLEDGEMENT

The authors would like to thank National Foundation of Environment (FNMA-Brazil), Brazilian Council of Scientific and Technological Development (CNPq, CNPq/PIBIC-Brazil) for fellowships and financial support, and Dr. Jales A. Cardoso (15ª Regional de Saude de Maringa-PR.) for supplying the amalgam samples.

REFERENCES

1.C.K.Blesius, F.D.Valle, and D.Clason,**Biological Therapy, 13**, (2), 1-10, (1995).

2.J.K.Nicholson, M.D.Kendall, and D.Osborn, **Nature,304**, 633-635,(1983).

3.G.Joselow, **Archives of Environmental Health 15**, 64, (1967).

4.H.Olson, **Scandinavian Journal of Work Environmental Health** 7 (4), 252-256, (1981).

5.K.Abraham, **Journal of Dental Research** 63 (1), 71-73, (1984).

6.G.D.Don, D.Robert, and J.W. Reinhardt. **Lancet** 1 (8123), 985-986, (1979).

7.W.D.Kuntz,**The American Journal of Obstetrics and Gynecology** 143 (4), 440-443, (1982).

8.J.Nozaki and H. Zhou, **Química Nova** 15 (1), 15-17, (1992).

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, Nº 8, 1999

HEIGHT MEASUREMENTS OF THE SPECTRUM AS AN ALTERNATIVE TO CONVENTIONAL SPECTROPHOTOMETRIC ANALYSIS OF A $KMnO_4$ - $K_2Cr_2O_7$ MIXTURE

Carmem D. Cardoso, Martha B. Adaime and Nádia S. Viaro
Departamento de Química - Universidade Federal de Santa Maria
Campus Camobi - Km 09 - CEP: 97.119-900 - Santa Maria - RS - Brasil

ABSTRACT

Quantitative spectrophotometric analysis is generally carried out plotting absorbance against concentration, for some concentration range, in accordance with Lambert, Bourger and Bee law. In this work, height measurement of the spectrum was used to analyse $KMnO_4$ and $K_2Cr_2O_7$ mixtures in two different known concentrations. The experiments were relatively simple to carry out, requiring only standard solutions of $KMnO_4$, $K_2Cr_2O_7$, sample mixtures of the two components, a visible light spectrophotometer, recorder, ruler and pencil. Comparison of the two methods showed that the height measurement method is more reliable and versatile as it has no requirement for the calculation of molar absorptivity, shows less relative error than the conventional method.

RESUMO

Análises espectrofotométricas quantitativas geralmente são efetuadas relacionando absorbância e concentração, segundo a lei de Lambert, Bourger e Beer. Neste trabalho, usa-se medidas da altura de espectros para analisar misturas de concentrações conhecidas de $KMnO_4$ e $K_2Cr_2O_7$. Os experimentos são relativamente simples de serem desenvolvidos, necessitando somente soluções padrões de $KMnO_4$, $K_2Cr_2O_7$, misturas dos dois componentes, fonte de luz visível para o espectrofotômetro, registrador, borracha e lápis. Comparando-se os dois métodos, observou-se que o método de medida de altura é mais seguro e versátil, pois não necesstita de cálculo de absortividade molar, mostrando menor erro relativo do que o método convencional.

Keywords
Height measurements, spectrophotometric analysis, $KMnO_4$-$K_2Cr_2O_7$ mixture, quantification

DOI: 10.48141/SBJCHEM.v7.n8.1999.87_1999.pdf

INTRODUCTION

During the development of a quantitative method fot the determination of an unknown concentration of a species using absorption spectrophotometry in the ultraviolet and visible regions, the first step is the choice of the maximun absorption wavelength where the measurements are made. At this wavelength the absorbance of the solution has a linear relation to its concentration within a defined concentration range, following the Lambert, Bourger and Beer law[1]. This is the classic method used in quantitative analysis.

To determine two compounds simultaneously by molecular absorption spectrophotometry, these determinations can be made based on absorption measurements at two selected wavelengths, from which a pair of simultaneous equations are obtained, taking into account that the absorbance is an additive property[2]. It is necessary also to measure (or otherwise obtain) the molar absorptivity of each pure compound at the two predetermined wavelengths. When there are more than two compounds in the mixture with superposition of absorption spectrum, more simultaneous equations are required and, consequently software is necessary to resolve them[3].

Another quantitative method for mixtures is by using graphs to solve the simultaneous equations. For mixtures of three compounds, the solution to the problem is obtained by a triangular diagram for mixtures of four compounds a tridimensional diagram - a tetrahedron is used[4].

As an alternative method, when mixtures are involved with overlapping spectra, many wavelengths must be determined so that linear diagrams can be made from which the concentrations can be determined. This method can be applied to mixtures of two or more components[4].

The measurement of the area of the spectrum and the absorbance band height at different points of the spectrum has also been used sucessfully[5].

The purpose of the present work was to show the effectiveness of the spectrum height measurement for the simultaneous quantitative analysis of a mixture of $KMnO_4$ and $K_2Cr_2O_7$ using the spectrophotometric method.

EXPERIMENTAL PROCEDURE

All the reagents were of analytical grade. Stock solutions of 0.01 mol L^{-1} $KMnO_4$ and 0.02 mol L^{-1} $K_2Cr_2O_7$ were prepared in previously boiled and cooled deionized water containing 5 mol L^{-1} H_2SO_4. The concentration of the $KMnO_4$ solution was determined titrimetically against $H_2C_2O_4$ 0.01 mol L^{-1} [6].

Then, 13 standard solutions of the individual components, were prepared in 50 mL volumetric flasks completing the volumes with deionized water.

Sample mixtures with known concentrations were also prepared as follows: A - 2 mL of 0.01 mol L^{-1} $KMnO_4$ solution, 5 mL of 0.02 mol L^{-1} $K_2Cr_2O_7$ solution and 5 mL of 5 mol L^{-1} H_2SO_4 solution; B - 1 mL of 0.01 mol L^{-1} $KMnO_4$ solution, 2 mL of 0.02 mol L^{-1} $K_2Cr_2O_7$ solution and 5 mL of 5 mol L^{-1} H_2SO_4 solution.

The absorption spectra of each standard solution was measured between 400 to 600 nm on a Perkin Elmer 124 spectrophotometer and recorded on a Perkin Elmer 56

recorder. A cell of 1 cm was used while the attenuation was 10 mV and the chart speed was 20 $mm.min^{-1}$.

Height measurements' were made at 1.1 cm (λ=560 nm) from the origin for the $KMnO_4$ spectra (h_1) and at 5.3 cm (λ=449 nm) of the origin from the $K_2Cr_2O_7$ spectra (h_3).

Table I lists the data used to make the plot of the $KMnO_4$ standard solutions while Table II lists the data used to make the plot of the $K_2Cr_2O_7$ standard solutions.

These heights were plotted against the respectives concentrations as shown in Figures 3 and 4.

The spectra of mixture A and B were also recorded from 400 to 600 nm of wavelength. Height measurements were made at the same positions as for the individual solutions.

RESULTS AND DISCUSSION

From height readings' at 1.1 cm from the origin of mixtures' spectra, according Figures 1 and 2, the $KMnO_4$ concentration can be determined directly from the linear plot of h_1 (height) x C (concentration), Figure 3.In this situation, there was no interference from the $K_2Cr_2O_7$ solution, giving a $KMnO_4$ concentration of 4.0×10^{-4} mol L^{-1} for mixture A and, for mixture B, a concentration of 2.0×10^{-4} mol L^{-1}.

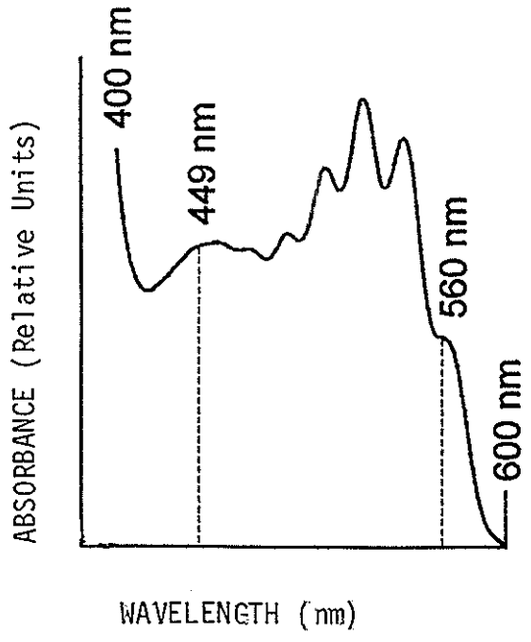


Figure 1. Absorption spectrum of the mixture A.

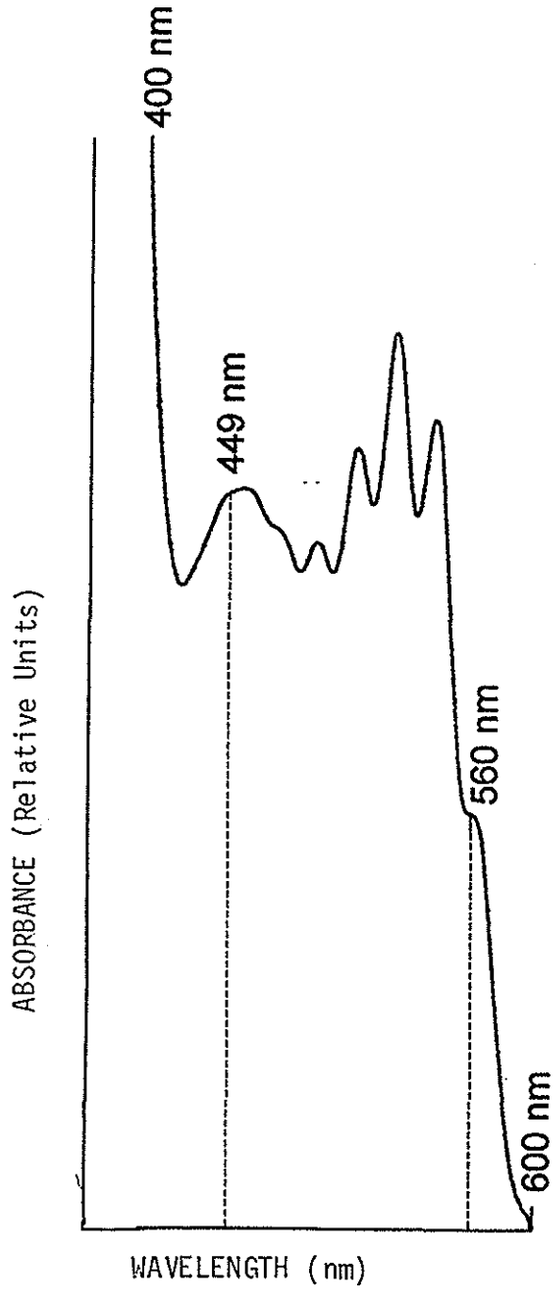


Figure 2. Absorption spectrum of the mixture B.

$KMnO_4$ standard solutions were prepared on the obtained concentrations from the mixtures and they were used to make the absorption spectra. The heights were measured on the same wavelength that was measured h_t. For mixture A, h_tA it was of 12.5 cm and h_2A it was of 0.8 cm and for mixture B, h_tB it was of 5.1 cm and h_2B of 0.35 cm.

Table I. Spectral height data of $KMnO_4$ standard solutions measured at 560 nm.

Volume $KMnO_4$ 0.01mol L^{-1} (mL)	Volume H_2SO_4 5 mol L^{-1} (mL)	Concentration (x 10^4 mol L^{-1})	Concentration (mg L^{-1})	h_1(height) (cm)
0.4	5	0.8	12.64	1.30
0.7	5	1.4	22.12	2.30
1.0	5	2.0	31.60	3.15
1.3	5	2.6	41.08	3.80
1.6	5	3.2	50.56	5.25
1.9	5	3.8	60.04	6.00
2.2	5	4.4	69.52	7.40
2.5	5	5.0	79.00	8.10
2.8	5	5.6	88.48	9.50
3.1	5	6.2	97.96	10.75
3.4	5	6.8	107.44	12.15
3.7	5	7.4	116.92	13.15
4.0	5	8.0	126.40	14.30

Table II. Spectral height data of $K_2Cr_2O_7$ standard solutions measured at 449 nm.

Volume $KMnO_4$ 0.02 mol L^{-1} (mL)	Volume H_2SO_4 5 mol L^{-1} (mL)	Concentration (x10^3 mol L^{-1})	Concentration (mg L^{-1})	h_3(height) (cm)
1.0	5	0.4	117.6	2.8
1.5	5	0.6	176.4	4.0
2.0	5	0.8	235.2	5.5
2.5	5	1.0	294.0	6.3
3.0	5	1.2	352.8	8.0
3.5	5	1.4	411.6	9.0
4.0	5	1.6	470.4	9.0
4.5	5	1.8	592.2	11.5
5.0	5	2.0	588.0	12.5
5.5	5	2.2	646.8	13.8
6.0	5	2.4	705.6	15.5
6.5	5	2.6	764.4	16.2
7.0	5	2.8	823.2	17.6

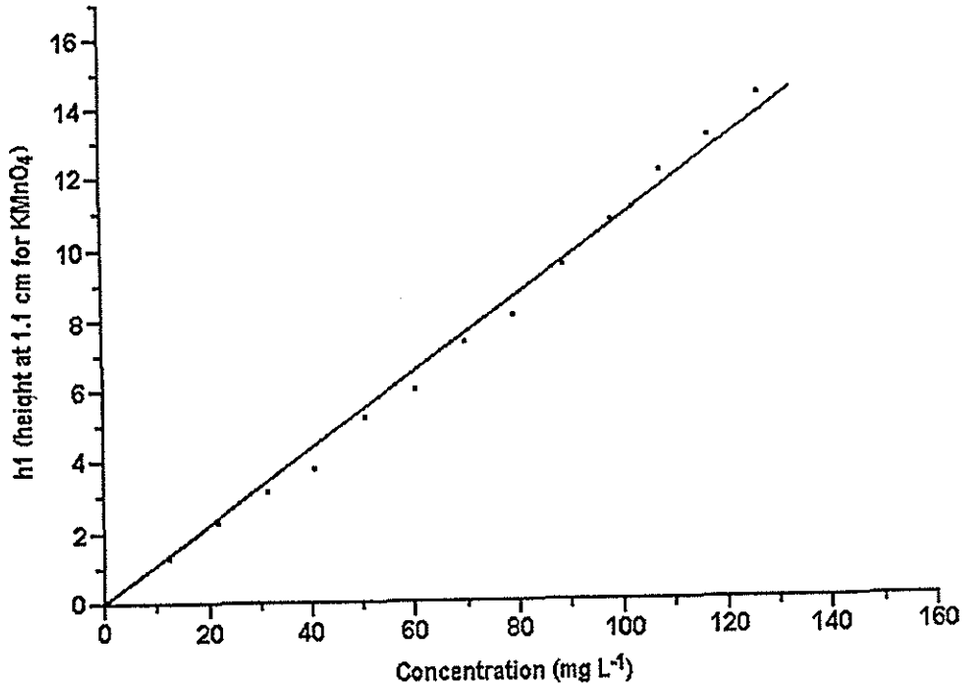


Figure 3. Plot of height (h_1) versus the concentration of $KMnO_4$ at 1.1 cm. Data from Table I.

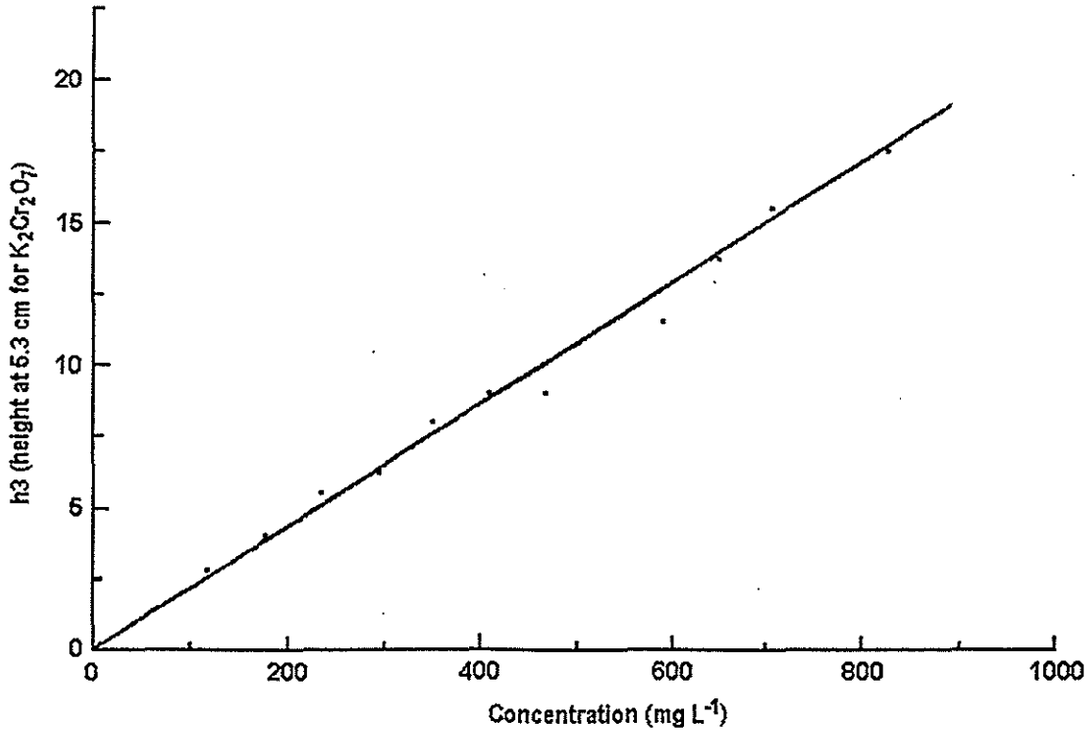


Figure 4. Plot of height (h_3) versus the concentration of $K_2Cr_2O_7$ at 5.3 cm. Data from Table II.

Table III. Results for the two-component mixtures by the conventional and the alternative methods.

Mixtures	Calculated concentration (x 10^4 mol.L^{-1})	Method 1 (x10^4 mol.L^{-1})	Relative Error (%)	Method 2 (x 10^4 mol.L^{-1})	Relative Error (%)
$KMnO_4$	4.0	4.0	0.0	5.0	+25.0
A					
$K_2Cr_2O_7$	20.0	18.0	- 10.0	27.0	+35.0
$KMnO_4$	2.0	2.0	0.0	2.8	+40.0
B					
$K_2Cr_2O_7$	8.0	7.3	- 8.8	10.0	+25.0

Method 1 - Alternative method (Height Measurement)
Method 2 - Conventional method using simultaneous equations

Considering the total height (h_t) measured at 5.3 cm for the mixtures as the sums of the contributions from $KMnO_4$ and $K_2Cr_2O_7$:

$$h_t = h_2 + h_3 \qquad (1)$$

Then, from: $h_3=h_t - h_2$, the values of h_3 were calculed (mixture A, h_3=11.7 cm and mixture B, h_3=4,75 cm). From Figure 4, the determined $K_2Cr_2O_7$ concentrations' for mixtures A and B were 1,8 x 10^{-3} mol L^{-1} and 7.3 x 10^{-4} mol L^{-1}, respectivelly.

Table III gives comparative data for the conventional and this alternative method.

The conventional method uses simultaneous equations, so as many equations as the number of components in the mixture are necessary, requiring values of molar absorptivity as well as absorbances and known concentrations. The relation height versus with this concentration brings the reality nearer than the relation absorbance versus concentration. With this alternative method is not necessary to calculate molar absorptivity and the relative error is smaller compared to the conventional method.

ACKNOWLEDGMENT

The authors would like to thank FIPE (Brazil) for financial support of this work and Dr. Carol Collins for useful suggestions.

REFERENCES

1. J. Robinson,. W.*Undergraduate Instrumental Analysis*; 4th ed.; Dekker Inc: New York,,USA,1987, p 54.

2. H. H. Willard; L. L. Merrit, Jr.; J. A. Dean and F. A. Settle, Jr., *Instrumental Methods of Analysis*, 6th ed.; Wadsworth Publishing Co; Belmont, California. USA, 1981, Vol. 1, p 77.

3. I. Giolito, *Medidas Fotométricas*, Faculdade de Farmácia e Bioquímica da USP, São Paulo, Brasil,1968, p 51.

4. A. C. Kenneth, and J. E. Chukwuenweniwe, *Analytical Chemistry*, 51(8), 1262-1266, (1979).

5. C. D. Cardoso; I. Ramires; I. Santos; M. B. Adaime and N. S. Viaro, *Química Nova*, 14(4), 49, (1991).

6. O. A. Ohlweiler, *Química Analítica Quantitativa*, Livros Técnicos e Científicos Editora , Rio de Janeiro, Brasil, 1976, Vol. 2, p. 571.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

COORDINATION COMPOUNDS OF Cu(II) AND Ni(II) WITH SCHIFF BASES DERIVED FROM FORMYLMENTHONE AND *o*-, *m*-, *p*-TOLUIDINE

Adalgiza Ciobanu*, Florica Zălaru**, D.Albinescu*, Christina Zălaru*

* Faculty of Chemistry, Organic Chemistry Dept., University of Bucharest, 90-92 Panduri Road, Romania
** Faculty of Chemistry, Inorganic Chemistry Dept., University of Bucharest, 23 Dumbrava Rosie, Romania

ABSTRACT

The Schiff bases obtained from formylmenthone and o-, m-, p-toluidine behave as bidentate ligands with O and N donor atoms in Ni(II) and Cu(II) complexes by the type NiL_2 and $CuL_2.(H_2O)_2$. The donor atoms and the possible geometry for the complexes were assigned by means of chemical, thermodifferential analyses and electronic, EPR and IR spectra.

RESUMO

As bases de Schiff obtidas de o,m, e p-toluidina e formilmentona agem com ligantes bidentados com átomos doadores de O e N e reagem com Ni(II) e Cu(II) formando complexos do tipo NiL_2 e $CuL_2(H_2O)_2$. A natureza dos átomos doadores e a geometria e estrutura dos complexos foi determinada usando análise química e termodiferencial e métodos de espectroscopia eletrônica, infravermelha e de REP.

KEYWORDS: Coordination compounds of Cu(II) and Ni(II), Schiff bases derived from formylmenthone

DOI: 10.48141/SBJCHEM.v7.n8.1999.95_1999.pdf

INTRODUCTION

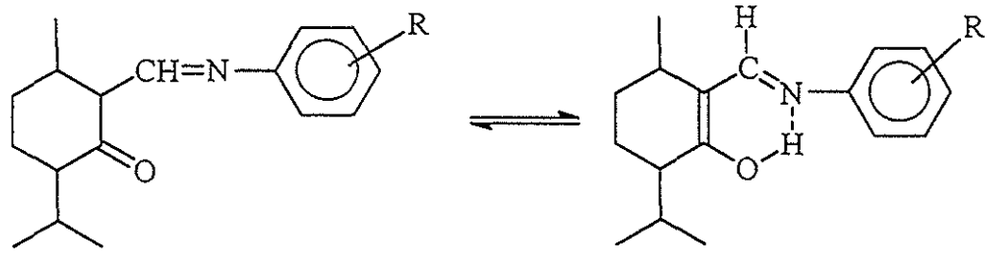
Our previous paper[1] has reported the preparation and characterisation of the coordination compounds of some "3d" metals with Schiff bases derived from 3-formylcarvone and *o*-, *m*-, *p*-toluidine. The position of the methyl substituent on the benzene ring has determined the type of the complex and monodentate or bidentate coordination of the ligands. It seemed desirable to investigate the coordination ability of the Schiff bases derived from 2-formylmenthone and *o*-, *m*-, *p*-toluidine on the same "3d" metals, for purposes of comparison (scheme 1).

Three new ligands able to generate complexes have been synthesized:

6 – isopropyl – 3 – methyl – 2 - [(2'-methylphenylamino) methylen] cyclohexan - 1 – one, (*ortho* – L);

6 – isopropyl – 3 – methyl – 2 - [(3'-methylphenylamino) methylen] cyclohexan - 1 – one, (*meta* – L);

6 – isopropyl – 3 – methyl – 2 - [(4'-methylphenylamino) methylen] cyclohexan - 1 – one, (*para* – L);



1a 1b

Scheme 1

where R = *o*-, *m*-, *p*- methyl.

The presence of the >C=O and >C=N – groups lying in *ortho*-position with respect to each other favours keto-enolic tautomerism (Scheme 1). This tautomerism has been attributed to an intramolecular hydrogen bond and might explain their chelating ability. NMR studies have shown that such Schiff bases with the carbonyl and azomethine groups lying in the *ortho*-position exist in solution as the enolic tautomer (1b) and that the tautomer distribution was solvent dependent[2,3].

EXPERIMENTAL

The ligands were prepared according to the literature and $Cu(CH_3COO)_2.H_2O$ and $Ni(CH_3COO)_2.4H_2O$ p.a. Merck were used.

The complexes were prepared by mixing warm aqueous methanol solution (50%) of metal acetate (1 mmol) and ligands (2 mmols). The resulting precipitates were filtered and washed with aqueous methanol solution (50%) and dried at room temperature. The metal content was obtained gravimetrically.

The electronic diffuse reflectance spectra within 300-1100 nm range were obtained with VSU-2P Zeiss-Jena Spectrophotometer, using MgO as a standard.

EPR spectra were recorded at room temperature on polycrystalline powders, on ART-5-IFA Spectrograph.

The klystron frequency was 9060 MHz and modulation of the magnetic field 100 KHz. The EPR spectral parameters were calculated against a Mn(II) standard.

Thermodifferential analyses were carried out with a Paulik-Paulik-Erdey Derivatograph Q 1500-D MOM. Conditions of measurements: temperature range up 1000°C, heating program: 10 degree/min., sensitivity DTA = 1/10, m_4 = 0.0180 g, S = 20 and m_5 = 0.0372 g, S = 50; atmosphere over sample air.

IR spectra (KBr pellets) were measured on a BIO-RAD FTS-135 Spectrometer.

RESULTS AND DISCUSSION

Characteristic data for the Cu(II) and Ni(II) complexes are presented in Table 1. The elemental and thermodifferential analyses are consistent with their formulation as anhydrous, monomeric Schiff bases chelates (NiL_2) or hydrated, monomeric complexes ($CuL_2.(H_2O)_2$) with water molecules either as coordinated or as crystalline water. All the metal chelates are brownish-red, readily soluble in organic solvents (chloroform, acetone, methanol), but sparingly soluble in water.

Thermodifferential analysis curves for compounds Cu(*para*-L)$_2$.$(H_2O)_2$ and Ni(*para*-L)$_2$ are shown in detail in Figures 1a and 1b.

Table I. Elementary analysis results for the complexes studied

No	Compound	Cu% Found/Calcd.	Ni% Found/Calcd.	H_2O% Found/Calcd.
1.	Cu(*ortho*-L)$_2$.(H_2O)$_2$	10.37/9.94	-	7.03/5.63
2.	Cu(*meta*-L)$_2$.(H_2O)$_2$	9.64/9.94	-	6.80/5.63
3.	Ni(*meta*-L)$_2$	-	10.14/9.80	-
4.	Cu(*para*-L)$_2$ (H_2O)$_2$	10.09/9.94	-	6.91/5.63
5.	Ni(*para*-L)$_2$	-	10.50/9.80	-

For compound Cu(*para*-L)$_2$.(H_2O)$_2$ (Fig.1a) the mass loss observed within 60-215°C range in the TG curve corresponds to the loss of two water molecules per molecule of each copper compound. The TG curve shows that the water molecules are liberated in two steps (weight loss at 170°C, found: 2.98%, calcd., for H_2O: 2.88%; weight loss at 215°C, found: 7.03%, calcd., for $2H_2O$: 5.76%). Hence, the two water molecules are present as coordinated water, in Cu(II) complexes.

The results indicate that compound Ni(*para*-L)$_2$ (Fig.1b) is stable up to 150°C, but rapidly looses ligands at 565°C (weight loss at 565°C, found: 88.86%, calcd., for 2L: 90.14%).

The electronic diffuse reflectance spectra of the complexes are given in Fig.2 and 3.

The spectra of the Ni(II) complexes (3 and 5, Fig.2) are similar and are consistent with tetrahedral Ni(II) complexes[4]. The spectra present the band ν_3 (660 nm) assignable to the $^3T_1(P) \leftarrow {}^3T_1$ transition weak band (760 nm, compound Ni(*meta*-L)$_2$) or the shoulder (760 nm, compound Ni(*para*-L)$_2$) are assigned as spin forbidden transitions to components of ^1D levels[4]. The band ν_1 assignable $^3T_2 \leftarrow {}^3T_1$ transition (near 500 nm) is covered by a broad and intense band due to the ligands (500 nm). It is present in the spectra of the copper complexes, also. The spectra of the copper complexes

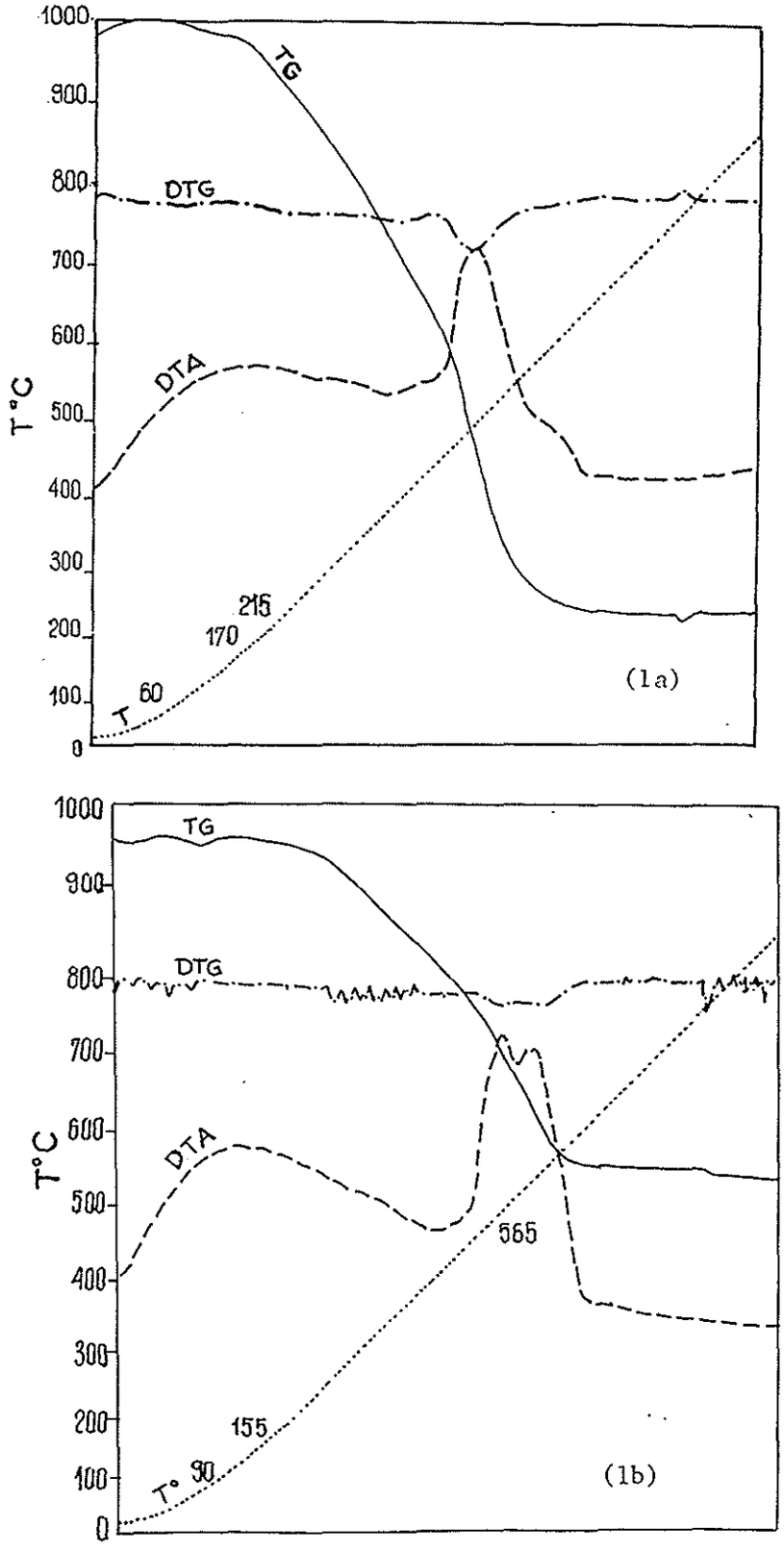


Fig. 1. Thermodifferential curve of Cu(*para*-L)$_2$ 2H$_2$O (1a) and Ni(*para*-L)$_2$ (1b)

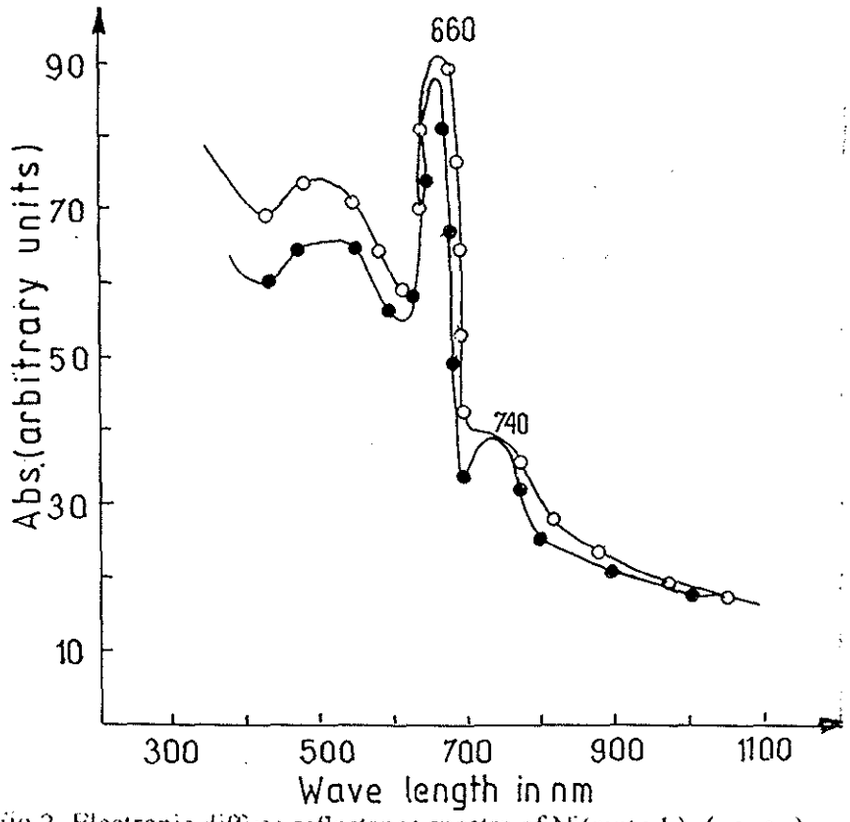


Fig.2. Electronic diffuse reflectance spectra of Ni(*meta*-L.)$_2$ (-•-•-) and Ni(*para*-L.)$_2$ (-o-o-)

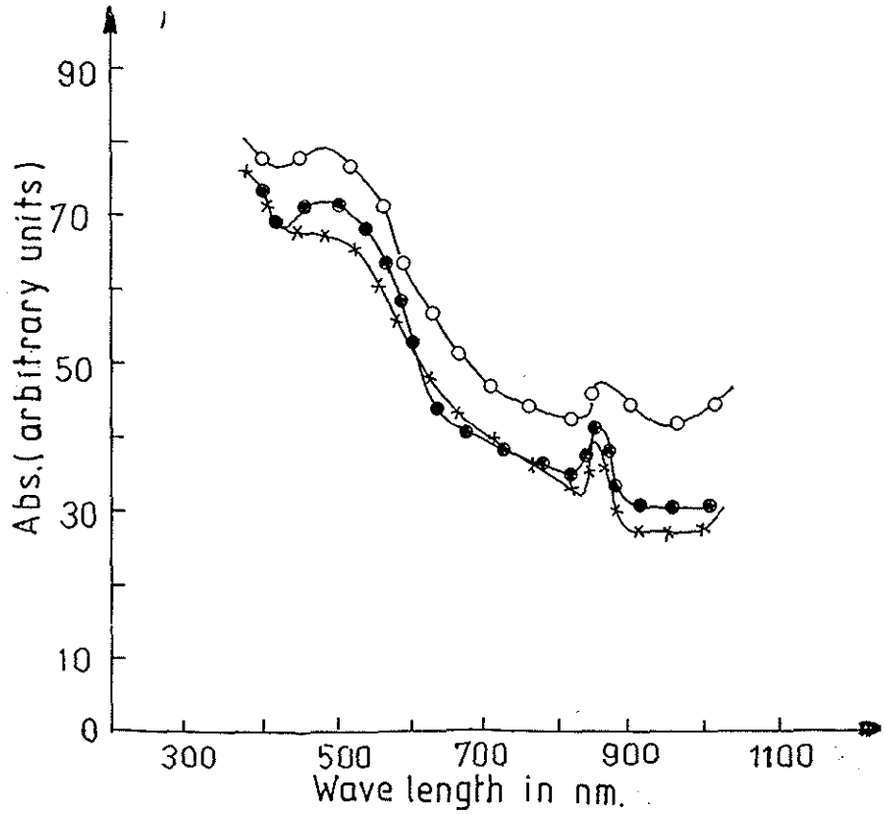
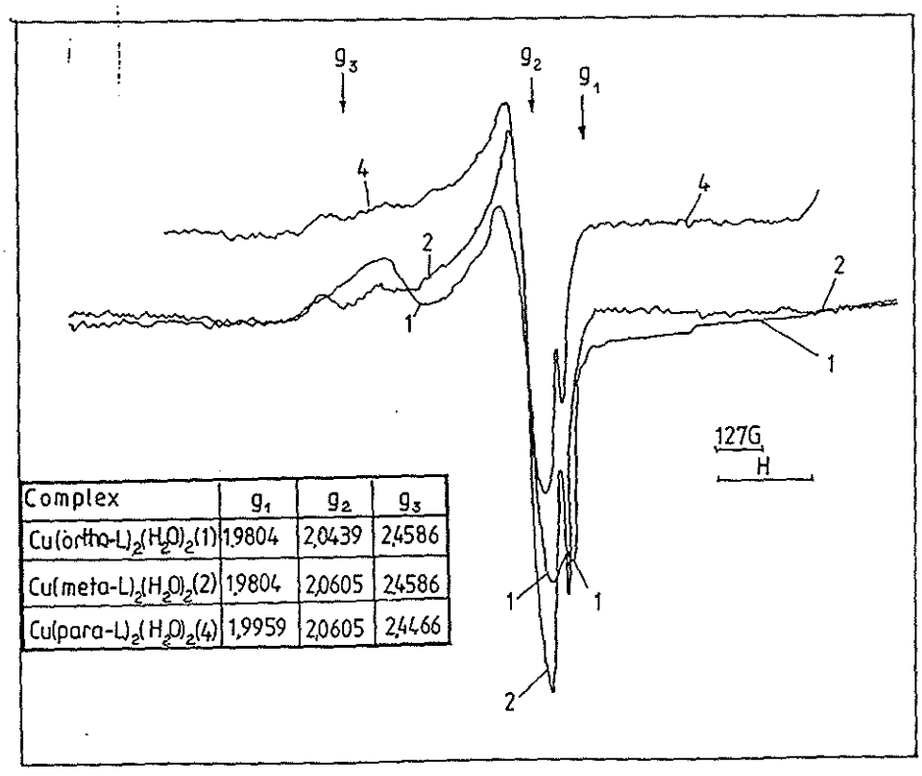


Fig.3. Electronic diffuse reflectance spectra of:
Cu(*ortho*-L.)$_2$.(H$_2$O)$_2$ (-x-x-);
Cu(*meta*-L.)$_2$.(H$_2$O)$_2$ (-• • -);
Cu(*para*-L.)$_2$.(H$_2$O)$_2$ (-o-o-)

(Cu(*ortho*-L)$_2$.(H$_2$O)$_2$, Cu(*meta*-L)$_2$.(H$_2$O)$_2$, Cu(*para*-L)$_2$.(H$_2$O)$_2$) in Fig.3 are similar and present a band at 800 nm that could be assigned to a *d-d* transition associated with a distorted octahedron[5-7]. EPR spectra of the three copper complexes recorded at room temperature on polycrystalline samples present a similar intense EPR signal characteristic monomeric species of Cu(II) ion with a third order anisotropy for the factor *g* resulting from distortion of octahedral geometry (Fig.4).

This anisotropy is compatible with Cu(II) ion in a compressed rhombic-octahedral geometry[6,7] with R > 1 and supports the electronic spectra. In a three *g* value spectrum with $g_1 < g_2 < g_3$, the value of $R = (g_2 - g_1)/(g_3 - g_2)$ may be significant[8]: if R > 1, a predominant dz^2 ground state is present and would be consistent with compressed axial or rhombic symmetry with slight misalignment of the axex. If R < 1, a predominant $dx^2 - y^2$ ground state is present[6-8].

The main IR bands and their assignments for the free ligands and the complexes are shown in Table 2.



Complex	g_1	g_2	g_3
Cu(ortho-L)$_2$(H$_2$O)$_2$(1)	1,9804	2,0439	2,4586
Cu(meta-L)$_2$(H$_2$O)$_2$(2)	1,9804	2,0605	2,4586
Cu(para-L)$_2$(H$_2$O)$_2$(4)	1,9959	2,0605	2,4466

Fig.4. EPR spectra and SPR spectral parameters of:
Cu(*ortho*-L)$_2$.(H$_2$O)$_2$ (1);
Cu(*meta*-L)$_2$.(H$_2$O)$_2$ (2);
Cu(*para*-L)$_2$.(H$_2$O)$_2$ (4).

Table II. The main bands in the IR spectra (cm^{-1}) and their assignments

No.	L/compound	ν_{OH}	$\nu_{C=O}$	$\nu_{C=N}$	H_2O coord.
	Ortho-L	3200-3320m	1700sh	1550, 1590, 1630vs	-
1.	Cu(*ortho*-L)$_2$.(H$_2$O)$_2$	3423s	-	1558, 1615vs	1111w
	Meta-L	3310, 3450m	1700vw	1560, 1620, 1640s	-
2.	Cu(*meta*-L)$_2$.(H$_2$O)$_2$	3395m	-	1557s, 1610vs	1094w
3.	Ni(*meta*-L)$_2$	3398m	-	1543s, 1510vs, 1635s	-
	Para-L	3300, 3400m	1700vw	1520, 1580, 1630s	-
4.	Cu(*para*-L)$_2$.(H$_2$O)$_2$	3399m	-	1514vs, 1545s, 1613s	1111w
5.	Ni(*para*-L)$_2$	3422m	-	1510, 1543, 1558m	-

s – strong; m – medium; w – weak; sh – shoulder; v – very

The keto-enolic tautomerism is supported by presence of the bands due to ν_{OH} and $\nu_{C=O}$. The band due to $\nu_{C=O}$ (1700 cm^{-1}) occurs as a very weak band or a shoulder. The band due to ν_{OH} occurs as a broad band (3200-3600 cm^{-1} range) with two unresolved peaks. The band due to $\nu_{C=N}$ occurs as a broad band (1500-1600 cm^{-1} range) with three unresolved peaks. A comparison of the position of the bands in spectra of the complexes with their position in the IR spectra of free ligands shows changes of the bands due to ν_{OH} and $\nu_{C=N}$. Upon coordination, the stretching frequencies, $\nu_{C=N}$ are shifted to lower values and stretching frequencies ν_{OH} are shifted to higher values. These changes are generally noticed upon coordination of the Schiff bases containing an N and O donor atoms, by the both donor atoms[9]. The new band near 1111 cm^{-1} occurring in the IR spectra of the three copper complexes only could be assigned to the coordinated water molecules in agreement with Fujita[10] and is be consistent with the results of the thermodifferential analysis.

On the basis of elemental, differential analyses and spectral measurements, we conclude that Cu(II) ion is hexacoordinated in a compressed rhombic geometry, while Ni(II) ion is tetracoordinated in a tetrahedral geometry.

The ligands acted bidentately with both O and N donor atoms by deprotonation of the OH group making evident the participation of the ligands in the enolic tautomeric form.

Cu(II) coordinates by atom donors, N and O in a plane and by shorter bonds to axial water molecules in a compressed rhombic geometry (Fig.5).

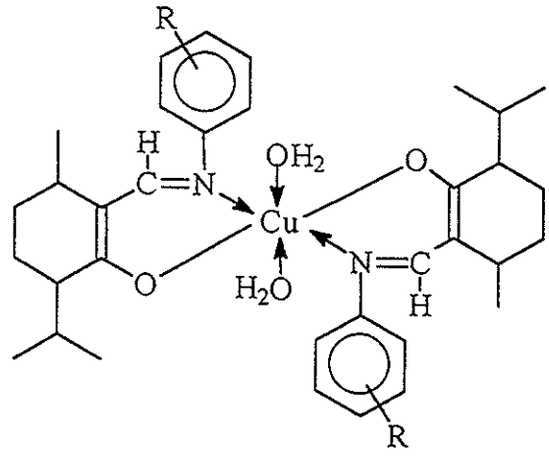


Fig.5. Structural formula proposed for the Cu(II) complexes

The tetrahedral environment of the Ni(II) ion suggested by electronic spectra is obtained by both donor atoms, N and O.

We may conclude that the metallic ion determines the type of the new complexes.

The arguments for the structure of the new chelates were fully supported by the spectral data (electronic, EPR and IR spectra).

REFERENCES

1. P.Grandclaudon, F.Zălaru, D.Albinescu, A.Ciobanu and A.Meghea, *An.Univ.Bucharest, 6, 11* (1997)
2. G.O.Dudek, *J.Am.Chem.Soc., 85*, 694 (1963)
3. G.O.Dudek and G.P.Volpp, *J.Am.Chem.Soc., 86*, 2697 (1964)
4. A.B.Lever, *"Inorganic Electronic Spectroscopy"*, Elsevier, Amsterdam, 182, 1968
5. B.J.Hathaway, *J.Chem.Soc.,* 1196 (1972)
6. B.J.Hathaway and D.E.Billing, *Coord.Chem.Rev., 143* (1970)
7. B.J.Hathaway, *Structure and Bonding, 57*, 85, 109 (1984)
8. D.E.Billing, R.J.Dudley, B.J.Hathaway and A.Tomlinson, *J.Chem. Soc.(A),* 691 (1971)
9. G.C.Percy and D.A.Thortnton, *J.Inorg.Nucl.Chem., 34*, 3357 (1972)
10. J.Fujita, K.Nakamato and M.Kobayshi, *J.Am.Chem.Soc., 78*, 3963 (1956)

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

PHYSICAL CHEMICAL STUDIES OF THE AGGREGATION AND CATALYTIC PROPERTIES OF THE SURFACTANT CETYLDIMETHYLETHYLAMMONIUM BROMIDE (CDEAB)

Lavinel G. Ionescu[a,b] Sílvia Dani[b] and Elizabeth Fátima de Souza[c]

[a] Departamento de Química Pura, Faculdade de Química, Pontifícia Universidade Católica do Rio Grande do Sul - PUCRS, Porto Alegre, RS, Brazil
[b] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Luterana do Brazil - ULBRA, Canoas, RS, Brazil

[c] Instituto de Ciências Biológicas e Química, Pontifícia Universidade Católica de Campinas - PUC-CAMPINAS, Campinas, SP, Brazil

ABSTRACT

The micellization of cetyldimethylethylammonium bromide (CDEAB) in water was studied by means of surface tensiometry. The critical micellar concentration (CMC) was determined at 25°, 32° and 40 °C and thermodynamic properties such as the free energy of micellization (ΔG^{o}_{mic}), enthalpy (ΔH^{o}_{mic}) and entropy (ΔS^{o}_{mic}) of micellization were measured. The CMC at 25 °C was 9.05 x 10^{-4} M and the corresponding values of the thermodynamic parameters were: ΔG^{o}_{mic} = -4.15 kcal/mol; ΔH^{o}_{mic} = -1.37 kcal/mol and ΔS^{o}_{mic} = + 9.33 e.u. Micelles of the surfactant CDEAB were good catalysts for the alkaline hydrolysis of p-nitrophenyl diphenyl phosphate (NPDPP) with a maximum catalytic factor of approximately 80, comparable to that of CTAB. Typical activation parameters measured for 0.005 M NaOH were: E_a = 9.0 kcal/mol; $\Delta H^{o\neq}$ = 8.4 kcal/mol; $\Delta G^{o\neq}$ = 19.2 kcal/mol and $\Delta S^{o\neq}$ = -36.3 e.u. The kinetic results were also analyzed in terms of the pseudo-phase ion exchange models (PPIE) and showed that the model is applicable and gave reasonable fits.

KEYWORDS: cetydimethylethylammonium bromide; micellization; micellar catalysis; phosphate esters

RESUMO:

A micelização do brometo de cetildimetiletilamônio (CDEAB) em água foi estudada por métodos de tensiometria superficial. A concentração micelar crítica (CMC) foi determinada a 25°, 32° e 40 °C e propriedades termodinâmicas tais como a energia livre (ΔG^{o}_{mic}) de micelização, entalpia (ΔH^{o}_{mic}) e entropia (ΔS^{o}_{mic}) de micelização foram medidas. A CMC a 25 °C foi 9,05 x 10^{-4} M e os valores correspondentes para os parâmetros termodinâmicos foram os seguintes: ΔG^{o}_{mic} = -4,15 kcal/mol; ΔH^{o}_{mic} = -1,37 kcal/mol e ΔS^{o}_{mic} = +9,33 e.u. Micelas do surfactante CDEAB foram bons catalisadores para a hidrólise alcalina do p-nitrofenil difenil fosfato, com um fator catalítico máximo de aproximadamente 80, comparável ao do CTAB. Parâmetros de ativação representativos medidos experimentalmente para 0,005 M NaOH foram: E_a = 9,0 kcal/mol; $\Delta H^{o\neq}$ = 8,4 kcal/mol; $\Delta G^{o\neq}$ = 19,2 kcal/mol and $\Delta S^{o\neq}$ = -36,3 e.u. Os resultados cinéticos também foram analisados em termos do modelo de pseudo-fase de troca iônica (PPIE) e mostraram que o modelo é aplicável e satisfatório.

DOI: 10.48141/SBJCHEM.v7.n8.1999.105_1999.pdf

INTRODUCTION

The present paper deals with the study of the micellization process of surfactant cetyldimethylethylammonium bromide or hexadecyldimethylethylammonium bromide [CDEAB, $CH_3CH_2N^+(CH_3)_2CH_2(CH_2)_{14}CH_3$ Br^-] and its use as a catalyst for the alkaline hydrolysis of p-nitrophenyl diphenyl phosphate.

In the past, as a part of our systematic investigation of the process of micellization we have studied many surfactants in water, non-aqueous solvents and water solutions containing various co-solvents or additives by means of a variety of experimental techniques including surface tensiometry, nuclear magnetic resonance (NMR) and quasi-elastic light scattering (QELS)[1-14]. The surfactant CDEAB was chosen because of its similarity to cetyltrimethylammonium bromide (CTAB).

Phosphate esters are compounds with interesting biological and physiological properties and are widely used as pesticides, drugs and nerve gases. Their accumulation and their effect in the environment are of paramount importance[15,16].

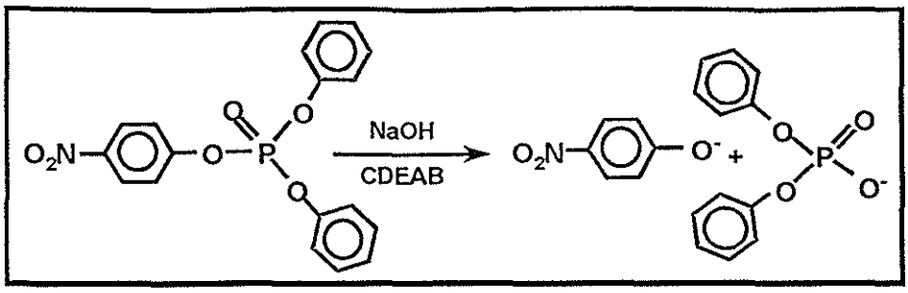
In studies reported in the literature we have shown that the hydrolysis of di- and tri-substituted phosphate esters is catalyzed by micelles of cetyltrimethylammonium bromide [$C_{16}H_{33}N^+(CH_3)_3$ Br^-], N,N-dimethyl-N-hydroxyethyldodecylammonium bromide [DHEDAB, n-$C_{12}H_{25}N^+(CH_3)_2CH_2CH_2OH$ Br^-] and N,N-dimethyl-N-hydroxyethylcetylammonium bromide [CHEDAB, n-$C_{16}H_{33}N^+(CH_3)_2CH_2CH_2OH$ Br^-][17]. The last two, CHEDAB and DHEAB are among the first examples of surfactants that form functional micelles.

Micellar aggregates of DHEDAB and CHEDAB are excellent catalysts for the hydrolysis of both lithium p-nitrophenyl ethyl phosphate (LiPNEP) and p-nitrophenyl diphenyl phosphate (NPDPP) in the presence of hydroxide ions, with over a 300-fold rate enhancement for the hydrolysis of the triaryl phosphate in the presence of CHEDAB. The dependence of the reaction rate on hydroxide ion concentration and the catalytic effect have been explained in terms of nucleophilic participation of the alkoxide ion of DHEDAB and CHEDAB, with pK_a of 12.4 and 12.9, respectively for the ionization of the hydroxyl groups[17]. For reactions with fluoride ion, the hydroxy-substituted surfactants are no better catalysts than the corresponding alkyltrimethylammonium bromides, suggesting that electrophilic catalysis is relatively unimportant. Cetylpyridinium bromide [CPBr, $C_5H_5N^+C_{16}H_{33}$ Br^-] is similar to CTAB at low hydroxide concentration and has a slightly more pronounced effect with fluoride ion. Zwitterionic surfactants such as lauryl carnitine chloride (LCCl) and palmityl carnitine chloride (PCCl) have little effect on the rate of hydrolysis of LiPNEP[18,19].

The addition of primary amines enhanced the rate of reaction in the presence of CTAB and CHEDAB for the triaryl phosphate, but much of the increase was due to attack by amine on the aryl group. In the absence of micelles, amines increased the overall rate of the reaction by attacking the aryl group without markedly catalyzing hydrolysis[20].

The micellar catalyzed oxidative cleavage of a carbon-carbon bond in Dicofol$^{(TM)}$[21] and the micellar catalyzed dehydrochlorination of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and some of its derivatives have also been subject of our investigations[22,23]. In recent studies we have reported results obtained for the hydrolysis of p-nitrophenyl diphenyl phosphate in aqueous solutions in the presence of micelles of diethyl heptadecyl imidazolinium ethyl sulfate (DEHIES) and CTAB, sodium hydroxide and dimethylsulfoxide (DMSO) and analyzed the effect of internal pressure of the medium, dielectric constant, donor number and polarity of the solvent and the effect of DMSO on micellization[24-28].

The present paper deals with the study of the hydrolysis of p-nitrophenyl diphenyl phosphate (NPDPP) in the presence of micelles of cetyldimethylethylammonium bromide (CDEAB) in aqueous solutions containing NaOH, as illustrated by the following scheme:



EXPERIMENTAL PROCEDURE

Materials. The p-nitrophenyl diphenyl phosphate (NPDPP) was prepared using standard methods[29,30]. A sample was also obtained from Prof. Fred Menger, Emory University, Atlanta, Georgia, USA. The surfactant cetyldimethylethylammonium bromide (CDEAB) was purchased from Chem. Service, West Chester, Pa., USA. The sodium hydroxide was analytical reagent grade and was purchased from Merck Co.

Surface Tension Measurements. All solutions were prepared volumetrically with deionized double distilled water and contained a series of at least fifteen different concentrations of CDEAB. The surface tension of the CDEAB-H_2O solutions was measured at 25°, 32° and 40 °C by means of a Fisher Model 21, Semi-Automatic Tensiometer. Ten milliliters aliquots of the solutions were placed in a Petri dish with a diameter of 6 cm. The temperature of the solutions was brought to the chosen temperature using a water bath and the Petri dish was kept at the desired temperature by placing it in a container through which water was circulated from the constant temperature bath. The tensiometer was set a constant height. The final surface tension of any solution was the average of at least three independent measurements.

The critical micellar concentrations (CMC's) were determined from plots of the surface tension of the solutions versus the concentration or log concentration of CDEAB. The marked change in the plots was taken as an indication of micelle formation and the inflection point was considered to correspond to the CMC.

The thermodynamic parameters ΔG°_{mic}, ΔH°_{mic} and ΔS°_{mic} were determined using standard equations[31,32] derived on the basis of the assumption that the process of micellization involves the formation of a distinct micellar phase at the CMC and that the concentration of monomers in solution is constant, once micelles are formed. The experimental accuracy in the values determined for ΔG°_{mic} is about ± 100 cal/mole. On the other hand, ΔH°_{mic} and ΔS°_{mic} are more approximate since they were calculated on the basis of measurements at three temperatures only.

Kinetic Measurements. The hydrolysis of p-nitrophenyl diphenyl phosphate was studied spectrophotometrically by measuring the rate of appearance of the p-nitrophenoxide anion at 4030 Å with a Varian DMS-80 spectrophotometer equipped with a temperature controlled cell compartment. The reaction was studied at 15°, 25° and 35°C at various concentrations of NaOH and CDEAB. The pseudo-first order rate constant (k_ψ), in s^{-1}, was determined from linear plots of logarithm of absorbance versus time and the second order rate constants (k_{2m}) in the micellar phase and (k_2^0) in the aqueous phase, in $s^{-1}M^{-1}$, were calculated

from k_ψ and the hydroxide ion concentration. Activation parameters such as the activation energy (E_a), the activation entalphy ($\Delta H^{o\neq}$) and the activation entropy ($\Delta S^{o\neq}$) were determined from experimental k_ψ values measured at three different temperatures using the following equations.

$$\ln k_\psi = \ln A - (E_a/R)\,(1/T) \quad (1)$$

$$\Delta H^{o\neq} = E_a - RT \quad (2)$$

$$\Delta S^{o\neq} = 4.576\,(\log k_\psi - 10.753 - \log T + E_a/4.576T) \quad (3)$$

$$\Delta G^{o\neq} = \Delta H^{o\neq} + \Delta S^{o\neq} \quad (4)$$

where, R corresponds to the gas constant and T to the absolute temperature.

RESULTS AND DISCUSSION

Some typical experimental results obtained for the surface tension of CDEAB in water solutions at 25° and 32°C are illustrated in Figure 1.

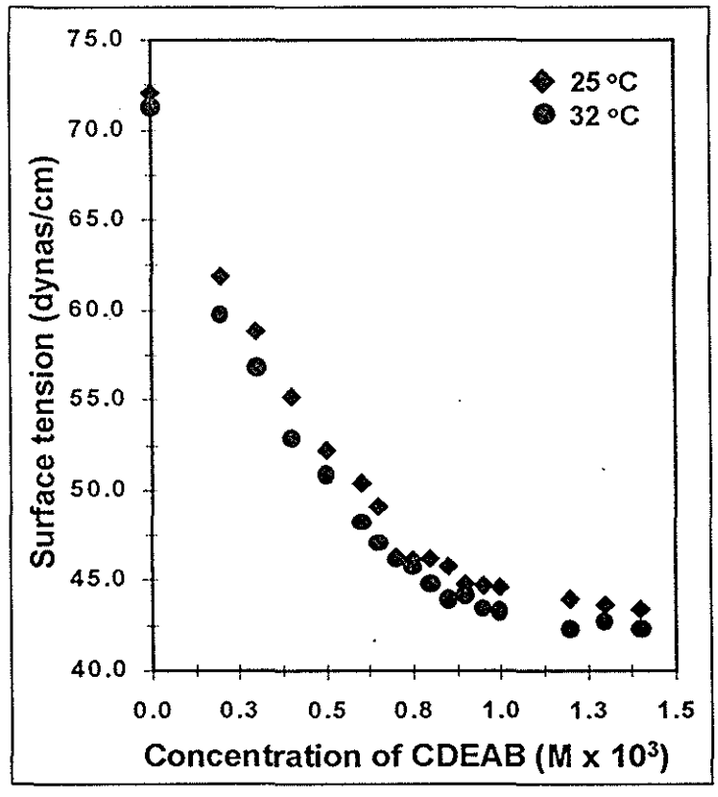


Figure 1. Plot of Surface Tension versus Concentration of Cetyldimethylethylammonium Bromide (CDEAB) in water at 25° and 32°C.

All plots of surface tension versus the concentration of CDEAB exhibited initial marked drops and subsequent leveled off. The inflection point in the given curve was taken as the CMC. At times, plots of surface tension versus the logarithm of the concentration of surfactant gave a better determination for the CMC. Such results are shown in Figure 2 for the same temperatures.

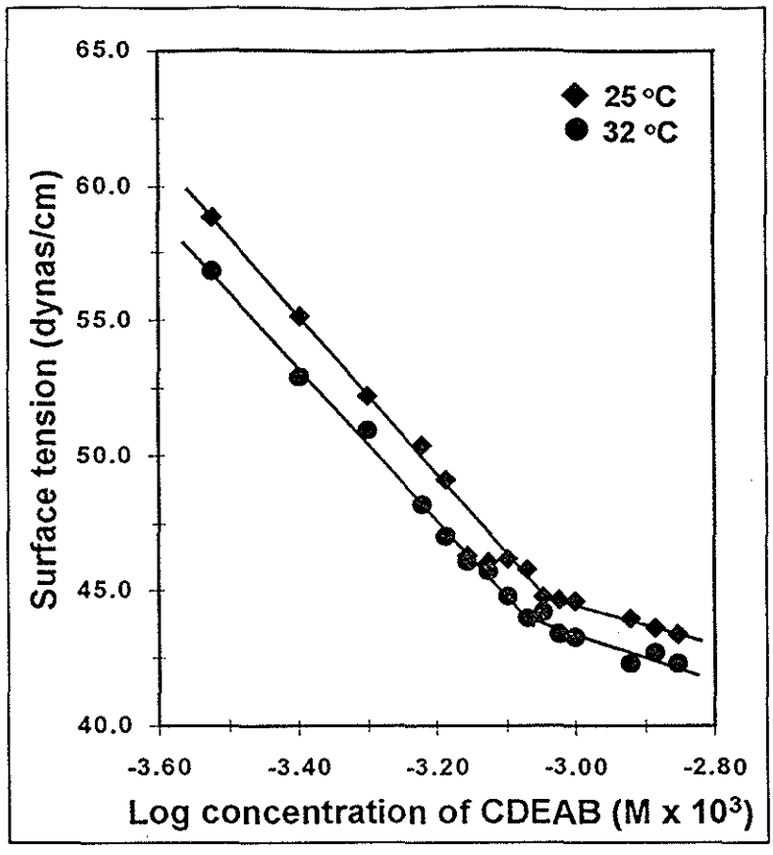


Figure 2. Plot of Surface Tension versus Logarithm of the Concentration (M x 10^{-3}) of Cetyldimethylethylammonium Bromide (CDEAB) in water at 25° and 32°C.

The experimental values determined for the critical micellar concentration (CMC) are summarized in Table I and compared to cetyltrimethylammonium bromide (CTAB). Table II illustrates the experimental values obtained for the thermodynamic functions, i.e., the standard free energy of micellization, ΔG°_{mic}, the enthalpy, ΔH°_{mic}, and the standard entropy of micellization ΔS°_{mic} at 25°C, again compared to CTAB[7,11,14]. As expected, the difference between the experimental values obtained for the CMC and the thermodynamic properties is very small; the difference in the structure of the two surfactants being only the substitution of one of the head CH_3- groups by an ethyl group.

Table I. Critical Micellar Concentration (CMC) of Cetyldimethyethyllammonium Bromide (CDEAB) in Aqueous Solutions Compared to CTAB[14]

Surfactant	Temperature (°C)		
	25	32	40
CDEAB	9.05×10^{-4} M	9.30×10^{-4} M	10.0×10^{-4} M
CTAB	9.20×10^{-4} M	--	10.0×10^{-4} M

Table II. Some Thermodynamic Properties for the Formation of Micelles of Cetyldimethylethylammonium Bromide (CDEAB) in Water at 25 °C Compared to CTAB[14]

Surfactant	Free Energy of Micellization at 25 °C ΔG°_{mic} (kcal/mole)	Enthalpy of Micellization ΔH°_{mic} (kcal/mole)	Entropy of Micellization at 25 °C ΔS°_{mic} (e.u.)
CDEAB	-4.15	-1.37	+9,33
CTAB	-4.14	-1.03	+10.43

Typical profiles for the pseudo-first order rate constant, k_{ψ}, as a function of the concentration of CDEAB for the hydrolysis of p-nitrophenyl diphenyl phosphate (NPDPP) at 25°C in aqueous solutions containing various concentrations of NaOH, ranging from 0.001 M to 0.010M are shown in Figure 3.

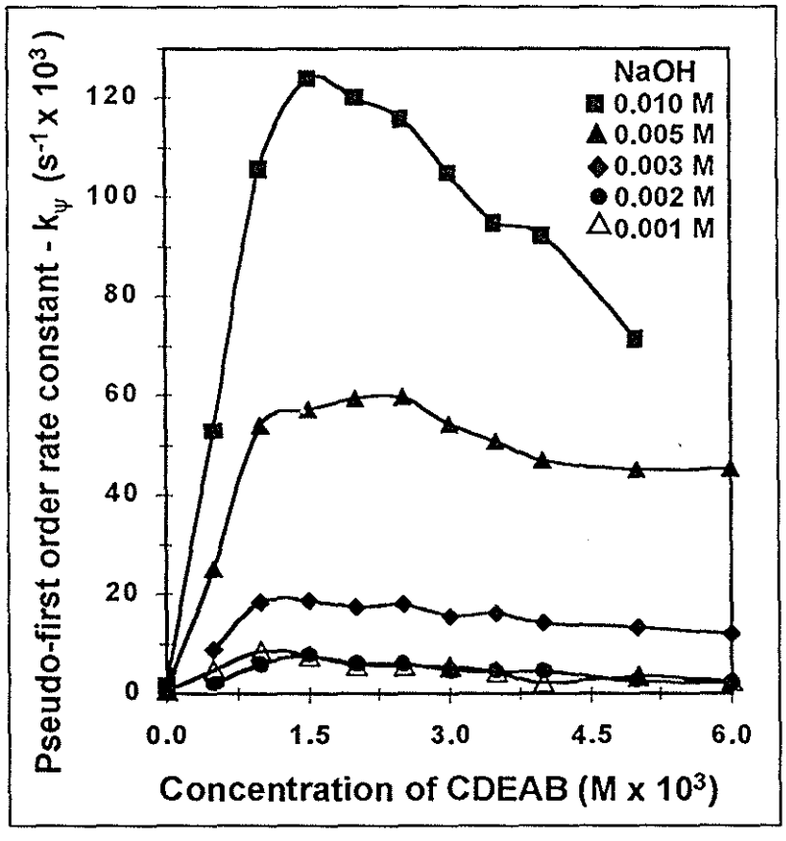


Figure 3. Rate Profiles for the Hydrolysis of p-Nitrophenyl Diphenyl Phosphate in Aqueous Solutions of NaOH and Various Concentrations of CDEAB at 25°C.

The experimental rate profiles obtained are characteristic of micellar catalyzed reaction in aqueous solutions with a maximum at 2.0 x 10^{-4} M CDEAB, similar to that measured for CTAB. The addition of CDEAB to the reaction medium causes an increase in the rate of hydrolysis up to a point (the maximum in rate) where there is total incorporation of the substrate

in the micellar phase. Subsequent addition of the surfactant causes a decrease in the reaction rate, probably due to the dilution of the reactive counter-ions in the Stern layer of a higher number of micelles.

Typical activation parameters measured for the reaction with CDEAB are shown in Table III. As can be seen from the analysis of the results the activation parameters for the two surfactants are very similar and comparable to others measured for micellar catalyzed reactions. The addition of the surfactant in the form of micelles causes a decrease of approximately 5 kcal/mol in the activation energy.

Table III. Activation Parameters for the Hydrolysis of p-Nitrophenyl Diphenyl Phosphate in Aqueous Solutions of 0.005 M NaOH in the Presence of CDEAB and CTAB[28] at 25 °C.

Surfactant	Concentration (M x 10^4)	E_a (kcal/mole)	$\Delta H^{o\neq}$ (kcal/mole)	$\Delta G^{o\neq}$ (kcal/mole)	$\Delta S^{o\neq}$ (e.u.)
--	--	+ 15.2	+14.6	+21.3	-22.2
CDEAB	18	+ 9.0	+ 8.4	+19.2	-36.3
CTAB	15	+11.4	+10.8	+18.7	-26.6
CTAB	20	+10.5	+ 9.9	+18.8	-29.9

Most of the models proposed for micellar catalysis[31-38] consider the partition coefficient for the substrate between the micellar and aqueous phase and the distribution of the reagents between the two phases. The hydrolysis of NPDPP with hydroxide ion in the presence of CDEAB may be considered a bimolecular reaction of OH^- ion and the substrate. Since the concentration of OH^- in the micellar phase is dependent on the concentration of both bromide ions and surfactant, a quantitative treatment of the reaction rate must consider ion exchange on or near the micellar surface. For the reaction under consideration, the model proposed by Quina and Chaimovich[38] reduces to Equation 5, that gives the theoretical dependence of the pseudo-first order constant, k_ψ, as a function of the total hydroxide ion concentration

$$k_\psi = \frac{\{(k_{2m}/V)\, K_S K_{OH/Br}\, [(Br)_m/(Br)_w] + k_2^0\}(OH)_T}{(1+K_S C_D)\,[1+K_{OH/Br}\,(Br)_m\,(Br)_w]} \qquad (5)$$

where, C_D is the concentration of micellized surfactant, V is the molar volume of the reactive region at the micelar surface, k_ψ is the pseudo-first order rate constant, k_{2m} is the second order rate constant in the micellar phase, k_2^0 is the second order constant in the aqueous phase, $K_{OH/Br}$ is the ion exchange constant, K_s is the binding constant for the substrate, $(Br)_m$ is the concentration of Br^- in micellar phase, $(Br)_w$ is the concentration of Br^- in aqueous phase, $(OH)_T$ is the total concentration of hydroxide ions and V is the molar volume of surfactant.

With substrates such as p-nitrophenyl diphenyl phosphate that are very insoluble in water and are solubilized by CDEAB the expression for $k_\psi$can be reduced to a simpler form given by Equation (6):

$$k_\psi = \frac{k_{2m}}{C_D V}\,(OH)_T\,\frac{K_{OH/Br}\,[(Br)_m/(Br)_w]}{[1+K_{OH/Br}\,(Br)_m\,(Br)_w]} \qquad (6)$$

The concentration of Br^- in the micellar and aqueous phases can be obtained using the following equations[39,40]:

$$A_1 = C_D + CMC + K_{OH/Br}\,(OH)_T + (1 - \alpha)\,C_D\,K_{OH/Br} \qquad (7)$$

$$(OH)_m = \frac{(-A_1) + [(A_1)^2 + 4\,(1 - K_{OH/Br})\,(OH)_T\,K_{OH/Br}\,(1 - \alpha)\,C\,]^{0.5}}{2\,(1 - K_{OH/Br})} \qquad (8)$$

$$(B_r)_m = (1 - \alpha)\,C_D - (OH)_m \qquad (9)$$

$$(Br)_w = \alpha\,C_D + CMC + (OH)_m \qquad (10)$$

where CMC is the critical micellar concentration, α is the degree of ionization of the micelle and $(OH)_m$ is the concentration of OH^- in the micellar phase.

We have calculated the theoretical values of k_ψ for the reaction discussed above using $\overline{V}$ = 0.37 l/mol; $K_{OH/Br}$ = 0.08; α = 0.20 and various concentrations of CDEAB and NaOH. The results obtained for the calculated values of k_ψ using different values for k_{2m} compared to the experimental data are illustrated in Figure 4.

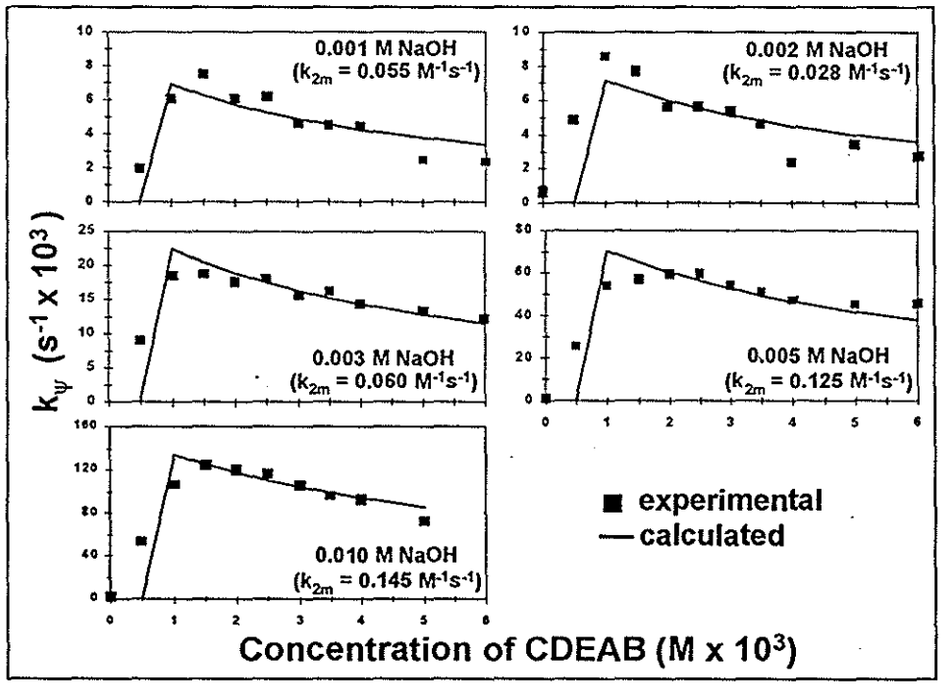


Figure 4. Experimental and Theoretical k_ψ Values for the Hydrolysis of p-Nitrophenyl Diphenyl Phosphate in Aqueous Solutions of NaOH and Various Concentrations of CDEAB at 25°C.

The best fits between the experimental and theoretical k_ψ values are obtained using a variable k_{2m}, between 0.028 $M^{-1}s^{-1}$ and 0.145 $M^{-1}s^{-1}$ indicating that the pseudo-phase ion exchange model (PPIEM) gives a reasonable agreement for this micellar catalyzed reaction.

ACKNOWLEDGMENTS

Support received from CNPq - National Research Council of Brazil, FINEP and Sarmisegetusa Research Group, Las Cruces and Santa Fe, New Mexico, USA is gratefully acknowledged.. EFS also acknowledges the financial support from FAPESP, São Paulo, Brazil.

REFERENCES

1. L. G. Ionescu and J. K. Tsang, *Rev. Roum. Biochim., 15*, 211 (1978).
2. L. G. Ionescu, T. Tokuhiro, B. J. Czerniawski and E. S. Smith, in "*Solution Chemistry of Surfactants*", K. L. Mittal, Ed., Plenum Press, New York, Vol. 1, p. 487 (1979).
3. T. Tokuhiro and L. G. Ionescu in "*Solution Chemistry of Surfactants*", K. L. Mittal, Ed., Plenum Press, New York, Vol. 1, p. 507 (1979).
4. L. G. Ionescu, T. Tokuhiro and B. J. Czerniawski , *Bull. Chem. Soc. Jpn., 52*, 922 (1979).
5. T. Tokuhiro, D. S. Fung and L. G. Ionescu,. *J. Chem. Soc. Faraday Trans. II, 75*, 975 (1979).
6. L. G. Ionescu and B. J. Czerniawski, *Rev. Roum. Biochim., 18*, 103 (1981).
7. L. G. Ionescu, and V. T. De Fávere in "*Solution Behavior of Surfactants: Theoretical and Applied Aspects*", K. L. Mittal and E. J. Fendler, Eds., Plenum Press, New York, Vol. 1, p. 407, 1982.
8. L. G. Ionescu, *Arch. Biol. Med. Exp., 12(2)*, 272 (1979).
9. L. G. Ionescu and D. S. Fung, *Bull. Chem. Soc. Jpn., 54*, 2503 (1981).
10. L. G. Ionescu, and D. S. Fung, *J. Chem. Soc. Faraday Trans. I*, 77, 2907 (1981).
11. L. G. Ionescu, L. S. Romanesco and F. Nome in "*Surfactants in Solution*", K. L. Mittal and B. Lindman, Eds., Plenum Press, New York, Vol. 2, p. 789, 1984.
12. L. G. Ionescu and P. E. De Brito Moreira, *Atual. Fís. Quim. Org., 2*, 79 (1984).
13. L. G. Ionescu, *Química Nova, 8(3)*, 191 (1985).
14. L. G. Ionescu, S. M. H. Probst, E. F. de Souza, *South. Braz. J. Chem., 6(7)*, 67-76 (1998).
15. F. A. Gunther, J. D. Gunther, *Chemistry of Pesticides*, Sringer Verlag, New York, 1971.
16. C. Salazar, G. M. Souza, C. P. D. Silva, *Manual de Inseticidas e Acaricidas - Aspectos Toxicologicos*, UFPel, Pelotas, Brazil, 1976.
17. C. A. Bunton, L. G. Ionescu, *J. Am. Chem. Soc., 95*, 2912 (1973).
18. L. G. Ionescu, D. A. Martinez, *J. Colo. Wyo. Acad. Sci., 7*, 13 (1974).
19. L. G. Ionescu, *Bull. N. Mex. Acad. Sci., 14*, 65 (1973).
20. C. A. Bunton, S. Diaz, J.M. Hellyer, I. Ihara, L. G. Ionescu, *J. Org. Chem., 40*, 2313 (1975).
21. F. Nome, E. W. Schwingel, L. G. Ionescu, *J. Org. Chem., 45*, 705 (1980).
22. F. Nome, A. Rubira, L. G. Ionescu, *J. Phys. Chem., 86*, 1181 (1982).
23. L. G. Ionescu, F. Nome, In *Surfactants in Solution*; K. L. Mittal, B. Lindman, Eds.; Plenum Press: New York, Vol. 2, p. 1107, 1984.
24. L. G. Ionescu, E. F. Souza, *South. Braz. J. Chem., 1*, 75 (1993).
25. L. G. Ionescu, E. F. Souza, In *Surfactants in Solution*; A. K. Chattopadhyay, K. L. Mittal, Eds.; Marcel Dekker: New York, Vol. 64, p. 123, 1996.
26. L. G. Ionescu, E. F. Souza, *South. Braz. J. Chem., 3*, 63 (1995).
27. L. G. Ionescu, D. A. R. Rubio, E. F. Souza, *South. Braz. J. Chem., 4*, 59 (1996).
28. E. F. Souza, L. G. Ionescu, *Coll. and Surfaces A, 149*, 609 (1999).
29. A. M. Ross, J. Toet, *Tev. Trav. Chim., 77*, 1946 (1958).
30. A. S. Kirby, J. Jounas, *J. Chem. Soc. B* , 1165 (1970).
31. N. Muller in "Reaction Kynetics in Micelles", E. H. Cordes, Ed., Plenum Press, New York, p.1-23, 1973.
32. D. G. Hall, *Trans. Faraday Soc., 66*, 1351, 1359 (1970).

33. S. Dani, Master's Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazsil, 1991, 169 pp.
34. J. H. Fendler, E. J. Fendler, *Catalysis in Micellar and Macromolecular Systems*, Academic Press: New York, 1975.
35. I. V. Berezin, K. Martinek, A. K. Yatsimirskii, *Russ. Chem. Rev., 42*, 787 (1973).
36. K. Martinek, A. K. Yatsimirskii, A. V. Levasov, I. V. Berezin, In *Solubilization and Microemulsions*; K. L. Mittal, Plenum Press: New York, Vol. 2, p. 489, 1977.
37. L. S. Romsted, In *Solubilization and Microemulsions*; K. L. Mittal, Ed., Plenum Press: New York, Vol. 2, p. 509, 1977.
38. F. Quina, H. Chaimovich, *J. Phys. Chem., 83*, 1844 (1979).
39. N. Funasaki, *J. Phys. Chem., 83*, 1998 (1979).
40. C. Otero, E. Rodenas, *Can. J. Chem., 63*, 2892 (1984).

The SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY (ISSN: 2674-6891; 0104-5431) is an open-access journal since 1993. Journal DOI: 10.48141/SBJCHEM.
http://www.sbjchem.com.
This text was introduced in this file in 2021 for compliance reasons.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

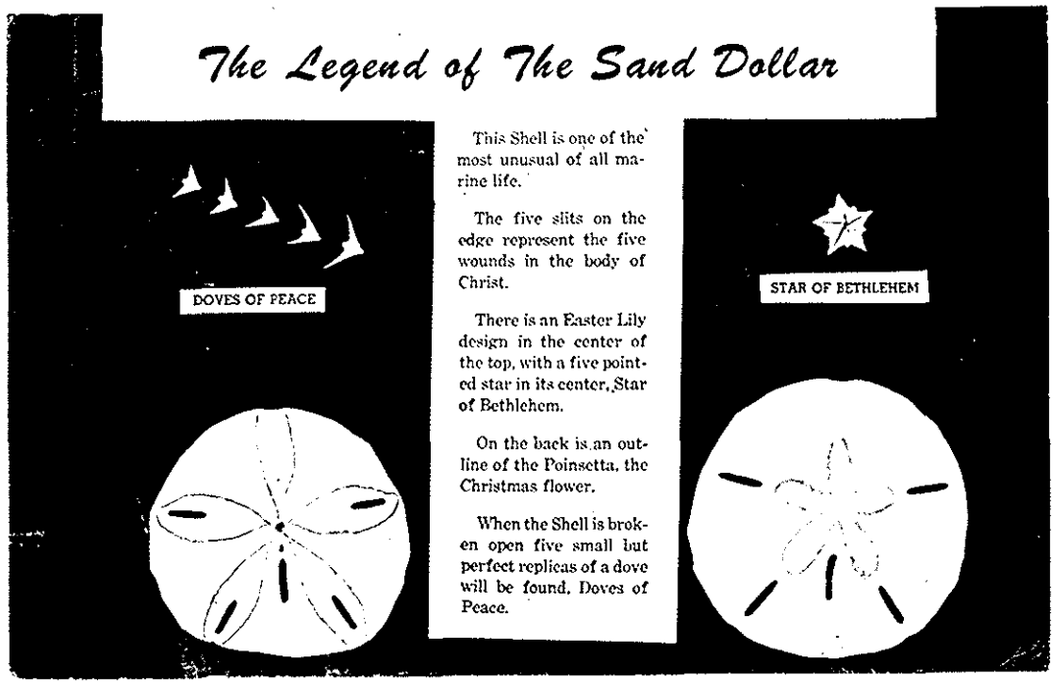
ISSN 0104-5431

VOLUME SEVEN, NUMBER EIGHT DECEMBER 1999

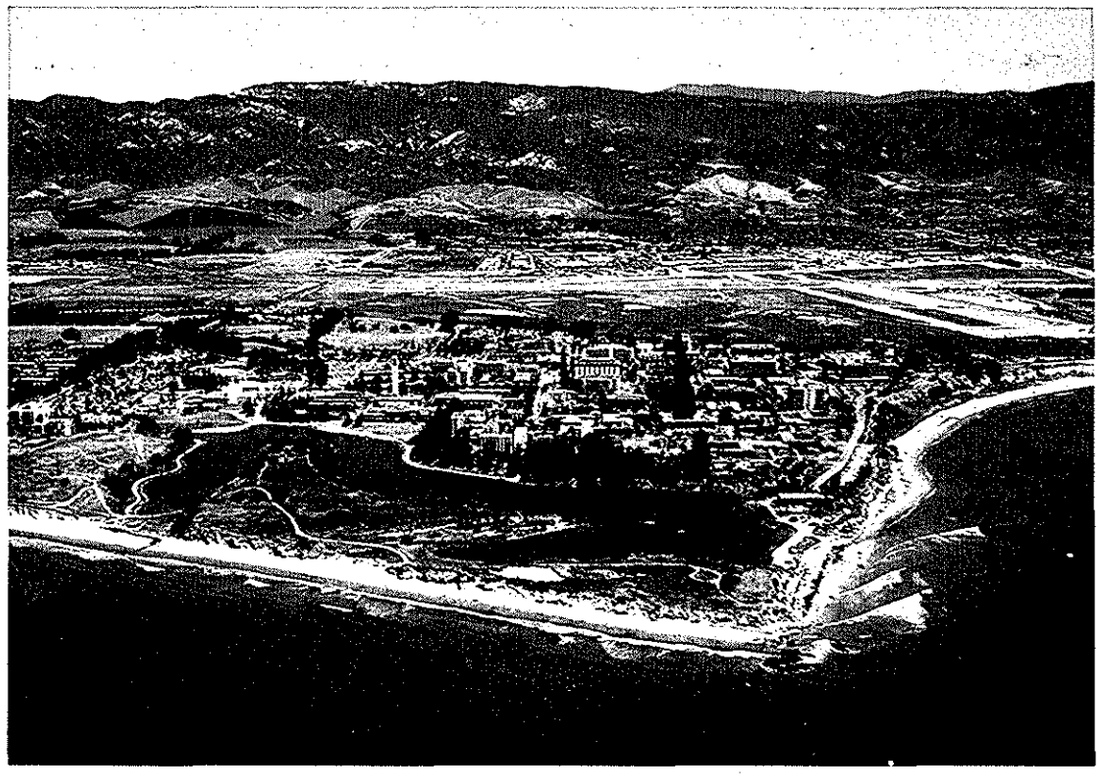
AUTHOR INDEX / ÍNDICE DE AUTORES

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

ISSN 0104-5431



A LENDA DA BOLACHA DE AREIA



UNIVERSITY OF CALIFORNIA, SANTA BARBARA, USA.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

ISSN 0104-5431

The *SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY* is an international forum for the rapid publication of original scientific articles dealing with chemistry and related areas. At the present there are no page charges and the authors will receive twenty five free reprints of their papers.

SPECIAL COMBINATION OFFER FOR NEW SUBSCRIBERS!

SUBSCRIPTION INFORMATION

PRICE: Brazil and Latin America: US$ 35.00 per issue.

Other Countries: US$ 50.00 per issue, including air mail delivery.

Persons or institutions outside Brazil should send subscription fee payable to Dr. L. G. Ionescu, c/o SBJC 8532 Howard Circle, Huntington Beach, California, USA 92647

ORDER FORM

☐ Please enter my subscription for _____ issues of Southern Brazilian Journal of Chemistry.

☐ Please send me _____ copies of the Southern Brazilian Journal of Chemistry Vol. 1,2,3,4,5,6 (1993-1998)
(Circle desired copies.)

☐ I enclose a check or money order in the amount of $_____

☐ Please send me a Pro Forma Invoice for the above order.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

Lavinel G. Ionescu, B.S., M.S., Ph.D., Editor
Caixa Postal 15032, Agronomia
Porto Alegre, RS BRASIL 91501-970

TEL. 55 51 485-1820 FAX 55 51 320-3612 or 55 51 477-1313

EMail: LGIPUCRS a MUSIC.PUCRS.BR
LAVINEL a MOZART.ULBRA.TCHE.BR

INFORMATION FOR AUTHORS

The Southern Brazilian Journal of Chemistry - SBJC will publish review articles, original research papers and short communications dealing with chemistry and interdisciplinary areas such as materials science, biotechnology, bioengineering and other multidisciplinary fields.

Articles report the results of a complete study. They should include an Abstract, Introduction describing the known art in the field Experimental or Materials and Methods, Results and Discussion, Acknowledgments (when appropriate) and References.

Short Communications should be limited to 1500 words, including the equivalent space for figures and/or tables and should include an Abstract and concise Experimental.

Manuscripts may be submitted on-line or in triplicate (original and two copies by registered mail) and are received with the understanding that the original has not been submitted for publication elsewhere. It is implicit that all the persons listed as authors have given their approval for the submission of the paper and that permission has also been granted by the employer, when necessary.

Manuscripts must be written in American or British English, single spaced, on A4 sheets (21 cm x 29.5 cm) and one side only and should be numbered beginning with the title page. Type must be 12 Arial or Times New Roman.

Margins of at least 3 cm should be left at the top and bottom and both sides of each page. The first page of the paper should contain only the title of the paper, the name(s) and addressees) of the author(s), an abstract of not more than 250 words and 4-8 keywords. We reserve the right to translate the abstract in Portuguese. Abstracts are required of all papers including reviews and short communications.

Figures and Tables with short explanatory titles, each on a separate sheet, should be adequate for direct reproduction and identified in pencil on the back of each page by Arabic numerals, corresponding to the order they appear in the manuscript. Tables and Figures (BMP or JPG format) may also be included directly in the text when convenient and the article may submitted in a quasi-final form in order to facilitate editorial work.

References should be numbered in the sequence they appear in the text, cited by superior numbers and listed at the end of the paper in the reference section in the numerical order they appear in the text. The style for references is shown below:

1. L. G. Ionescu and D. S. Fung, *J. Chem. Soc. Faraday Trans. I, 77*, 2907-2912 (1981).
2. K. L. Mittal, Ed., "*Solution Chemistry of Surfactants*", Plenum Press, New York (1984), Vols. 1-3, pp. 1-2173.

IUPAC Rules should be used for the name of chemical compounds and preference should be given to 51 units.

Authors are invited to send manuscripts by registered air mail to the EDITOR - SBJC, C.P. 15032, Agronomia, Porto Alegre, RS BRASIL 91501, or by e-mail to **lavinel@ibest.com.br** or **lavinel@pop.com.br**.

VISIT OUR SITE: http://www.sbjchem.he.com.br

SCIENCO
SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

ISSN 0104-5431

The *SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY - SCIENCO* publishes original research articles in chemistry and related interdisciplinary areas and is intended to fill a gap in terms of scientific information for Southern Brazil.

Occasionally the journal will include review papers and articles dealing with chemical education and philosophy and history of science. It will be published mainly in English, with abstracts in Portuguese and only occasional papers in other languages. At the present there are no page charges and the authors will receive twenty five reprints of their papers free of charge.

We have set high standards for the articles to be published by ensuring strong but fair refereeing by at least two reviewers. We hope that this journal will provide a forum for dissemination of high quality research in chemistry and related areas and are open to any questions and suggestions.

The Editor

SUBSCRIPTION INFORMATION

Brazil and Latin America:
US$ 35.00 per issue.

Other Countries: US$ 50.00 per issue, including air mail delivery. Persons or institutions outside Brazil should send subscription fee payable to Dr. L. G. Ionescu, c/o SBJC, 8532 Howard Circle, Huntington Beach, California, USA 92647

MAILING ADDRESS

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY - SBJC
Lavinel G. Ionescu, B.S., M.S., Ph.D., Editor
C.P. 15032, Agronomia
Porto Alegre, RS BRASIL 91501-000
Tel. (051) 485-1820 FAX (051) 339-1564

Endless Column, 1937, cast iron
CONSTANTIN BRÂNCUSI

FINANCIAL SUPPORT

SARMISEGETUSA RESEARCH GROUP

SANTA FE, NEW MEXICO, U.S.A.