DCC-33 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B1—B2 (1972)

Preparation and Properties of Carrier Bound Hyaluronidase

O. Ščedrov

Laboratory of Biochemistry, Faculty of Technology University of Zagreb, 41000 Zagreb, Croatia, Yugoslavia

Prepn. and properties of carrier bound hyaluronidase (hyluronate glycanohydrolase, *EC* 3.2.1.35) have been described. Two lyophilized hyaluronidase samples were used: one from bull testis (activity 500 I. U./mg), and the other from ram testis (activity 767 I. U./mg). Aminochloro-*s*-triazinyl derivs. of DEAE-cellulose and Sephadex G-200 (both prepd. in the Laboratory of Biochemistry), Enzacryl AA (Koch-Light product), and the copolymers consisting of acrylamide, *N,N'*-methylene-bis-acrylamide and maleic acid (Boehringer product), cyclized and noncyclized, were used as carriers. The hyaluronidase activity was detd. by turbidimetric method according to Dorfman with minor modifications.

The spectrofluorometric method for assaying hyaluronidase activity described by Guilbault et al. [*Anal. Biochem.* 18 (1967) 241] was found to be unreliable. The conditions used in the prepn. of carrier bound enzyme were as follows: enzyme-carrier weight ratio 1:5, attachment time 16 hours, temperature 4—6^0 C, 0.20 *M* phosphate or citric acid-phosphate buffers with varying pH.

The noncyclized Boehringer carrirer was found to be the most favourable for hyaluronidase attachment. It was ascertained that the amt. of the carrier bound enzyme depends on the pH of the buffer used as well as on the enzyme purity. By changing the pH of the buffer in any direction from the optimum value, a decreasing amount of bound enzyme could be noticed. Under optimum conditions 32$^0/_0$ of the enzyme could be bound corresponding to 49 I.U./mg carrier.

The properties of carrier bound hyaluronidase were compared with that of sol. hyaluronidase. The expts. showed that the carrier bound hyaluronidase retained 13.5$^0/_0$ of its initial activity in the soln. after 36 days at room temp. and that the activity was reduced to 45$^0/_0$ after 10 minutes heating at 80^0 C. Thus the bound hyaluronidase was more stable than the soluble one. Dodecyl sulfate in concn. of 10^{-3} *M* inhibits the soluble enzyme completely, but does not affect the bound hyaluronidase. The pH dependence of the bound hyaluronidase activity was also examd. The optimum was found at a pH of 6.2. Kinetic measurements showed that substrate decompn. was faster with the bound hyaluronidase than with the soluble one. The bound enzyme can be used many times as a catalyst.

Examiners: Prof. P. Mildner, Prof. M. Proštenik, and Prof. A. Režek.

Oral examination: March 31, 1972.

Dissertation deposited at the University Library, Zagreb and Faculty of Technology, University of Zagreb.

(127 pages, 9 tables, 8 figures, 127 references, original in Croatian)

O. ŠČEDROV

DCC-33

1. Preparation and Properties of Carrier Bound Hyaluronidase

I. Ščedrov O.

II. Laboratory of Biochemistry, Faculty of Technology, University of Zagreb, 41000 Zagreb, Croatia, Yugoslavia

Enzymes, carrier bound
Hyaluronate glucanohydrolase, carrier bound
Hyaluronidase, carrirer bound
  from bull testes
  from ram testes

DCC-34 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B3—B4 (1972)

The Interaction of EDTA with Some Radionuclides in Sea Water and Sodium Chloride Solutions

Lj. Marazović-Musani

Laboratory for Electrophoresis, Center for Marine Research,
»Ruđer Bošković« Institute, 41000 Zagreb, Croatia, Yugoslavia

The chelation of some radionuclides in sea water and in NaCl soln. with EDTA was investigated by high voltage paper electrophoresis. In order to compare the behaviour of investigated radionuclides in sea water-EDTA systems with their behaviour in NaCl-EDTA systems 0.55 *M* sodium chloride soln. was used as supporting electrolyte. EDTA concn. was varied from 10^{-6}—10^{-2} *M*, pH being adjusted to 8.0. The behaviour of radionuclides was followed by measuring the dependence of their electrophoretic mobilities on the EDTA concn. in the systems and on the aging of the systems from 0—10 days.

From the data obtained it is evident that, depending on the EDTA concn., the investigated radionuclides behave in three characteristic ways. In sea water and in NaCl soln. without EDTA as well as at low EDTA concns. some of the investigated radionuclides behave as cations: ^{45}Ca, ^{51}Cr (in NaCl soln.), ^{54}Mn, ^{58}Co, ^{65}Zn, $^{88(90)}$Y (in NaCl soln.), ^{90}Sr and ^{109}Cd, with different electrophoretic mobilities, and the other do not move in the electric field: ^{51}Cr (in sea water), $^{55(59)}$Fe and $^{88(90)}$Y (in sea water). At very high EDTA concns. all the radionuclides are found to be complexed with EDTA. In an intermediate range between low and high EDTA concns. the electrophoretic mobilities of the investigated radionuclides show either continuous change (fast interaction with EDTA): ^{45}Ca, ^{54}Mn, ^{65}Zn, $^{88(90)}$Y (in NaCl solns.), ^{90}Sr and ^{109}Cd, or their complexed forms can be separated from uncomplexed ones (slow interaction with EDTA): ^{51}Cr, $^{55(59)}$Fe, ^{58}Co and $^{88(90)}$Y (in sea water). Special case represents ^{32}P behaving as anion in both systems.

From exptl. results apparent stability consts. of the metal-EDTA complexes were calcd. for the two types of interaction with EDTA (slow and fast processes).

Part of thesis was published and reported: *Rapp. Comm. int. Mer Médit.* **20** (1972) 701.

Examiners: Dr Z. Pučar, Prof. P. Strohal, and Prof. M. Mirnik.

Oral examination: June 19, 1972;

Degree confered: October 31, 1972.

Thesis deposited at University Library, Zagreb and Institute »Ruđer Bošković«, Zagreb and Rovinj.

(243 pages, 35 figures, 19 tables, 237 references, original in Croatian)

LJ. MARAZOVIĆ-MUSANI

DCC-34

1. The Interaction of EDTA with Some Radionuclides in Sea Water and Sodium Chloride Solutions

I. Marazović-Musani Lj.

II. Laboratory for Electrophoresis, Center for Marine Research, »Ruđer Bošković« Institute, Zagreb, Croatia, Yugoslavia

Cadmium-109
Calcium-45
Cobalt-58
Chromium-51
EDTA
  complexes with radionuclides
Electrophoresis, high voltage paper
Iron-55 (59)
Manganese-54
Phosphorus-32
Sea water
  complexes of radionuclides in
Stability constants
  of some radionuclides with EDTA
Strontium-90
Yttrium-88 (90)
Zinc-65

DCC-35 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B5—B6 (1972)

Comparative Studies of Cholinesterases in their Reaction with Inhibitors and Substrates

V. Simeon

Biochemical Laboratory, Institute for Medical Research, Yugoslav Academy of Sciences and Arts, 41000 Zagreb, Croatia, Yugoslavia

The interaction of acetylcholinesterase (EC 3.1.1.7) and cholinesterase (EC 3.1.1.8) with inhibitors and substrates was studied. Two organophosphates [di-(2-chloroethyl)-3-chloromethylcoumarin-7-yl phosphate and methylsulphate of diethyl-3-trimethyl-ammonium-phenyl-phosphate] and two carbamates (3-isopropylphenyl-*N*-methylcarbamate and bromide of 3-trimethylammonium-*N,N*-dimethylcarbamate) were used as inhibitors. Acetylthiocholine, acetylcholine, benzoylcholine, acetyl-β-methylcholine, butyrylcholine, *o*-nitrophenylbutyrate and triacetine served as substrates.

The effect of temp. on the reaction of enzymes with inhibitors and substrates was measured. Further, the influence of substrate on the degree of reversible inhibition was studied.

The kinetics of progressive inhibition of both enzymes by carbamic acid esters show that these compds. do not form a measurable amt. of the Michaelis complex between enzyme and inhibitor. The same is found for the interaction of serum cholinesterase with the two organophosphates. However, the kinetics of interaction of acetylcholinesterase with organophosphates show the existence of a reversible complex between enzyme and inhibitor. From the influence of substrates on the degree of reversible inhibition it has been concluded that the reversible inhibition occurs outside the active site of acetylcholinesterase.

The rate consts. of inhibition for acetylcholinesterase and human serum cholinesterase increase with increasing temp. obeying the Arrhenius equation. The rate consts. of inhibition of horse serum cholinesterase with all the four inhibitors increase from 10—30 °C, but decrease from 30—40 °C. This is attributed to a conformational change in the enzyme.

The Michaelis consts. for acetylcholinesterase and cholinesterase do not change with temp. from 10—40 °C, while the K_m value for horse serum cholinesterase and acetylthiocholine is constant from 10—30 °C and increases from 30—40 °C. This is an evident analogy between the reaction of serum cholinesterase with inhibitors and substrates.

The thesis was partly published in *Biochem. J.* **130** (1972) 515.

Examiners: Dr. E. Reiner, Prof. P. Mildner, and Prof. Dj. Deželić

Oral examination: January 13, 1972.

Degree conferred: January 26, 1972.

(105 pages, 29 tables, 19 figures, 73 references, original in Croatian)

V. SIMEON

DCC-35

1. Comparative studies of Cholinesterases in their Reaction with Inhibitors and Substrates

I. Simeon V.

II. Institute for Medical Research, Yugoslav Academy of Sciences and Arts, 41000 Zagreb, Croatia, Yugoslavia

Acetylcholine acetylhydrolase
Acetylcholinesterase
Acylcholine acylhydrolase
Carbamic acid, esters of, cholinesterase inhibition by
Cholinesterase
Enzyme inhibition, temp. dependence of
Organophosphorus compds., cholinesterase inhibition by

DCC-36 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B7—B8 (1972)

Structure — Reactivity Relationship. The Azide Probe as a Measure of the Carbonium Ion Stability

D. Kocijančić-Kovačević

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

The azide competition factors ($k_{N_3^-}/k_{SOH}$) were used for studying the relative relationship between the structure and selectivity of the intermediates in the solvolytic reactions of benzhydryl bromide and chloride, 2-adamantyl tosylate, 2-methyl-2-adamantyl chloride, 1-adamantyl bromide, cyclohexyl, 3β-cholestanyl and 3α-cholestanyl brosylates.

The results obtained cannot be simply explained by a structure and selectivity relationship. Tertiary substrates, 2-methyl-2-adamantyl chloride and 1-adamantyl bromide, gave lower $k_{N_3^-}/k_{SOH}$ values than secondary ones, cyclohexyl and 3α-cholestanyl brosylates. This is opposite from what should be expected on the basis of the stability of respective carbonium ions, and could be explained by the reaction of azide ion and the ion pair(s) of secondary systems.

The competition factors do not depend only on the structure of the substrates but also upon other various parameters such as temp., solvent, ionic strength of the soln. and concn. of sodium azide.

Since the mechanism of solvolytic reactions where ion pairs are intermediates, is very complex and involves several steps, significant mechanistic conclusions cannot be obtained on the basis of competition factors.

A part of this work was published in *Tetrahedron* 28 (1972) 2469.

Examiners: Prof. D. Sunko, Dr. T. Cvitaš, and Dr. A. Deljac

Oral examination: July 14, 1972.

Thesis deposited at the Faculty of Science, University of Zagreb.

(126 pages, 19 tables, 11 figures, 92 references, original in Croatian)

D. KOCIJANČIĆ-KOVAČEVIĆ

DCC-36

1. Structure — Reactivity Relationship. The Azide Probe as a Measure of the Carbonium Ion Stability

I. Kocijančić-Kovačević D.

II. Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Azide probe
Carbonium ion, stability of

DCC-37 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B9—B10 (1972)

Site of Protonation of Some Substituted Phenols and Alkylphenyl Ethers in SbF_5/HSO_3F

M. Eckert-Maksić

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Protonation of a variety of substituted phenols and alkylphenyl ethers was studied in mixture of SbF_5 with HSO_3F (»magic acid«) by low temp. N.M.R. spectroscopy. The site of protonation was found to be strongly dependent upon the nature and the position of the substituents.

p-Alkyl substituted phenols and alkylphenyl ethers behave as ambident bases under exptl. conditions used, protonating either on the oxygen atom or in the *ortho* position of the aromatic ring. The ratio of ring to oxygen protonation was found to be a sensitive function of: 1) the concn. of antimony pentafluoride in fluorosulfuric acid; 2) temp.; 3) substituent changes on both the ring and the oxygen atom; 4) the concn. of the base (*e. g.* SO_2 or H_2O).

The results indicate, that the ambident behaviour of the examd. bases is quite sensitive to their solvent environment. Thus, this system provides an opportunity to study the ability of different superacid systems to interact with various types of cations derived from a single parent molecule.

A part of this work was published in *Tetrahedron Letters* (1972) 1477.

Examiners: Prof. D. Sunko, Prof. J. W. Larsen, and Prof. D. Hadži.

Oral examination: September 13, 1972.

Thesis deposited at the Faculty of Science, University of Zagreb.

(131 pages, 8 tables, 40 figures, 85 references, original in Croatian with detailed summary in English)

M. ECKERT-MAKSIĆ

DCC-37 (Univ. Zagreb)

1. Site of Protonation of Some Substituted Phenols and Alkylphenyl Ethers in SbF_5/HSO_3F

I. Eckert-Maksić M.

II. Institute »Ruđer Bošković«,
41000 Zagreb, Croatia, Yugoslavia

N.M.R. spectroscopy, low temp.
  of alkylphenyl ethers
  of phenols, substituted
Protonation, in super acids
  of alkylphenyl ethers
  of phenols, substituted
Quenching, of ionic solns.

DCC-38 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B11—B12 (1972)

Conformational Analysis of Transition States in the Solvolysis of Cyclohexyl Derivatives. The Cholestanyl System

M. Tarle

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

In the course of this work transition states geometries in reactions of cholestanyl and epicholestanyl arenesulfonates were investigated by means of secondary deuterium isotope effets and by product analyses.

Ample evidence was presented that both epimeric brosylates solvolyze through the »chair« conformation in the transition state. The results obtained from this work were compared with the data in the literature dealing with various substituted cyclohexyl esters. It was concluded that only one substituent at the six membered ring is not sufficient to ensure the conformational homogeneity during the solvolytic process.

From the measurement of the β-deuterium effects on epicholestanyl brosylate it was deduced that hydrogen participation occurs in the slow step of the reaction. Comparing the values of the β-isotope effects and the products of the reactions of both labeled and unlabeled cholestanyl and epicholestanyl brosylates the conclusion was made that elimination is not involved in the kinetic step of the reaction, but occurs after the stable reaction intermediates were formed.

Measurements of α-deuterium effects confirmed the dependence of these effects on the degree of rehybridization of the reacting carbon atom in the rate detg. step. In this way the reduced value of α-effect caused by hydrogen participation in solvolysis of epicholestanyl arenesulfonates was explained. Here the degree of bridging in the transition state is not far advanced. The slightly reduced value of the α-effect in the solvolysis of cholestanyl derivs. was attributed to the intimate ion-pair formation in the kinetic reaction step.

Examiners: Prof. S. Borčić, Prof. D. Sunko, and Dr. K. Humski

Oral examination: November 3, 1972

Thesis deposited at the Faculty of Pharmacy and Biochemistry, University of Zagreb

(172 pages, 31 tables, 72 figures, 139 references, original in Croatian)

M. TARLE

DCC-38

1. Conformational Analysis of Transition States in the Solvolysis of Cyclohexyl Derivatives. The Cholestanyl System

I. Tarle M.

II. Institute »Ruđer Bošković«,
41000 Zagreb, Croatia, Yugoslavia

Cholestanyl derivs., solvolysis of
Conformational analysis, in solvolysis
Isotopic effect, secondary deuterium
  in solvolysis of cholestanyl derivs.

DCC-39 (Univ. Zagreb)

Croat Chem. Acta CCACAA 44 (4) B13—B14 (1972)

Water Sorption and Storage Stability of Tomato Powder and Dry Tomato Soup Mixtures

M. Bošković

Laboratory for the Technology of Fruit and Vegetable Products, Department of Biotechnology, Faculty of Technology, University of Zagreb, Zagreb, Croatia, Yugoslavia

Optimum stability of foam-mat and freeze-dried tomato powder stored in air at 23° C was found at water activity (a_w) of 0.15., 0.20 (*i. e.*, relative humidity 15—20%) and water content of 1.2—1.5%. Shelf-life was 6. 8 months.

Using humidostats, 4 water sorption isotherms (—12; 3; 20 and 40 °C) of foam-mat dried tomato powder were measured. The product had low water capacity and B. E. T. monolayer value.

Heats of sorption — calculated from the isosteres covering an interval between 20 and 34 mg equilibrium sorbed water/g solid — ranged from —1.2 to —0.4 kcal/kg.

Caurie's method [*J. Food Technol,* 5 (1970) 301.] for the detn. of optimum storage conditions to attain stability, involving a log-lin isotherm modification, was evaluated. Calculated values were found to be too high, and monolayer values too low to serve as the indicators of optimum a_w.

Colour changes of product were followed. Retention of the total liposoluble colour at various water activities (a_w-prophiles) was measured. Positions of the a_w-optima coincided with those for the overall product stability. Colour retention was highest at 20° and lowest at 3° and 40 °C storage, respectively.

Results of liposoluble colour determinations were compared with the measurements of colour values with the photoelectric tristimulus colorimeter Hunterlab D 25 and with the visual observations. Different sensitivity was found toward colour changes caused by the various degradative processes (non-enzymatic browning, oxidative fading) but the results were congruent.

Experimental dry soup mixture (tomato powder, dextrine, vegetable shortening, Na-glutaminate, sugar and salt) was generally unstable, with lower sorption capacity, inferior colour retention and shorter storage life. It is recommended to either pack tomato powder separately or compounded with the inert constituents only.

Examiners: Prof. T. Lovrić, Prof. P. Mildner, and Prof. M. Filajdić.

Oral examination: March 29, 1972.

Degree conferred: May 26, 1972.

Thesis presented to and deposited at the Faculty of Technology, Univ. of Zagreb.

M. BOSKOVIC

DCC-39

1. Water Sorption and Storage Stability of Tomato Powder and Dry Tomato Soup Mixtures

I. Bošković M.

II. Laboratory for the technology of of fruit and vegetable products, Department of Biotechnology, Faculty of Technology, University of Zagreb, Zagreb, Croatia, Yugoslavia

Dehydrated foods, storage
Foam-mat drying
Sorption, on tomato powder

DCC-40 (Univ. Zagreb)

Croat. Chem. Acta CCACAA 44 (4) B15—B16 (1972)

Kinetics of the Hydrolysis of Glucuronic Esters and Glucuronic Ethers by β-Glucuronidase from Animal and Bacterial Sources

J. Tomašić

Tracer Laboratory, Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

Comparative studies on the actions of β-glucuronidase from bovine liver and *E. coli* towards synthetic 1-O-acyl-β-D-glucupyranuronic acids (I) (acyl = = benzoyl, veratroyl, indol-3-ylacetyl, α-ethylbutyryl) and glucuronic ethers (II) (aglycon = 3,4-dimethoxyphenol, 3,4-dimethoxybenzyl alcohol, *p*-nitrophenol, phenolphthalein) as substrates have been undertaken and kinetic data obtained have been correlated. The synthesis of hitherto undescribed I and II has been presented.

For direct detn. of glucuronic acid in the presence of labile glucosiduronic acids a new quant. method has been developed. The method is based on the colour reaction of glucuronic acid and benzidine in acetic acid, and the assay is carried out under mild conditions which do not affect the C-1 ester bond.

The enzyme activity has been examd. with respect to the pH of the medium and the substrate and enzyme concn. Comparison of the kinetic data (K_m and V_{max}) indicates: 1. Affinities of I and II for the enzyme are not primarily dependent on the type of sugar-aglycon linkage. 2. Relative activities of mammalian β-glucuronidase toward the eight substrates examd. do not parallel the activities of β-glucuronidase from *E. coli;* the latter was found to be much more effective in hydrolysing I than the former.

The thesis was partly published in: *Anal. Biochem.* 45 (1972) 164—75; *Croat. Chem. Acta* 44 (1972) 493.

Examiners: Dr. D. Keglević, dr. E. Reiner, and dr. Z. Štefanac.

Oral examination: June 28, 1972.

Dissertation deposited at the University Library, Zagreb, and Institute »Ruđer Bošković«, Zagreb.

(90 pages, 5 tables, 16 figures, 89 references, original in Croatian).

J. TOMAŠIĆ

DCC-40

1. **Kinetics of the Hydrolysis of Glucuronic Esters and Glucuronic Ethers by β-Glucuronidase from Animal and Bacterial Sources**

I. Tomašić J.

II. Tracer Laboratory, Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

Glucuronic acid, determination with benzidine
Glucuronic esters, chemical synthesis of,
—, enzymic hydrolysis of
Glucuronic ethers, chemical synthesis of,
—, enzymic hydrolysis of
β-Glucuronidase, of *E. coli* origin,
—, from bovine liver,
—, kinetic studies with

MCC-44 (Univ. Zagreb)
Master of Science Thesis

Croat. Chem. Acta CCACAA 44 (4) B17—B18 (1972)

Study of the Sporulation of *Bacillus thuringiensis* var. *thuringiensis*

*S. Rendić**

Laboratory of Biochemistry, Technological Faculty, University of Zagreb,
41000 Zagreb, Croatia, Yugoslavia

In the course of the submerged cultivation of bacteria *Bacillus thuringiensis* var. *thuringiensis* in medium contg. starch, molasses, yeast and inorg. salts in fermentors of 30, 300 and 3000 l. capacity, lysis occurred at the end of the exponential phase of growth. Lysis of the cells could not be detected during the cultivation in flasks contg. 300 ml of the medium. After lysis, new vegetative cells were formed which usually sporulated when the cells reached the exponential phase of growth.

It was found that the synthesis of proteases in bacteria begins at the end of the exponential phase of growth, *i. e.* at the beginning of the process of sporulation. The presence of rapidly metabolised growth substrate (particularly sol. sugars) at the end of the exponential phase of growth acts as a catabolic repressor on the synthesis of proteases and enzymes of the Krebs cycle; therefore, the lysis was followed by a drop of pH and accumulation of acetate in the medium. The cells were commited to sporulation without lysis when in the fermentation broth no free sol. sugars could be detected or when an anaerobic shock during the cultivation was applied.

The thesis was reported in part at 6th *FEBS Meeting*, Madrid, 1969.

Examiners: Prof. P. Mildner, Prof. G. Tamburašev, Prof. S. Ban.

Oral examination: July, 9th 1970

Thesis deposited at the Technological Faculty, University of Zagreb.

(81 pages, 8 figures, 7 tables, 61 references, original in Croatian)

S. RENDIĆ

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* Present address: Research Department »Pliva«, Pharmaceutical and Chemical Works, Zagreb.

MCC-44

1. **Study of the Sporulation of *Bacillus thuringiensis* var. *thuringiensis***

I. Rendić S.

II. Laboratory of Biochemistry, Tehnological Faculty, University of Zagreb, 41000 Zagreb, Croatia Yugoslavia

Anaerobic shock
Bacillus thuringiensis var. *thuringiensis*
Lysis
Proteases, synthesis of
Sporulation

MCC-45 (Univ. Zagreb)
Master of Science Thesis

Croat. Chem. Acta CCACAA 44 (4) B19—B20 (1972)

Precipitation of Calcium Phosphates. Metastabile Equilibria and Kinetics

B. Purgarić

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Pptn. of slightly soluble calcium phosphates, prepd. by direct mixing of the solns. of calcium chloride (2×10^{-4} — 1.8×10^{-1} *M*) and sodium phosphate (1.6×10^{-3} — 1.2×10^{-1} *M*, preadjusted to pH = 7.4) was studied at 37 °C. The pptn. boundary was detd. 24 hours after mixing and isolated ppts. were identified on the ground of morphology (light and electron microscopy), chem. anal., X-ray and electron diffraction, IR spectroscopy, and refractive indices.

The results show that over the widest range of reactant concns. calcium deficient apatites (DA) with molar Ca/P ratios between 1.42 and 1.52 were formed, while octacalcium phosphate (OCP) was detected only at the pptn. boundary. At relatively high concn. products ($Ca_T \cdot P_T > 10^{-4}\,M^2$) mixtures of dicalcium phosphate dihydrate (DCPD) and apatites were obtained.

The kinetics of the transformation of the initially formed poorly cryst. calcium phosphate ppts. into DA was followed at 37° C both at constant pH = 7.4 and at a variable pH. In both cases pptn. occurred in at least two separate steps; the reaction was faster at constant pH.

The pptn. kinetics of DCPD from equimolar solns. of calcium chloride and sodium phosphate (2.3×10^{-2} — 4×10^{-2} *M*), which were obtained by direct mixing of the reactants, was followed quant. in 0.15 *M* sodium chloride solns. at constant pH (pH = 5) and temp. (25° C). The concns. of all calcium and phosphate contg. ions and complex species which were present in soln. at any given reaction time were calcd. from exptl. data by a computer program. From these data the order and rate const. of the process of crystal growth of DCPD were detd. At initial reactants concns. 2.3×10^{-2} *M*, 2.5×10^{-2} *M*, and partly 2.7×10^{-2} *M*, where agglomeration was negligible, the rate of the process was proportional to the square of the supersatur. Values of the rate consts. varied between 10^7 and 10^8 $l^3\,mol^{-3}\,min^{-1}$.

Anal. of the exptl. data according to the Christiansen-Nielsen theory shows that crystal growth of DCPD under the conditions given is most probably controlled by a fourth order process of surface nucleation through a polynuclear layer mechanism.

The thesis was reported in part at the *Regional Meeting of Chemists*, Zagreb, Croatia, Feb. 26—28, 1969. and at the *VII European Symposium on Calcified Tissues*, Montecatini, Italy, March 23—26, 1970. (Proceedings: *Calc. Tiss. Res.* 4 (Suppl.) (1970) 142).

Part of the results was published: *Croat. Chem. Acta* 41 (1969) 37; *Calcif. Tiss. Res.* 8 (1971) 142.

Examiners: Dr. H. Füredi-Milhofer, Prof. B. Težak, and Prof. R. Wolf.

Oral examination: February 11, 1972.

Thesis deposited at the University Library and Institute »Ruđer Bošković«, Zagreb.

(171 pages, 13 tables, 34 figures, 159 references, original in Croatian)

B. PURGARIĆ

MCC-45

1. Precipitation of Calcium Phosphates. Metastable Equilibria and Kinetics

I. Purgarić B.

II. Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Apatites
Calcium,
  di-, phosphate dihydrate
  octa-, phosphate
  phosphates, pptn. of
Crystal growth

MCC-46 (Univ. Zagreb)
Master of Science Thesis

Croat. Chem. Acta CCACAA 44 (4) B21—B22 (1972)

The Mechanism of the Formation and Transformation of Slightly Soluble Calcium Phosphates

Lj. Brečević

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Pptn. of calcium phosphates from aq. solns, of calcium chloride ($2.5 \times 10^{-4} — 9 \times 10^{-2}$ *M*) and sodium phosphate ($1 \times 10^{-3} — 6 \times 10^{-2}$ *M*; $[NaOH]/[H_3PO_4] \approx 1.75$; pH ≈ 7.4) was studied at 25^0 C. The pptn boundary, as obserbed 24 hours after mixing and the distribution of free and complexed calcium at this boundary were detd. Light and electron microscopy, X-ray and electron diffraction, chem. anal., refractive indices and IR spectroscopy were used to characterize the ppts.

Over the widest range of reactant concns. octacalcium phosphate (OCP) or slightly hydrolyzed OCP was formed. At low supersatns. (at the pptn. boundary) ppts. predominantly amorphous to electron diffraction were obtained. At relatively high concn. products ($[Ca_{tot.}] \times [P_{tot.}] > 10^{-4}$) mixtures of dicalcium phosphate dihydrate (DCPD) and apatites were obtained.

The dependence of the characteristics of the ppts. and the »final« pH values on the initial concns. of the pptg. components has been demonstrated by means of pptn. diagrams.

The kinetics of formation and transformation of calcium phosphate ppts. obtained by mixing equal volumes of solns. (6×10^{-3} *M* in total calcium and/or total phosphate) was investigated. Changes of pH, turbidity and the number of particles formed (> 5 μm) were followed simultaneously as a function of time. Ppts. were isolated at various time intervals and characterized by the above mentioned methods. Initially a ppt. with a molar Ca/P ratio of 1.5, amorphous to X-ray and electron diffraction was formed, IR spectra indicated the presence of PO_4^{3-} and HPO_4^{2-} ions. After a period of metastability, pptn. of a cryst. material within and/or above the amorphous phase occurs. The cryst, ppt. showed the characteristics of OCP, while in the later stages hydrolysis into calcium deficient apatites (DA) was observed.

Parts of this thesis are published in: *Calcif. Tiss. Res.* **8** (1971) 142—153; *ibid.* **10** (1972) 82—90.

Examiners: Dr. H. Füredi-Milhofer, Prof. B. Težak, and Prof. M. Herak.

Oral examination: February 11, 1972.

Thesis deposited at the Central Chemical Library, Zagreb and Institute »Ruđer Bošković«, Zagreb.

(77 pages, 6 tables, 15 figures, 98 references, original in Croatian)

LJ. BREČEVIĆ

MCC-46

1. The Mechanism of the Formation and Transformation of Slightly Soluble Calcium Phosphates

I. Brečević Lj.

II. Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Apatites
Calcium,
  di-, phosphate dihydrate
  octa-, phosphate
  phosphates, pptn. of
Crystal growth

MCC-47 (Univ. Zagreb)
Master of Science Thesis

Croat. Chem. Acta CCACAA 44 (4) B23—B24 (1972)

Investigations of Complex Reaction Mechanisms by Cyclic Chronopotentiometry

D. Čukman

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Cyclic chronopotentiometry (CCP) has been proved as a suitable technique in studying complex electrochem. — chem. reaction mechanisms. It is useful both as a diagnostic and as a quant. measurement technique for distinguishing between various possible reaction path. Combined with numerical prediction based on digital simulation of various mechanism, CCP is capable of accurate detn. of rate coeffs. of chem. reactions coupled to electron transfer.

Previously reported studies of the Uranium (VI)—(V)—(IV) system in acidic media take as confirmed that the reduction of U (VI) to U (V) is followed by the disproportionation reaction of U (V) to U (VI) and U (IV). Reported rate coeffs. differ depending on the techniques used. Results of measurements of rates and diagnosis of mechanism of the reduction of uranium on mercury electrode in acidic (perchlorate and nitrate) media show complex behavior of that system.

To explain the exptl. results digital simulation of a number of possible mechanisms was attempted. In the range of pH from 0.5 to 1.5 the disproportionation reaction is certainly not the only chemical reaction path. Neither the formation of a complex between the U (V) and the U (VI) species nor the E—C—E mechanism scheme were supported by exptl. evidence. There is the possibility that some U (V) species, an intermediate in the reaction sequence, is specifically adsorbed on mercury electrode. However, no single, straightforward reaction scheme fits the exptl. data at all the concn. of U (VI) and pH studied.

Part of the work was reported at the *3rd Yugoslav Congress on Pure and Applied Chemistry*, Ljubljana 1972, and *23rd Meeting of I. S. E.*, Stockholm 1972.

Examiners: Dr. V. Pravdić, Dr. M. Herak, and Dr. M. Branica

Oral examination: March 9, 1972.

Thesis deposited at the Institute »Ruđer Bošković«, Zagreb, and the Central Chemical Library of the Croatian Chemical Society, Zagreb,

(70 pages, 15 tables, 16 figures, 78 references, 2 computer programs, original in Croatian)

D. ČUKMAN

MCC-47

1. Investigations of Complex Reaction Mechanism by Cyclic Chronopotentiometry

I. Čukman D.

II. Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Chronopotentiometry, cyclic
Digital computer simulation
Disproportionation reactions
  of uranium
Electrochemical study
  of uranium
Uranium
  electrochemical study of

MCC-48 (Univ. Zagreb)
Master of Science Thesis

Croat. Chem. Acta CCACAA 44 (4) B25—B26 (1972)

Solvolyses in Fluoroalcohols: Solvent and Structural Effects

I. Szele

Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

The solvolysis rates of 7-methyl-norbornyl tosylate (MNBOTs) were found to be remarkably insensitive towards changes in the Grunwald-Winstein Y value in 2,2,2-trifluoroethanol (TFE)—water mixts.

The β-deuterium isotope effects for MNBOTs in TFE—water mixts. are exceptionally high, and have to be explained in terms of the unique structure of this substrate. The isotope effects are decreasing with increasing nucleophilicity of the solvent.

It was also found that 2-methyl-2-adamantyl *p*-nitrobenzoate showed in TFE—water mixts. the smalles as yet recorded m value for a tertiary substrate (0.040).

7-Methyl-*anti*-1-norbornenyl *p*-nitrobezoate showed in the same solvent an m value of 0.213 which is consistent with the decreased solvent dependence in the solvolysis rates of anchimerically assisted substrates.

Since 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) seemed to be another interesting solvolytic solvent Y values were detd. for HFIP—water mixts. Here, unexpectedly, the *t*-BuCl solvolysis rates did not change monotonously but manifest a minimum at 66.7 mole per cent of water. This result implies the existence of a dihydrate, the mixts. of which with either water or HIFP have to be considered as separate binary solvent system. The solvolysis rates of MNBOTs in HFIP—water mixts. desreased with increasing water content(!), but showed a sharp inflection in the log *k vs.* mole per cent water plot. The position of this inflection coincides with the minimum of *t*-BuCl solvolysis rate.

The β-deuterium isotope effects for MNBOTs in this solvent are, in accordance with its low nucleophilicity, the highest as yet observed effects for this substrate.

A structure for the HFIP $2\,H_2O$ complex is preposed. The kinetic results are discussed in terms of this structure and its possible interactions with the transition states of the investigated tertiary substrates.

A part of this work was pubilshed in *Tetrahedron Letters* (1972) 1827, 3617.

Examiners: Prof. D. Sunko, Dr. Z. Majerski, and Dr. T. Cvitaš

Oral examination: September 12, 1972

Thesis deposited at the University of Zagreb.

(77 pages, 9 tables, 8 figures, 68 references, original in Croatian)

I. SZELE

MCC-48

7. Solvolysis in Fluoroalcohols: Solvent and Structural Effects

I. Szele I.

II. Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Isotopic effect, secondary deuterium
mY — Relationship
Norbornane-7-yl-, 7-methyl-, tosylate
Solvolysis, in fluorinated alcohols

MCC-49 (Univ. Zagreb)
Master of Science Thesis

Croat. Chem. Acta CCACAA 44 (4) B27—B28 (1972)

Synthesis and Solvolysis of Deuterated 7-Norbornenyl and 7-Norbornyl Derivatives

M. Tomić

»INA-Naftaplin«, Zagreb and Institute »Ruđer Bošković«, 41000 Zagreb,
Croatia, Yugoslavia

The following compds. and their undeuterated analogues were prepd.: *anti*-7-norbornenyl-7-d_1, *anti*-7-norbonenyl-1,2,3,4-d_4, 7-methyl-d_3-7-norbornyl, 7-methyl-7-norbornyl-1,4-d_2, 7-methyl-7-norbornyl-1,2-*endo*, 3-*endo*, 4-d_4, 7-methyl-7-norbornyl-1,2,2,3,3,4-d_6, tosylates, and 7-methyl--d_3-*anti*-7-norbornenyl *p*-nitrobenzoate. Solvolysis rates were measured and the corresponding secondary deuterium isotope effects were calcd.

The α-deuterium effect with *anti*-7-norbornenyl-7-$d_1$tosylate was only slightly reduced in magnitude ($k_H/k_D = 1.13$).

The solvolysis of 7-methyl-d_3-*anti*-7-norbornenyl *p*-nitrobenzoate showed a markedly small β-deuterium effect ($k_{CH_3}/k_{CD_3} = 1.09$) compared to the value for the corresponding satd. compd. ($k_{CH_3}/k_{CD_3} = 1.89$).

Deuterium on the double bond in the *anti*-7-norbornenyl-1,2,3,4-d_4 tosylate gave a small inverse effect (about 2% per D) which is most probaly not only of inductive origin.

The results show that the neighboring double bond participates in the rate detg. step and that α-effects are less sensitive to the change in geometry of the transition state than β- and γ-effects.

A part of this work was published in *Tetrahedron Lett.* (1972) 1827.

Examiners: Prof. D. Sunko, Dr. T. Cvitaš, and Prof. S. Borčić.

Oral examination: October 4. 1972.

Thesis deposited at the Faculty of Science, University of Zagreb.

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M. TOMIĆ

MCC-49

1. Synthesis and Solvolysis of Deuterated 7-Norbornenyl and 7-Norbornyl Derivatives

I. Tomić M.

II. »INA-Naftaplin«, Zagreb

III. Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

Isotopic effects, deuterium, in solvolysis
  of 7-norbornyl, 7-methyl-, tosylate
  of 2-norbornene-7-yl, *anti*, tosylate
  of 2-norbornene-7-yl, 7-methyl, *anti*-, p-nitrobenzoate
7-Norbornanol, 7-methyl-, tosylate
  1,4-d_2
  -1,2,2,3,3,4-d_6
  -1,2-*endo*, 3-*endo*, 4-d_4
  -methyl-d_3
2-Norbornene-7-ol, *anti*-, tosylate
  -7-d_1
  1,2,3,4-d_4
2-Norbornene-7-ol, 7-methyl-, *anti*-*p*-nitrobenzoate
  -methyl-d_3

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»G. Ciamician« Chemical Institute, University of Bologna, Via Selmi 2, Bologna 40126, Italy and »Ruđer Bošković«, Institute, Center for Marine Research, Rovinj, Yugoslavia

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Department of Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, and Division of Endocrinology, Department of Medicine, University Hospital Rebro, Zagreb, Croatia, Yugoslavia

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BCC-1085

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Institute of Inorganic and Analytical Chemistry, University of Zagreb, Yugoslavia

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BCC-1086

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BCC-1087

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Department of Chemistry, The John Hopkins University, Baltimore, Maryland 21218 and Belfer Graduate School of Science, Jeshiva University, New York, New York 10033 and Institute für Physikalische Chemie der Johann Wolfgang Goethe-Universität zu Frankfurt am Main, Germany

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Department of Chemistry, The University of Chicago, Chicago, Ill. 60637

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Zavod za kemiju i biokemiju, Medicinski fakultet, Zagreb

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Laboratory of Analytical Chemistry, Faculty of Science, and Institute of Inorganic and Analytical Chemistry, University of Zagreb, Zagreb, Croatia, Yugoslavia

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Department of Chemistry, Illinois State University, Normal. Ill. 61761

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Ruđer Bošković Institute, 41000 Zagreb, Croatia, Yugoslavia

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Evans Chemical Laboratories, The Ohio State University, Columbus, Ohio 43210 and the Department of Chemistry, Virginia Polytechnic Institute and State University, Blackburg, Virginia 24064

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Institut za kontrolu i ispitivanje lijekova SRH, Zagreb

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Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37916

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The Ohio State University, Columbus, Ohio 43210

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Institute of Electrochemistry and Electrochemical Technology, Zagreb, University of Zagreb, and Institute of Physical Chemistry, Faculty of Chemical Technology, Split, University of Zagreb, Yugoslavia

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Institute of Electrochemistry and Electrochemical Technology, Faculty of Technology, Zagreb, University of Zagreb, Yuogslavia

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Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia, Yugoslavia and Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

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Institute of Colloid and Surface Science and Department of Chemistry, Clarkson College of Technology, Potsdam, New York 13676

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Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

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Department of Physical Chemistry, »Ruđer Bošković« Institute, Zagreb, Yugoslavia

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Medical Faculty, University of Zagreb, Hospital »Dr. M. Stojanović«, Zagreb, (Yugoslavia)

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Institute of Forensic Medicine and Criminalistics, Faculty of Medicine, Zagreb

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Institute of Electrochemistry and Electrochemical Technology, Faculty of Technology, University of Zagreb, Zagreb, Yugoslavia

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Department of Chemistry and Institute of Colloid and Surface Science, Clarkson College of Technology, Potsdam, New York 13676

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Institute for Inorganic Technology, Faculty of Chemical Technology, Split, University of Zagreb, Yugoslavia and Institute for Electrochemistry and Electrochemical Technology, Faculty of Technology, University of Zagreb, Zagreb, Yugoslavia

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Zavod za farmakognoziju, Farmaceutsko-biokemijskog fakulteta, Zagreb

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M. Randić

Department of Physics, University of Utah, Salt Lake City, Utah 84112

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Institute »Ruđer Bošković«, 41001 Zagreb, P.O.B. 1016, Croatia, Yugoslavia

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Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

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Faculty of Science and Mathematics, Departments of Chemistry and Physics, University of Zagreb, Zagreb, Marulićev trg 19, Croatia (Yugoslavia)

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Physikalisches Institut der Naturwissenschaftlich-mathematischen Fakultät, Sarajevo and Institute Ruđer Bošković, Zagreb, Yugoslavia

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Center for Marine Research, Institute »Ruđer Bošković«, Zagreb, Yugoslavia

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Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

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Laboratory of Analytical and Physical Chemistry, Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Zagreb, and Laboratory of Physical Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia, Yugoslavia
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Ruđer Bošković Institute, Zagreb, and INA-NAFTAPLIN, Zagreb, Yugoslavia
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Center for Marine Research, »Ruđer Bošković« Institute, Zagreb, Croatia, Yugoslavia
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Tracer Laboratory, Institute »Ruđer Bošković«, Zagreb, Yugoslavia

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Chemistry Department, Revelle College, University of California, San Diego, La Jolla, California

σ-πConjugation Occurrence and Magnitude

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