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HPLC'92 in Baltimore

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After the very successful '*15th International Symposium on Column Liquid Chromatography, HPLC'91*', in Basel it was not easy to organize the 16th meeting of this series. Prof. *Fred Regnier* of Purdue University, West Lafayette, Indiana, took the challenge, and in certain aspects he could set a standard which had never been reached before. There were two outstanding features which gave HPLC'92 a specific character: all posters were (or should have been) mounted for the whole week, and all lectures, with the exception of the few plenary lectures, needed to be presented also as a poster.

In total there were 109 lectures (too many, in my opinion, as four parallel sessions were necessary) and 346 posters. The Baltimore Convention Center offered excellent facilities for the presentation of both types of contributions. Distraction and recreation could be found in the nearby Inner Harbor with several exciting museums, including the famous Baltimore Aquarium, shops, restaurants, and all types of harbor cruises.

A total number of 1200 participants met in Baltimore and enjoyed a high quality symposium. Yet, a markedly high number of missing posters made it obvious that the financial situation of many companies is worse than one or two years ago, and that less people are allowed to visit symposia. As a surprise, even Queen Isabel was present and had moved court because 'here is the place where the great discoveries will be announced'. In fact, the symposium was opened by *Christopher Columbus* and his companions who reported the Queen about their journey to a new world; they had found their way although the Tryptic Map which was available to them was lousy.

The Future of HPLC

As usual, one of the cornerstones of the symposium was the plenary lecture by *Csaba Horváth* [1] who is able to define and express clearly what HPLC is, and why we need it. In his opinion, the three most important topics of research in this field are molecular recognition, surface chemistry, and chromatography theory. Today's important goals of HPLC are peptide screening (in simple terms, to find information about peptides) and oligonucleotide chemistry (to find oligonucleotides which can bind to a target protein); the needed knowledges are the adsorption behavior of proteins, the characterization of sorbents, the development of multicolumn separation systems, and, as a very general goal, the development of techniques which allow to increase the speed of separation.

An example of how modern tryptic mapping is performed is shown in *Fig. 1* [2]. The authors performed the separation of cytochrome c peptides at 75°, at a flow rate of 1.6 column volumes per min and with steep gradients. The time per chromatogram could be reduced by a factor of four without impairing the resolution. Deamination of peptides with amide-based residues (glutamine) was not observed.

A different approach for fast analysis is the replacement of immunoassays (which are slow, *i.e.* in the range of hours) by antibody-type chromatography [3]. The

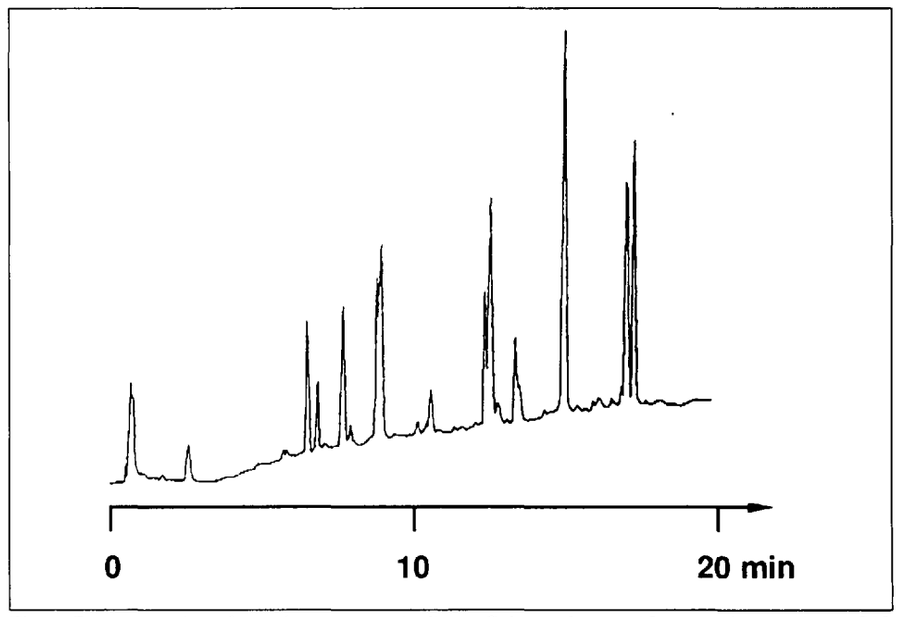

Fig. 1. *Fast separation of cytochrome c tryptic digest.* Column: 2 mm x 15 cm; stationary phase: *C18 Delta-Pak* 5 µm; mobile phase: H_2O/CH_3CN with 0.1% CF_3COOH, gradient 2% B/min; flow rate: 0.72 ml/min; temperature: 75°; detection: UV-VIS 190–425 nm [2].

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stationary phase used is of the perfusion type and bears covalently bonded antibodies. 'Immunodetection' is performed by eluting the compound of interest with a specific eluent. The assay allows the real-time detection of biomolecules because one run takes some seconds only under ideal conditions but in any case less than two min.

Packing Materials

New approaches were the continuous-type of stationary phase and 'fabric chromatography'. In the first case, the column packing consists of one single piece of polymer which was synthesized *in situ* [4][5]; this allows very rapid chromatography, *e.g.*, the separation of four proteins in 20 s. A most surprising type of stationary phase is a rolled fabric which is woven from a yarn with suitable adsorptive properties [6]. This approach was just published (*J. Chromatogr.* **1992**, *598*, 169), and the authors claim that fabric based materials show promise for preparative protein separations.

A number of posters discussed the use of zirconia ZrO_2 as a pH- and temperature-stable stationary phase. It can also be prepared in microspherical form [7] and derivatized. As the search for new materials is going on, even chitin was presented as a HPLC phase [8]. This material showed high mechanical strength and good size-exclusion properties for hydrophilic oligomers. Hydroxyapatite can be synthesized in 'sea urchin' form, *i.e.* as a radiating acicular structure which allows the rapid flow of the mobile phase without high back pressure [9].

Less exotic but most interesting approaches were the presentation of highly purified silica which has excellent adsorption properties, *e.g.*, more linear adsorption isotherms than traditional silicas [10], and the synthesis of highly monodispersed styrene-divinylbenzene polmers with very narrow pore size distribution [11] (*Anal. Chem.* **1992**, *64*, 1232).

Just the week before the symposium *Regis* introduced a commercially available stationary phase called 'Buckyclutcher' which allows the separation of C_{60} and C_{70} buckminsterfullerenes [12].

Separation of Enantiomers

Special attention was given to the protein-type chiral stationary phases. Several research groups try to find possible retention mechanisms. On α-acid glycoprotein evidence for a multiple-site, competitive and allosteric interaction mechanism was found [13]. Ovomucoid protein was cut into isolated domains, and preliminary results showed that some domains are able to separate some racemates [14]. On human serum albumin (HSA) type columns, it was tried to differentiate chiral and achiral amounts of retention [15] (*Chromatographia* **1992**, *33*, 546), a knowledge which may also be highly interesting for the design of 'brush-type' chiral stationary phases. A completely different approach, coming from clinical research, is the determination of the breakthrough curve of chiral drugs on a HSA column [16] which gives information about the binding properties of protein and drug. Similarly, stereoselective enzyme-substrate and enzyme-inhibitor interactions can be studied by the frontal analysis of DL-tryptophan ethyl ester on stationary phases with immobilized trypsin and α-chymotrypsin [17].

A surprising result was found in a study for the enantioselective separation of β-blockers on a tyrosine-derived chiral stationary phase. With supercritical CO_2 (and modifier) as mobile phase, the separation factors were much higher than with hexane (and modifier), *i.e.*, than with normal-phase HPLC conditions [18] (*Chirality* **1992**, *4*, 252). The reason, therefore, is the complexing action of CO_2 which forms two H-bridges with the OH and NH_2 groups of β-blockers; the resulting structure is rather rigid which is favorable for chiral recognition. Another surprise was the fact that two diastereoisomers of pravastatin lactone, a compound with nine chiral centers, only one of them differs in the case presented here, could not be separated on a number of chiral stationary phases (also not on bare silica), but that baseline separation was easily achieved on *Shandon Hypercarb* (a graphite type achiral phase) with methanol as eluent [19].

Sample Preparation

Pre-column solid phase derivatization is an attractive approach for sample preparation. It can, *e.g.*, be performed on-line for the sensitive analysis of amphetamines in plasma by tagging them with a fluoreneacetyl group which is picked up during the passage through a polymeric solid-phase reagent [20]. The tag can even be a chiral moiety thereby allowing the formation of diastereoisomers for subsequent enantioselective analysis [21].

Electrodialysis allows the automated isolation and concentration of ionic analytes from complex matrices [22]. An application of this elegant technique is the analysis of sulfonamide antibiotics in milk, serum, or tissue.

Detection

LC-MS was a prominent technique at HPLC'92. As an example, it is possible to obtain molecular mass and structure information from a 4 picomole sample of Calmodulin tryptic digest [23] by using a 0.2-mm i.d. packed column. Since the flow rate is only 2 µl/min, no splitting of the eluate is necessary; the suitable ionization method is electrospray.

As the pressure for sensitive, novel methods for detection comes from trace analysis, it is obvious that new developments in this field are often coupled to microchromatographic techniques (since sample dilution is much lower in narrow than conventional-diameter columns). Trace analysis of amino-acid enantiomers in microfossils was performed on a 50- or 100-µm i.d. packed capillary with laser-induced fluorescence detection [24]. For excitation, the HeCd or Ar ion laser, both with wavelengths above 300 nm, could be used, because the amino acids were derivatized prior to chromatography with *o*-phthaldialdehyde and a glucopyranose derivative.

By means of a coulometric electrode array system, it was possible to identify and quantitate 36 different compounds in beverages and juices in one single run [25]. A number of these phenols could not yet be identified, and so far they seem not have been known as constituents in these types of samples.

Optimization and Fundamentals

Pharmacia has introduced an expert (and validation) system as part of their new HPLC system *Smart* for the separation of proteins [26]. This seems to be a highly useful expert system, as it gives excellent proposals how to perform the separation of a given mixture based on physical properties of the proteins involved. The weakest point of it is the fact that it is only available when buying a *Smart* chromatograph! A simple, yet sophisticated expert system comes from Bern; the group of Prof. *Clerc* has developed *Carthago* which predicts retention times of small molecules [27]. It is not commercially available, but whoever is interested may ask at the Institute of Pharmacy (as long as it exists!) for a copy.

Multivariate analysis of the many parameters which may influence a chromatographic separation is perhaps the most useful approach to understand what is going on; therefore, it can be used for optimization as well as for theoretical studies. Now the necessary computer power is easily accessible, and several contribu-

tions presented most interesting results. To mention just one of them, it was possible to correlate retention behavior (in micellar liquid chromatography) and biological activity of 26 *para*-substituted phenols [28].

For years now, among the most interesting theoretical work is done by *Georges Guiochon* and his group. With preparative HPLC as starting point, it is obvious that clarity about the process comes from knowledge of the adsorption isotherms. A classical 'chiral' problem, the separation of *Tröger*'s base on cellulose triacetate, has now been solved, and it was shown that the two enantiomers have clearly differing isotherms [29]. It follows that the adsorption of the two forms occurs at different sites of the cellulose.

Preparative HPLC

Another *Guiochon* study showed that, in elution or displacement chromatography of a two-component mixture (all three compounds involved have *Langmuir*-type isotherms), the maximum production rate is always higher in overloaded elution mode than in displacement mode [30]! This means that plucky overloading, giving more than poor peak shapes, can be the key for successful preparative separations instead of searching for a displacer.

If one decides to perform displacement chromatography, it is not easy to find a suitable displacer. How this can be achieved scientifically and not by hoping for good luck was presented in a beautiful contribution about enantioselective preparative HPLC on a number of chiral stationary phases of differing types [31].

In a comparison of microparticulate silicas for the packing of preparative columns it was found that the 5-μm packing was superior to the 12-μm one in terms of both plate number and stability [32].

A tricky and rapid method for the preparative isolation of proteins comes from *Fred Regnier*'s group: SNAP is selective non-adsorptive preparative chromatography [33]. This is performed at the isoelectric point of the protein which is to be isolated. The solute is directed in multiple cycles through anion- and cation-exchange columns of the perfusion type. The protein is not adsorbed, whereas small ionic molecules are.

Supercritical Fluid Chromatography

Only seven contributions were presented in the SFC session. Perhaps the topic is no longer 'modern' enough, and perhaps the field of really useful applications is known today. A hint that SFC is not dying at all is the fact that *Hewlett-Packard* is present again on the market with a chromatograph which can be used for both packed and capillary columns [34] (after an early SFC adventure, *Hewlett-Packard* was not engaged in this technology for a number of years). In a second poster, the same authors claimed that 'the significant reduced viscosity of supercritical fluid mobile phases allows the user to exploit efficiency as a major resoving tool. Particles as small as 1.5 μm or columns as long as 2 m are easily employed in packed column SFC' with retention times not longer than several min [35].

Not in the SFC section but under 'Fundamentals', a noteworthy poster could be found where CH_3OH/H_2O HPLC mobile phases were mixed with up to 50% CO_2 [36]. The separations obtained were still of the HPLC-type, but the benefits were increased speed of analysis and improved column efficiency, while the solvation power of the eluent (poor in SFC and one of its main problems) was maintained. The only necessary adaption of the HPLC chromatograph is a fused silica capillary at the detector outlet which acts as a restrictor.

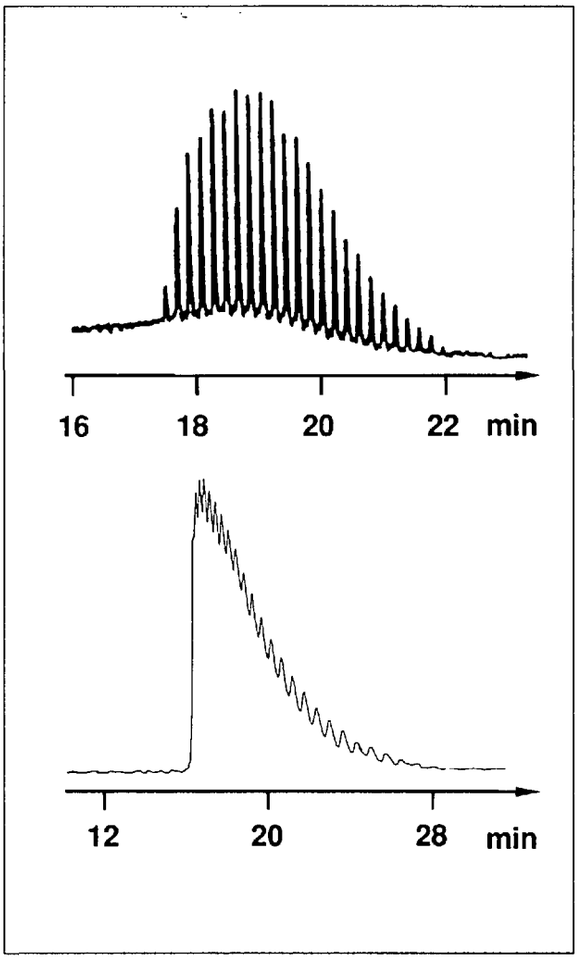


Fig. 2. *Separation of polydeoxyadenylic acids with chain lengths from 40mer to 60mer.* Top: by capillary gel electrophoresis. Below: by gradient HPLC [40].

Biopolymers

Some of the highly sophisticated techniques used, when biopolymers and HPLC meet, have been already mentioned at the very beginning of this review. One field of growing interest are HPLC-based immunoassays which are flexible and can be automated [37]. The HPLC column acts as the storeroom for the antibodies; by injection of the sample they pick up a certain amount (but not all) of labeled analytes and the remaining analytes can be detected in the eluate.

HPLC in the version of frontal immunochromatography can also be used for the investigation of protein conformations [38]. Displacement chromatography was successfully utilized for the separation of closely related protein variants [39].

Capillary Electrophoresis

The HPLC symposium was always open to alternative separation methods, and although special meetings about capillary electrophoresis are organized now a large number of contributions used this technique. This policy should be continued, because the contact between research-

ers using only one of the methods is very stimulating. There is no doubt that, at least for certain applications, capillary electrophoretic techniques give a separation performance which is an order of magnitude better than HPLC. An example is shown in *Fig. 2* where the same polynucleotide mixture is separated by both techniques [40] (*J. Chromatogr.* **1991**, *558*, 273). However, the two methods are not only rivals, but they can used simultaneously in two-dimensional separations. By coupling of reversed-phase or size exclusion liquid chromatography with capillary zone electrophoresis (CZE), it was possible to multiply the information obtained [41]. Because one CZE run lasts 15 s only it is possible to perform up to four CZE separations during the elution of a single HPLC peak.

A lot of basic research is still needed in the field of capillary electrophoresis. It was possible to get video recordings of the flow profile within a capillary [42]. Most promising is the use of secondary equilibria or microemulsions in order to make possible the separation of complex mixtures, but these phenomena are not yet really understood [43].

HPLC'93 in Hamburg

This was a personal 'tour d'horizon', and a great number of other fascinating contributions, especially applications, could be found in Baltimore. It can be expected that this will also be the case 1993 in Hamburg where the next symposium of this series will take place. Prof. *Klaus Unger* had the pleasure to close HPLC'92 with the invitation for HPLC'93. It will be held from May 9 to 14 at the Kongresszentrum Hamburg. The secretariat is: Gesellschaft Deutscher Chemiker, Abteilung Tagungen, Postfach 90 04 40, D-W–6000 Frankfurt 90.

[1] C. Horváth, 'Molecular chromatography: Separation by seeing, recognizing and interacting at the molecular level'.
[2] P. Young, H. Richardson, T. Wheat, G. Vella, 'Fast peptide separations at elevated temperatures by microbore HPLC'.
[3] N.B. Afeyan, B. Dorval, L. Khatchaturian, 'Immunological detection of biomolecules in real time'.
[4] Q.C. Wang, J.M.J. Frechet, 'Novel continuous rods of macroporous polmer as a HPLC separation media'.
[5] S. Hjerten, J. Mohammad, K. Nakazato, 'Compressed continuous beds – A novel inexpensive, easy-to-prepare, high-resolving type of chromatographic support'.
[6] A. Velayudhan, Y.Yang, R. Hendrickson, C. Ladisch, M.R. Ladisch, 'Protein chromatography and scale-up for process applications'.
[7] Z. el Rassi, J. Yu, 'Microspherical zirconia-based stationary phases'.
[8] J.D. Macfarlane, H. Suzuki, H. Watanabe, S. Moriguchi, K. Itoyama, H. Seo, 'A novel hydrophilic HPLC support made from chitin'.
[9] R.B. Wilhelmy, 'WFP hydroxyapatite: A new 'sea urchin' support structure for improved chromatographic performance'.
[10] J.J. Kirkland, J.J. DeStefano, 'Normal-phase HPLC with highly purified porous silica microspheres'.
[11] Q.C. Wang, K. Hosoya, F. Svec, J.M.J. Frechet, 'Polymeric porogens in the preparation of novel monodispersed macroporous polymeric separation media for HPLC'.
[12] C.J. Welch, 'Progress in the design of selectors for buckminsterfullerene'.
[13] A.F. Aubry, F. Gimenez, I.W. Wainer, 'Effect of mobile phase pH and dimethyloctylamine on the retention and stereoselectivity of antimalarial agents on a α_1-acid glycoprotein: Evidence for a multiple-site chiral mechanism'.
[14] T.C. Pinkerton, J. Haginaka, 'Enantioselectivity of ovomucoid bonded-phase HPLC columns produced with isolated protein domains'.
[15] R. Kaliszan, T.A.G. Noctor, W.P. Purcell, I.W. Wainer, 'Chiral and achiral aspects of quantitative structure-retention relationships on a human serum albumin based HPLC column'.
[16] B. Loun, D.S. Hage, 'Characterization of thyroxine-albumin binding using high-performance affinity chromatography'.
[17] W.K. Chui, T. Alebic-Kolbah, I.W. Wainer, 'Enzyme-based HPLC columns as probes of enzyme/substrate and enzyme/inhibitor interactions'.
[18] L. Siret, N. Bargmann, A. Tambuté, M. Caude, 'Direct enantiomeric separation of β-blockers on tyrosine-derived chiral stationary phases by SFC with carbon dioxide'.
[19] J.E. McCune, B.C. Willoughby, A.S. Colborn, I.M. Mutton, 'Separation of the enantiomers of BCH189: A novel nucleoside analogue'.
[20] F.X. Zhou, I.S. Krull, B. Feibush, 'Direct injection analysis of amine drugs in plasma by solid-phase derivatization on a 9-fluoreneacetyl tagged reagent'.
[21] I.S. Krull, A.J. Bourque, M. Szulc, A. Seger, F.X. Zhou, B. Feibush, 'Solid phase derivatization of drugs in biological fluids'.
[22] J.D. Brewster, E.G. Piotrowski, 'Electromembrane sample treatment in multi-residue HPLC analysis of antibiotics'.
[23] K. Seta, T. Okuyama, 'Micro column HPLC system for HPLC/electrospray/mass spectrometry of biochemical samples'.
[24] M.L. Johansson, S. Folestad, A. Tivesten, 'Microanalytical determination of amino acid enantiomers using liquid chromatography with laser-induced fluorescence detection'.
[25] G. Achilli, G.P. Cellerino, G.V. Melzi d'Eril, 'Identification and determination of phenolic constituents in natural beverages and plant extracts using a coulometric electrode array system'.
[26] U.B. Axiö-Fredriksson, A. Danielsson, B.R. Österlund, 'Consultation with and validation of an expert system in the strategic and tactical planning of protein purification'.
[27] H.J. Helmlin, J.T. Clerc, 'Computer-assisted retention time prediction and gradient optimization with a spreadsheet program'.
[28] P.S. Joyner, J.K. Strasters, M.G. Khaledi, 'Quantitative structure-activity-retention relationships in micellar liquid chromatography'.
[29] A. Seidel-Morgenstern, G. Guiochon, 'Determination of isotherms and calculation of elution profiles for the enantiomers of *Tröger*'s base on cellulose triacetate'.
[30] A. Felinger, G. Guiochon, 'Optimization in overloaded elution and displacement chromatography'.
[31] P.L. Camacho, C. Piggee, M. Miner, G. Vigh, 'Chiral displacement chromatographic separations in the normal-phase mode'.
[32] K. Duff, R. Ludwig, 'Packing and evaluation of small particle preparative columns'.
[33] T.K. Nadler, F.E. Regnier, 'Rapid multiple cycle preparative separations with perfusion chromatography sorbents'.
[34] T.A. Berger, W.H. Wilson, 'A new supercritical fluid chromatograph'.
[35] T.A. Berger, W.H. Wilson, 'Considerations for method development in packed column SFC'.
[36] Y. Cui, S.V. Olesik, 'Further study of enhanced fluidity mobile phase in HPLC'.
[37] D.S. Hage, D.H. Thomas, 'Chromatographic automation of competitive binding immunoassays: Theory and characterization of a sequential injection method'.
[38] C. Xu, F.E. Regnier, 'The utility of frontal immunochromatography for protein conformational analysis'.
[39] W.S. Hancock, R.C. Chloupek, J.M. Jacobson, 'The use of displacement chromatography to separate variants of recombinant DNA-derived human growth hormone (rhGH)'.
[40] Y. Baba, M. Tsuhako, 'A comparative study on HPLC and capillary gel electrophoresis in high-resolution separations of polynucleotides RNA and DNA'.
[41] J.W. Jorgenson, J.P. Larmann, A.W. Moore, A.V. Lemmo, 'Two dimensional separation by LC-CZE'.
[42] T. Tsuda, 'Flow profile of electroosmosis in capillary zone electrophoresis'.
[43] D.B. Westerlund, M. Stefansson, I. Belterstein, 'Capillary electroseparation of peptides and glycoconjugates'.

CHIMIA 46 (1992) Nr. 9 (September)

INFORMATION

Gold for Switzerland at the Chemistry Olympiad 1992

The 24th International Chemistry Olympiad, conducted in 20 languages in Pittsburgh and Washington from July 11–22, subjected participants to a gruelling five-hour written examination and a five-hour test of practical laboratory techniques. The highest-scoring 10% were awarded gold medals, the next 20% silver, and the next 30% bronze.

Switzerland was at its 6th participation, with a team composed of *Laurent Cavin* (Pully/VD), *Lukas Hintermann* (Klingnau/AG), *André Rouge* (Romanel-Lausanne) and *Marco Ziegler* (Sulgen/TG), which had all obtained their 'Matur/Maturité' in 1992 in a Swiss high school (Gymnasium/Gymnase).

Theoretical and practical problems to be solved in the 1992 Olympiad are described in the appendix. The Swiss delegation behaved remarkably well, since it obtained three medals. *Lukas Hintermann* won a gold medal (94.14 points out of max. 100), and both *Marco Ziegler* and *André Rouge* got a bronze medal. This is the best result our small country has ever achieved so far, since preceding best results were two silver medals in 1991, one bronze medal in 1990, and none before. In the final grading scheme only three candidates had a better score than *Lukas Hintermann*, the best of all being *Ye Zheng*, a Chinese.

It can be worth noticing that our gold medallist has the merit of having set up his own chemistry laboratory at home in Klingnau. And as he was not allowed to buy corrosive chemicals in the drugstore, he managed to develop ingenious organic syntheses of his own by using commercial Na_3PO_4 instead of NaOH and commercial $NaHSO_4$, instead of H_2SO_4: 'nécessité fait loi', as they say in France.

The present Olympiad was so tough that plenty of results were approximately equal. As an example, *Marco Ziegler* got a bronze medal with 89.02 points, *i.e.* only 5.02 points behind *Lukas Hintermann*. Presenting too many significant figures could make you lose a gold medal.

Beside the competition itself, a rich cultural and entertaining program was organized before and after the competition: visits to the Carnegie Science Museum, the Westinghouse Cray Supercomputer Center in Pittsburgh, not to mention chemical magic shows and amusements parks. In Washington, the students visited the prestigious museums of the Smithsonian Institution; they were officially received in their respective embassies (except Switzerland, whose cultural attaché was in vacation), and at the Department of the State. In the Washington ceremony at which the awards were announced, Dr. *W. N. Lipscomb* of Harvard University, winner of the 1976 Nobel prize in chemistry, told participants that everyone was a winner: 'The Chemistry Olympiad is a useful and important competition, not least because it brings together bright chemistry students from all all over the world to exchange ideas'.

Next Olympiads will happen in Italy 1993, then in Norway, Canada, and Australia. Switzerland has been approached to host the competition later on.

f.l.t.r. *Lukas Hintermann*, *Marco Ziegler*, *André Rouge*, and *Laurent Cavin*

Appendix 1. Theoretical problems

1. (10 points). Microscopic organisms called diatoms are an abundant food source in the oceans producing carbohydrates from carbon dioxide and water by photosynthesis:
   $6\,CO_2 + 6\,H_2O$ + solar energy $\rightleftarrows$ $C_6H_{12}O_6 + 6\,O_2$
   a) During their first five years of life blue whales gain 75 kg of mass per day by feeding on krill. The whale must consume ten times this mass of krill each day. The krill must consume 10.0 kg of diatoms to produce 1.0 kg of krill. Assuming that the mass gain in the first five years of a whale's life is due to the consumption of carbohydrates ($C_6H_{12}O_6$), calculate the volume of CO_2 at STP (0°, 1.00 atm, 101 kPa) that must be used by the diatoms to produce the carbohydrates required by a blue whale in its first five years of life.
   b) i) There is 0.23 ml of dissolved CO_2 per liter sea water (at 24° and 1.00 atm, 101 kPa). If diatoms can completely remove carbon dioxide from the water they process, what volume of water would they process to produce the carbohydrates required by a blue whale during its first five years of life?
   ii) What fraction of the total volume of the oceans will be needed to supply the carbon dioxide for the first five years of growth of 1000 blue whales? The volume of the oceans is $1.37\ 10^{18}\ m^3$.
   c) 3% of the mass of a 9.1×10^4 kg adult whale is nitrogen. When a 9.1×10^4 kg blue whale dies, what is the maximum mass of ammonium ion that become available to marine organisms?
   d) 18% of a 9.1×10^4 kg whale's mass is carbon. Carbon can be returned to the atmosphere as carbon dioxide, and then removed from the atmosphere by weathering of rocks containing calcium silicate.
   $CaSiO_3 + 2CO_2(g) + 3H_2O \rightleftarrows Ca^{2+}(aq) + 2HCO_3^-(aq) + H_4SiO_4(aq)$
   What are the maximum number of grams of $CaSiO_3$ that can be weathered by the carbon dioxide produced from the decomposition of 1000 blue whales, the number estimated to die annually ?
2. (12 points). Many streams drain in areas where coal or metallic ores are mined. These streams have become acidic and contain dissolved iron and sulfate ions, due to sulfur-containing ores being exposed to oxygenated waters. The most common sulfur-containing mineral is pyrite, FeS_2, in which the oxidation state of iron is +2. As the iron-rich streams mix with other waters, the dissolved iron precipitates as goethite, FeO(OH), which coats the stream bottom, while the water remains acidic.
   a) Draw the electron dot structure in the S_2^{2-} ion.
   b) Write a balanced equation to show how hydrogen ion (H^+) is generated during the oxidation of pyrite in a stream to form a solution of iron(II) ions and sulfate ions.
   c) Write a balanced equation to show how many additional hydrogen

ions are generated, when the iron(II) ions are oxidized to form goethite, FeO(OH).

d) Calculate how many moles of pyrite would be required to bring 1.0 liter of pure water to a pH of 3.0, if the pyrite was completely converted into FeO(OH) and hydrogen ions. Neglect any HSO_4^- formed.

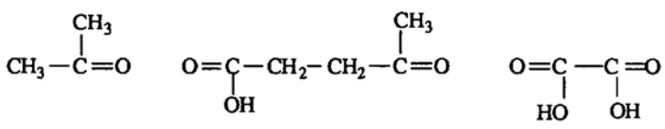
e) The concentration of iron(II) Fe(II) in a stream is 0.00835M. At a very narrow point in the stream it empties into a large pond, with a flow rate of 20.0 liters each minute. The water is sufficiently aerated that 75% of the Fe(II) is oxidized to Fe(III). The pH of the pond is high enough that the iron(III) precipitates immediately as $Fe(OH)_3$, which on aging becomes Fe_2O_3. What mass of Fe_2O_3 will be deposited in two years?

3. (8 points). Coniferyl alcohol, $C_{10}H_{12}O_3$, is isolated from pine trees.

a) Coniferyl alcohol is not soluble in water or aq. $NaHCO_3$. A solution of Br_2 in CCl_4 is decolorized when added to coniferyl alcohol forming **A** ($C_{10}H_{12}O_3Br_2$). Upon reductive ozonolysis coniferyl alcohol produces vanillin, (4-hydroxy-3-methoxybenzaldehyde), et **B** ($C_2H_4O_2$).
Coniferyl alcohol reacts with benzoyl chloride (C_6H_5COCl) in the presence of base to form **C** ($C_{24}H_{20}O_5$). This product rapidly decolorizes $KMnO_4$, and is insoluble in dilute NaOH. Coniferyl alcohol also reacts with cold HBr to form **D** ($C_{10}H_{11}O_2Br$).
Hot HI converts ArOR to ArOH and RI. Coniferyl alcohol reacts with excess hot HI to give CH_3I and **E** ($C_9H_9O_2I$). CH_3I in aqueous base reacts with coniferyl alcohol to make **F** ($C_{11}H_{14}O_3$), which is not soluble in strong base, but decolorizes Br_2/CCl_4.
Draw the structures of compounds **B - F**, and coniferyl alcohol

b) Compound **A** can exist as a number of stereoisomers. Draw **A**. Label each chiral center in **A** with an asterisk (*). For all stereoisomers, draw *Fischer* projections and label each chiral center with the proper *R* or *S* designation.

4. (8 points). Upon oxidation, a terpene alcohol **A** ($C_{10}H_{18}O$) can give either a ten-carbon aldehyde or a ten-carbon acid. Reaction with 2 moles of bromine gives **B** ($C_{10}H_{18}OBr_4$). When **A** undergoes oxidative ozonolysis, three products are obtained:



Compound **A** can also be reacted with HBr to give a number of products, including two acyclic bromides of formula $C_{10}H_{17}Br$. Give the structures of **A**, **B**, and of the two acyclic bromides $C_{10}H_{17}Br$. Using structural formulas, propose a mechanism for the formation of the two bromides.

5. (8 points). Nitrogen dioxide is found in the atmosphere. It can dimerize to give $N_2O_4(g)$.

a) With an electron dot structure, show the bonding in $NO_2(g)$ and $N_2O_4(g)$ using the concept of resonance. $NO_2(g)$ is paramagnetic. and $N_2O_4(g)$ is not paramagnetic.

b) At 298 K, the $\Delta G°$ of formation for $N_2O_4(g)$ is 98.28 kJ·mol^{-1}, whereas that of $NO_2(g)$ is 51.84 kJ·mol^{-1}. Starting with one mole of $N_2O_4(g)$, at 1.00 atm and 298 K, calculate what fraction will be decomposed, if the total pressure is kept constant at 1,00 atm, and the temperature is maintained at 298 K.

c) If $\Delta H°$ for the reaction $N_2O_4(g) \rightleftarrows 2NO_2(g)$ is +58.03 kJ·mol^{-1}. at what temperature would the fraction of $N_2O_4(g)$ decomposed be double that calculated in part (b)? Assume that entropy and enthalpy may be taken invariant with temperature.

d) The dissociation of $N_2O_4(g)$ to give $NO_2(g)$ is a first-order process with a specific rate constant of 5.3×10^4 s^{-1} at 298 K. How long would it take for 20% of the original $N_2O_4(g)$ to decompose?

e) The association of $NO_2(g)$ to give $N_2O_4(g)$ is a second-order process with a specific rate constant of 9.8×10^6 L·mol^{-1}.s^{-1} à 298 K. Calculate the concentration equilibrium constant K_c at 298 K for the reaction:
$2NO_2(g) \rightleftarrows N_2O_4(g)$.

6. (10 points). The level of carbon dioxide in the atmosphere is increasing. The partial pressure of CO_2 is expected to be about 4.40×10^{-4} atm (4.44×10^{-2} kPa) in the year 2020. The following data are available for this question: 1. *Henry*'s law constant for CO_2 in water at 25° and 1.00 atm (101 kPa) total pressure is 0.0343 mol l^{-1}. atm^{-1} (3.39×10^{-4} mol m^{-3} Pa^{-1}). 2. Thermodynamic values, in kJ mol^{-1} à 25°, are:

	$\Delta G_f°$	$\Delta H_f°$
CO_2(aq)	–386.2	–412.9
H_2O(liq)	–237.2	–285.8
HCO_3^-(aq)	–587.1	–691.2
H^+(aq)	0.0	0.0

For these questions, use a temperature of 25° and 1.00 atm (101 kPa) total pressure.

a) Calculate K and the enthalpy change for the reaction:
$CO_2(aq) + H_2O(l) \rightleftarrows H^+(aq) + HCO_3^-(aq)$

b) Calculate the concentration, in mol·l^{-1}, of the carbon dioxide dissolved in distilled water equilibrated with the atmosphere in the year 2020. Calculate the pH of this solution.

c) If the temperature of an equilibrated solution of CO_2 in water is increased, and the concentration of dissolved CO_2 is maintained constant, the pH of the solution changes. Predict whether the pH will increase or decrease

7. (12 points) When the fresh-water rivers that run into Chesapeake Bay flood after heavy rains in the spring, the increase in fresh water cause a decrease in the salinity in the areas where oysters grow. The minimum concentration of chloride ion needed in oyster beds for normal growth is 8 ppm (8 mg·l^{-1}).

a) After on week of heavy rain, the following analysis is done on water from the bay. To a 50.00 ml of bay water is added a few drops of a K_2CrO_4 solution as an indicator. The sample is then titrated with 16.16 ml of a 0.00164M $AgNO_3$ solution. At the end a brick-red precipitate forms
$K_sAgCl = 1.78 \times 10^{-10}$
$K_sAg_2CrO_4 = 1.00 \times 10^{-12}$

i) What is the molar concentration of chloride ion in the sample. Does the water contain sufficient chloride ion for normal growth of oysters?

ii) Write a balanced equation that describes the reaction of the sample with the titrant. Write then a balanced equation that describes the color change at the end-point of the titration.

iii) The concentration of chromate ion at the end-point is 0.020M. Calculate the concentration of chloride ion in the solution when the red-orange precipitate forms.

iv) For this titration to work most effectively, the solution being must be neutral or slightly basic. Write a balanced equation that described the competing reaction that would occur in acidic medium that would influence the observed end-point.

b) Typically a buffer is added to the solution being titrated to control the pH. Suppose the pH of the sample of bay water was determined to be 5.100, too acidic to perform the analysis accurately. Select a buffer from the ones listed that would enable you to establish and maintain a pH of 7.200. Make the assumption that the buffer does not react with the sample or titrant.
Using the buffer system you selected, calculate the mass of weak acid and of conjugate base you would need to dissolve in distilled water to prepare 500 ml of a stock solution buffered at a pH 7.2.

c) The chloride ion concentration in another 50.00 ml sample of bay water was determined by the *Volhart* method. In this method an excess of $AgNO_3$ is added to the sample. The excess Ag^+ is titrated with standardized KSCN, forming a precipitate of AgSCN. If the excess Ag^+ from the addition of 50.00 ml of 0.00129M $AgNO_3$ to the water sample required 27.46 ml of 1.41×10^{-3}M KSCN for titration, calculate the concentration of chloride ion in the bay water sample.

d) In natural waters with higher concentrations of Cl^-, the Cl^- can be determined gravimetrically by precipitating AgCl. However, AgCl is susceptible to photodecomposition as shown below:
$AgCl(s) \rightarrow Ag(s) + \frac{1}{2}Cl_2(g)$
Furthermore, if this decomposition occurs in the presence of excess Ag^+, the following additional reaction occurs :
$3\,Cl_2(g) + 3\,H_2O + 5\,Ag^+(aq) \rightarrow 5\,AgCl(s) + ClO_3^-(aq) + 6\,H^+(aq)$
If a 0.0100 g of a 3.000 g sample of AgCl in contact with the solution containing Ag^+(aq) undergoes photo-decomposition, calculate the final mass of the solid as a result of these reactions.

8. (10 points). You are provided, on a separate sheet, with three *Pourbaix* diagrams, one for water, one for nitrogen, and one for manganese.

a) Write the formula of the species of nitrogen that is predominant in oxygenated lakes of normal pH (pH about 6.0). Write the formula of the species of manganese that is predominant in highly O_2-depleted lakes rich in organic compounds that are strongly contaminated with bases (pH about 12.0).

b) People often find that clear, slightly acidic (pH about 5.0) water drawn from wells deposits a black manganese-containing solid on standing in toilet bowls. Write the chemical formula for the black solid. Write the formula for the species of manganese found in the well water, while it is still undergound.

c) According to *Pourbaix* diagrams, some of the chemical forms of manganese should oxidize NH_3(aq) or NH_4^+(aq) to N_2(aq). In the following list, circle the formulas of all of the forms of Mn that do this: Mn, $Mn(OH)_2$, Mn^{2+}, Mn_3O_4, Mn_2O_3, MnO_2, MnO_4^{2-}, MnO_4^-.

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e) *Pourbaix* diagrams can also be applied qualitatively to solids. Is it likely to be dangerous to gring together in a mortar and pestle potassium nitrate and manganese metal, and then potassium nitrate and manganese dioxide?

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Forschung nach Alternativmethoden zu Tierversuchen; Jahresbericht

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d) Ammonium permanganate, NH_4MnO_4, is a well-known salt, but ammonium manganate, $(NH_4)_2MnO_4$, is not well-known. Write and balance the equation for the decomposition of NH_4MnO_4 to give MnO_2 and N_2, and then for the decomposition of $(NH_4)_2MnO_4$ to give Mn and N_2.

e) *Pourbaix* diagrams can also be applied qualitatively to solids. Is it likely to be dangerous to gring together in a mortar and pestle potassium nitrate and manganese metal, and then potassium nitrate and manganese dioxide?

f) The standard potential, $E°$, for the reduction of MnO_4^- to MnO_2 is +1.692 V. Applying the *Nernst* equation, calculate the potential, at 25°, for the reduction of 0.00100M MnO_4^- at a pH=4.0.

9. (12 points). Pheromones are chemicals or mixtures of chemicals secreted by insects and some animals for communication. In the problem below you will find a number of pheromones undergoing reactions which were used in determining their structures. In each case, give the structure or structures of the carbon containing products produced. Show geometric (*cis/trans*) isomers where appropriate in part (e).

a) Isoamyl acetate + NaOH + Δ.
b) i) 2-heptanone + I_2 + NaOH.
ii) 2-heptanone + $NaBH_4$ / CH_3CH_2OH.
iii) 2-heptanone + CH_3CH_2MgBr + H_3O^+.
c) 2,6-dimethyl-10-methylene-do deca-2,6,11-triene +
i) + O_3 + Zn, H_3O^+.
ii) + O_3 + H_2O_2 + H_3O^+.
d) 3-methyl-4-hydroxy-hexane +
i) + H_2O/H_3SO_4 + Δ.
ii) + $Na_2Cr_2O_7$, H_3O^+.
iii) + CH_3COOH + H^+ + Δ.
e) 1-methylcyclohexene +
i) Br_2 /CCl_4.
ii) $KMnO_4$ /OH^-, cold.
iii) H_3O^+.
iv) HBr, peroxide.
v) BH_3/ether, then H_2O_2, OH^-.
vi) H_2, Pt.

Appendix 2.
Practical problem

In this experiment each candidate had to measure the pH and to determine the solubility of calcium carbonate in an aqueous solution saturated with carbon dioxide and in a solution free of carbon dioxide. The calcium was to be determined by complexometric titration with EDTA solution (to be standardized). The pH was measured by a glass electrode and a pH meter (to be calibrated). The most difficult part was measuring the pH in the solution free of carbon dioxide, since the pH is high and the calcium concentration is extremely low. Details about the procedure will not be given here, but can be obtained from the author.

M. Cosandey
Gymnase de Chamblandes
1009 Pully

Tagung des EURACHEM/Schweiz vom 21. August 1992, ETH-Zürich

Qualitätssicherung in analytischen Laboratorien

'Qualitätssicherung in analytischen Laboratorien' und 'Akkreditierung für den europäischen Markt': Diese beiden Themen lockten am 21. August rund 300 Teilnehmerinnen und Teilnehmer – vorwiegend im Bereich der analytischen Chemie tätig – an die von der EMPA St. Gallen organisierte EURACHEM-Tagung an der ETH-Zürich.

EURACHEM ist eine Interessengemeinschaft europäischer, auf dem Gebiet der analytischen Chemie tätiger Laboratorien. Beinahe alle Mitgliedstaaten der EG und der EFTA sowie der CEC (Commission European Communities) sind darin vertreten. EURACHEM versteht sich als europäisches Forum für die analytische Chemie. Am 21. August 1992, veranstaltete EURACHEM/Schweiz eine Tagung zum Thema 'Qualitätssicherung'. Für analytische Laboratorien innerhalb Europas werden die Qualitätssicherung und der Aufbau eines eigentlichen *QS-Systems* immer wichtiger, besonders im Hinblick auf die gegenseitige Anerkennung von Prüfresultaten. Ebenso interessieren sich diese Labors im Moment besonders für Fragen im Zusammenhang mit dem Aufbau und der Harmonisierung nationaler Akkreditierungs-Systeme sowie für die Zertifizierung von Prüflaboratorien in der Schweiz.

Ständerat Dr. *Otto Piller*, Direktor des Eidgenössischen Amtes für Messwesen (EAM), stellte in seinen Ausführungen an der EURACHEM-Tagung das schweizerische Akkreditierungssystem und das EAM als schweizerische Akkreditierungsstelle vor. Der freie Warenverkehr innerhalb des Europäischen Wirtschaftsraumes (EWR) setze ausser der Harmonisierung der technischen Normen und Vorschriften auch die Durchsetzung der allseitigen Anerkennung von Prüfverfahren und Prüfzertifikaten, Konformitätsnachweisen sowie Bauartzulassungen voraus. Dies basiere auf einem vertrauenswürdigen europäischen Prüf- und Zertifizierungswesen. Stellen, die im künftigen EWR eichen, kalibrieren, prüfen, überwachen und zertifizieren wollten, müssten deshalb nach einheitlichen Kriterien akkreditiert werden.

Auf die wirtschaftliche Bedeutung technischer Handelshemmnisse, wie sie etwa die unterschiedlichen nationalen Produkte- und Prüfvorschriften für ein- und dasselbe Produkt bedeuten, ging Dr. *Markus Huber*, Chef der Sektion für die Beseitigung technischer Handelshemmnisse im Bundesamt für Aussenwirtschaft, ein. Die ökonomische Bedeutung technischer Handelshemmnisse lasse sich nur schwer quantifizieren, die negativen Auswirkungen auf den grenzüberschreitenden Handel seien vermutlich grösser, als gemeinhin angenommen. Das Nebeneinander unterschiedlicher technischer Vorschriften in Europa führe zu einer Marktsplitterung, die an der Schwelle zum nächsten Jahrtausend im höchstindustrialisierten Erdteil der Welt als Anachronismus anmuten müsse: Für die Unternehmer bedeutet sie eine Mittelvergeudung durch Doppelarbeit bei Forschung und Entwicklung. Für die Behörden wiederum bedeuteten nicht-harmonisierte, technische Vorschriften Doppelarbeit bei Prüfungs- und Zulassungsverfahren, die sich in Mehrkosten für den Hersteller niederschlagen. Die Verbraucher letzlich bekämen in der Regel höhere Kosten durch höhere Preise zu spüren.

Dass die beiden oben erwähnten sowie die anderen kompetenten Referenten (s. Tagungsprogramm) die fachlichen Anliegen der Anwesenden im Kern trafen, machte sich in den anschliessenden Diskussionen bemerkbar. Ein gutes Zeichen für EURACHEM/Schweiz und die EMPA, in Zukunft weitere Tagungen zu aktuellen Themen zu organisieren.

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Forschung nach Alternativmethoden zu Tierversuchen; Jahresbericht

Die Stiftung Forschung 3R (vormals Stiftung Finanz-Pool 3R) hat ihren Jahresbericht über das fünfte Jahr ihrer Tätigkeit veröffentlicht. Sie präsentiert sich neu unter dem Namen 'Stiftung Forschung 3R'. Der Stiftungsrat möchte mit dem neuen Namen dem Zweck der Stiftung deutlicher Ausdruck verleihen.

Der Stiftung wurden im vergangen Jahr vom Bund und von der Interpharma (*Ciba-Geigy, F. Hoffmann-La Roche, Sandoz Pharma*) Fr. 800 000.– für die Unterstützung von Forschungsprojekten über Alternativmethoden zu Tierversuchen zur Verfügung gestellt. Im Jahre 1991 genehmigte die Stiftung vier neue Projekte. Damit sind bisher 23 Projekte mit einem Gesamtbudget von Fr. 3,5 Millionen zustande gekommen. 13 Projekte konnten inzwischen abgeschlossen werden.

Für die Unterstützung geniessen Projekte Priorität, die ins Schwerpunktprogramm der Stiftung passen. Dieses umschreibt für eine Periode von vier Jahren jene Forschungsgebiete, wo die Entwicklung von Alternativmethoden besonders dringlich erscheint.

Einen der Schwerpunkte bildet gegenwärtig die Erforschung von praxistauglichen Möglichkeiten zur *in vitro* Herstellung von monoklonalen Antikörpern. Diese für verschiedene Zweige der Forschung, der Diagnostik und der Therapie wichtigen hochspezifischen immunbiologischen Stoffe werden noch vielfach in belastenden Tierversuchen gewonnen. Insbesondere für mittlere Mengen, die in der Forschung oft gebraucht werden (1/10–2 g), fehlt es noch an optimalen *in vitro* Produktionsmöglichkeiten.

Die Stiftung versucht daher mit einer Validierungsstudie, an welcher etwa 30 Institute teilnehmen können, der Forschung und Entwicklung auf diesem Gebiet Impulse zu verleihen. Interessierte Forscher und Forscherinnen sind eingeladen, sich an der Validierungsstudie zu beteiligen.

Auskünfte:
Frau *Susi Eppenberger*, Präsidentin
Telefon 074 5 23 88
Frau Dr. med. vet. *Regula Vogel*
Bundesamt für Veterinärwesen
Telefon 031 970 84 87

Geburtstage

Zum 60. Geburtstag von Dr. phil. nat. *Hans Peter Stauffer*

Ende August feierte *Hans Peter Stauffer*, Professor für Chemische Analytik und weitere Fächer an der Abteilung Chemie der Ingenieurschule Burgdorf, seinen 60. Geburtstag. Dr. *Stauffer* ist eine Persönlichkeit besonderer Prägung, wodurch es schwer fällt ihn mit Kollegen zu vergleichen. Am ehesten könnte man einige Parallelen zu seinem ehemaligen Lehrer und Freund, dem verstorbenen Dr. *Max Lüthi* ziehen: Ausser der weitgehend übereinstimmenden Tätigkeit als Dozent, Redaktor (u.a. *Chimia*), Vorstandsmitglied des Schweiz. Chemiker Verbandes (Dr. *Lüthi* war Präsident), fallen ähnliche Charakterzüge auf, zum Beispiel das ausgeprägte Organisationstalent oder der feine Sinn für gehobene Lebensqualität, Humor und gute Umgangsformen. Dazu kommt ein grosser Erfahrungsschatz aus erfolgreicher früherer Praxis. Kein Wunder, dass das Urteil von *Hans P. Stauffer* sowohl in der Abteilung Chemie, wie auch in der Aufsichtskommission unserer Ingenieurschule, wo er die Dozenten vertritt, grosses Gewicht hat. Dr. *Stauffer* ist ein kompetenter und geschätzter Lehrer; seine Vorlesungsskripten werden in Praktikums- und Diplomberichten aller Chemiefächer immer wieder zitiert. Es fällt auch auf, wie die Studierenden trotz hoher Ansprüche des Dozenten den Unterricht in Chemischer Analytik gern besuchen. Das zugehörige Instrumentalanalytische Praktikum gewährt den Studenten viel Selbständigkeit, verbunden mit eigener Verantwortung. Die letztere richtig gebrauchen zu lernen, ist wohl das wertvollste 'Nebenprodukt' dieses fortschrittlichen Praktikumsbetriebs. Lieber *Hans Peter*, im Namen von uns Kollegen und vieler Studierender, gegenwärtiger wie ehemaliger, wünsche ich Dir für die kommenden Jahre Glück und Befriedigung im Beruf. Wir schätzen Dein charmantes Wesen und werden gern auch weiterhin Deinen Rat und Deine klaren Formulierungen annehmen.

Matthias Brönnimann

Veranstaltungen

28th ESF/EUCHEM Conference on Stereochemistry, Bürgenstock

Bürgenstock, Switzerland, May 2–May 8, 1993

President: Prof. *W. Oppolzer*
Department of Chemistry
University of Geneva
30, quai Ernest-Ansermet
CH–1211 Geneva 4

Secretariat: Prof. *A. Pfaltz*
Institute of Organic Chemistry
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Bücher

Bei der Redaktion eingetroffene Bücher

E. Osawa, O. Yonemitsu, Editors
'Carbocyclic Cage Compounds: Chemistry and Applications'
VCH Verlagsgesellschaft mbH,
D–6940 Weinheim, 1992

C. Eppelsheim, R. Flüglein
'Schwermetallanalytik'
Vogel Verlag und Druck AG,
D–8700 Würzburg 1, 1992

Wen Hao, H.S. Elbro, P. Alessi
'Polymer Solution Data Collection; 1: Vapor-Liquid Equilibrium'
DECHEMA Chemistry Data Series, Vol. XIV, Part 1
DECHEMA, Deutsche Gesellschaft für Chemisches Apparatewesen, Chemische Technik und Biotechnologie e.V.,
D–6000 Frankfurt/Main, 1992

'Firmenhandbuch Chemische Industrie', Bundesrepublik Deutschland, 15
Ausgabe 1992/93
ECON Verlag GmbH,
D–4000 Düsseldorf, 1992

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