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 Csaha 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 multicol-umn 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

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Fig. 1. Fast separation of cytochrome c tryptic digest. Column: 2 mm x 15 cm; stationary phase: C18 Delta-Pak 5 μm; mobile phase: H2O/CH3CN with 0.1% CF3COOH, gradient 2% B/min; flow rate: 0.72 ml/min; temperature: 75°; detection: UV-VIS 190–425 nm [2].
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 ZrO₂ 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 polymers 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 'Buckyclucher' which allows the separation of C₁₆₀ and C₂₀ 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 P₄₅-trypophan 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 CO₂ (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] (Chiral 1992, 4, 252). The reason, therefore, is the complexing action of CO₂ which forms two H-bridges with the OH and NH₂ 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 fluorene-acetyl 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].

Electro dialysis 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-phthalaldehydes and a glycopyanosyl 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 overloaded, 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 and that Langmuir-type 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 CH₃OH/H₂O HPLC mobile phases were mixed with up to 50% CO₂ [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.

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 be 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. Yella, ‘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 polycarbonate’.
[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. Ioyama, H. Sco, ‘A novel hydrophilic HPLC support made from chitin’.
[9] R.B. Wilhelmy, ‘WPO hydroxyapatite: A new ‘sorptive’ 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. Noso, F. Svec, J.M.J. Frechet, ‘Polymeric porogens in the preparation of novel monodispersied macroporous polymeric separation media for HPLC’.
[12] C.J. Welch, ‘Progress in the design of selectors for buckminsterfullerene’.
[13] A.P. Aubry, F. Gimenez, I.W. Wainer, ‘Effect of mobile phase pH and dimethylpolylamine on the retention and stereoselectivity of antimalarial agents on an α,α-dicarboxylic protein: Evidence for a multiple-site chiral mechanism’.
[14] T.C. Pinkerton, J. Haginaka, ‘Enantioselectivity of ovomucoid bonded-phase HPLC column produced with isolated protein domains’.
[15] R. Kulinski, 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-Kollab, I.W. Wainer, ‘Enzyme-based HPLC columns as probes of enzymemembrane and enzyme/inhibitor interactions’.
[18] L. Siret, N. Bargmann, A. Tambuit, 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. Collborn, I.M. Mutton, ‘Separation of the enantiomers of BCH189: A novel nucleoside analogue’.
[20] F.X. Zhou, I.S. Knuff, B. Feibush, ‘Direct injection analysis of amine drugs in plasma by solid-phase derivatization on a 9-fluoreneacetyl tagged reagent’.
[21] I.S. Knuff, A.J. Bourque, M. Szulc, A. Segger, 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-resolution HPLC analysis of antibiotics’.
[23] K. Seta, T. Okuyama, ‘Microcolumn HPLC system for HPLC/electrospray/mass spectrometry of biochemical samples’.
[24] M.L. Johansson, J. Folestad, J.M. Sege, ‘Microanalytical determination of amino acid enantiomers using liquid chromatography with laser-induced fluorescence detection’.
[25] G. Acklin, G.P. Cellerino, G.V. Melzi, ‘Identification and determination of phenolic constituents in natural beverages and plant extracts using a coulometric electrode array system’.
[26] U.B. Axil-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. Helmin, 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 isomers 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, V. Ghig, ‘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 chromatography’.
[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 immunnoassays: 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. Jorgensen, 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’.
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, was attended by 20 teams, each consisting of four students. 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 '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 medalist 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 NaHPO₄ instead of NaOH and commercial NaHSO₄ instead of Na₂SO₄; 'nécessaire 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 0.92 points behind Lukas Hintermann. Presenting too many significant figures could make you lose a gold medal.

After the competition itself, a rich cultural and entertaining program was organized before and after the competition: visits to the Carnegie Science Museum, the Western Cray Supercomputer Center in Pittsburgh, not to mention chemical magic shows and amusement 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 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.

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:

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{H}_2\text{O} + \text{solar energy} \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} \]

(a) During their first five years of life blue whales gain 73 kg of mass per day by feeding on krill. The whale must consume ten times this mass of krill each day. The whale 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 (\( \text{C}_6\text{H}_{12}\text{O}_6 \)), calculate the volume of \( \text{CO}_2 \) at STP (0°C, 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) There is 0.23 ml of dissolved \( \text{CO}_2 \) per liter sea water (at 24°C and 1.00 atm, 101 kPa). If diatoms can completely remove carbon dioxide from the water they process, what volume of water would they require to produce the carbohydrates required by a blue whale during its first five years of life?

(c) What is the maximum mass of ammonium ion that can be available in marine organisms?

2. (12 points). Many streams drain the continents, and carry dissolved iron and sulfate ions, due to sulfur-containing ores being exposed to oxygenated waters. The most common sulfur-containing mineral is pyrite, FeS₂, 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 becomes acidic. How many grams of FeS₂ can be weathered by the carbon dioxide produced from the decomposition of 1000 blue whales?

\[ \text{CaSiO}_3 + 2\text{CO}_2(g) + 3\text{H}_2\text{O} \rightarrow \text{Ca}^{2+}(aq) + 2\text{HCO}_3^-(aq) + \text{H}_2\text{SiO}_3(aq) \]

What are the maximum number of grams of CaSiO₃ that can be weathered by the carbon dioxide produced from the decomposition of 1000 blue whales, the number estimated to die annually?
ions are generated, when the iron(II) ions are oxidized to form goethite, FeO(OH).

d) Calculate how many moles of pyrite must 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 HSO₄⁻ formed.

c) The concentration of iron(II) Fe(II) in a stream is 0.00835 M. 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 Fe₂O₃. What mass of Fe₂O₃ will be deposited in two years?

3. (8 points). Coniferyl alcohol, C₁₅H₁₁O₂, is isolated from pine trees.

a) Coniferyl alcohol is not soluble in water, but dissolved in NaHCO₃. A solution of Br₂ in CC1₄ is decolorized when added to coniferyl alcohol forming A (C₁₅H₁₁O₂Br₂). Upon reduction ozonolysis coniferyl alcohol produces vanillin, (4-hydroxy-3-methoxybenzaldehyde), B (C₇H₇O₃).

Coniferyl alcohol reacts with benzoyl chloride (C₇H₇O₂Cl) in the presence of base to form C (C₁₅H₁₀O₂). This product rapidly decolorizes KMnO₄ and is insoluble in dilute NaOH. Coniferyl alcohol also reacts with cold HBr to form D (C₁₅H₁₂O₂Br). Hot H₂O converts ArOBr to ArOH and RI. Coniferyl alcohol reacts with excess hot H₂O to give CH₃ and E (C₁₅H₁₂O₂). CH₃ in aqueous base reactions with coniferyl alcohol to make F (C₁₅H₁₁O₂), which is not soluble in strong base, but decolorizes Br₂CCl₄.

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 (C₁₅H₂₀O) can give either a ten-carbon aldehyde or a ten-carbon acid. Reaction with 2 moles of bromine gives B (C₁₅H₁₀O₂Br₂). 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 C₁₅H₁₁Br₂.
- Coniferyl alcohol also reacts with cold HBr to form D (C₁₅H₁₂O₂Br).
- Coniferyl alcohol also reacts with cold HBr to form D (C₁₅H₁₂O₂Br).

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

- a) Calculate K and the enthalpy change for the reaction: CO₂(aq) + H₂O(l) ⇌ H⁺(aq) + HCO₃⁻(aq)
- b) Calculate the concentration, in mol⁻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 CO₂ in water is increased, and the concentration of dissolved CO₂ 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 flow 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 (mg/l.).

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 AgNO₃ solution. At the end a white precipitate of AgCl(s) is observed. Calculate the concentration of chloride ion in the sample.

b) If 0.0100 g of a 3.000 g sample of AgCl is in contact with the solution containing Ag⁺(aq) undergoes photo decomposition as shown below: AgCl(s) ⇌ Ag⁺(aq) + Cl⁻(aq)

Furthermore, if this decomposition occurs in the presence of excess Ag⁺, the following additional reaction pathway is available: Cl⁻(aq) + 3 H₂O ⇌ H⁺(aq) + Cl₂(aq) + H₂O(l)

If a 0.010 g of 3.000 g sample of AgCl in water sample. To a 50 ml 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 Pour- haus 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 O₂-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.

c) According to Pour- haus diagrams, some of the chemical forms of manganese should oxidize NH₄⁺(aq) or NH₃(aq) to NO₂⁻(aq).

In the following list, circle the formulas of all of the forms of Mn that do this: Mn, MnO, MnO₂, Mn₃O₄, Mn₂O₃, Mn₅O₈, Mn₂O₄²⁻.
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

Forschung nach Alternativmethoden zu Tierversuchen; Jahresbericht

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

Der Stiftung wurden im vergan- genen Jahr von Bund und von der Interpharma (Ciba-Geigy, H. Hoff- man-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 ge- kommen. 13 Projekte konnten in- zwischen abgeschlossen werden.

Für die Unterstützung genannter Projekte Priorität, die ins Schwer- punktprogramm der Stiftung pas- sen. Dieses umschreibt für eine Per- iod e von vier Jahren jene For- schungsprojekte, die Entwicklung von Alternativmethoden besonders dringlich erscheint.
Tagung des EURACHEM/Schweiz vom 21. August 1992, ETH-Zürich

Qualitätssicherung in analytischen Laboratorien

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

EURACHEM ist eine Interessenorganisation europäischer, auf dem Gebiet der analytischen Chemie täglicher Laboratorien. Beinahe alle Mitgliedstaaten der EG und der EFTA sowie der CEC (Commission Europäische Community) sind dann 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 eigenen QS-Systems immer wichtiger, besonders im Hinblick auf die gegenwärige Anerkennung von Prüfergebnissen. 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.

Ständer der Dr. Otto Pillet, Direktor des Eidgenössischen Amtes für Messwesen (EAM), stellte in seinen Ausführungsansätzen des EURACHEM-Tagung 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 mit aktuellen Themen zu organi-

Kontaktperson: Dr. P. Radiviva
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CH-9001 St. Gallen
Telefon 071 3001 01

Forschung nach Alternativmethoden zu Tierversuchen; Jahresbericht

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Für die Unterstützung der Projekte Priorität, die ins Schwerpunktsprogramm der Stiftung passen. Dieses umschreibt für eine Periode von vier Jahren jene Forschungsthemen, wo die Entwicklung von Alternativmethoden besonders dringend erscheint.

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

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

Auskunft: Frau Susi Eppenherger, Präsidentin
Telefon 074 5 23 88

Frau Dr. med. von der Regula Vogel
Bundesamt für Veterinärwesen
Telefon 031 970 84 87

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EURACHEM ist eine Interessenorganisation der EU, der EFTA und der EFTA sowie der CEC (Commission European Communities) sind daran vertreten. EURACHEM versteht Qualitätssicherung in analytischen Laboratorien als eines der wichtigen Themen für die Zukunft der analytischen Chemie. Die Tagung fand am 21. August 1992 in der ETH-Zürich statt.

Forschung nach Alternativmethoden zu Tierversuchen;
Jahresbericht

Die Stiftung Forschung 3R (vermehrte 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 Stiftungsrat möchte mit dem neuen Namen dem Zweck der Stiftung deutlicher Ausdruck verleihen.

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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. Geburtsstag. Dr. Stauffer ist eine Persönlichkeit besonderer Prüfung, wodurch es schwer fällt ihn mit Kollegen zu vergleichen. Anmerkungen könnte man einige Parallelen zu seinem ehema- ligen Lehrer und Freund, dem verstorbenen Dr. Max Lithi ziehen: Ausser der weitgehend übereinstim- menden Tätigkeit als Dozent, Redaktor (u.a. Chimia), Vorstandsmit- glied des Schweiz. Chemiker Ver- bandes (Dr. Lithi war Präsident), fallen ähnliche Charakterzüge auf, zum Beispiel das ausgeprägte Organisatiosntalent oder der feine Sinn für gehobene Lebensqualität, Hu- mor und gute Umgangsformen. Dazu kommt ein grosser Erfahrungs- schatz aus erfolgreicher früherer Praxis. Kein Wunder, dass das Ur- teil von Hans P. Stauffer sowohl in de- r Abteilung Chemie, wie auch in der Aufsichtskommission unserer Ingenieurschule, wo er die Dozen- ten vertritt, grosses Gewicht hat. Dr. Stauffer ist ein kompetenter und geschätzter Lehrer, seine Vorle- sungsskripten werden in Prakti- kums- und Diplomberichten aller Chemiefächer immer wieder zitiert. Es fällt auch auf, wie die Studien- den trotz hoher Ansprüche des Do- zenten den Unterricht in Chemischer Analytik gern besuchen. Das zuge- hörige Instrumentalanalytische Praktikum gewährt den Studenten viel Selbständigkeit, verbunden mit eigener Verantwortung. Die letztere richtig gebrauchen zu lernen, ist wohl das wertvollste 'Nebenprodukt' die- ses fortschrittlichen Praktikumsbe- triebis. Lieber Hans Peter, im Na- men von uns Kollegen und vieler Studierender, gegenwartiger wie ehemaliger, wünsche ich Dir für die kommenden Jahre Glück und Be- friedigung im Beruf. Wir schätzen Dein charmantes Wesen und wer- den gern auch weiterhin Deinen Rat und Deine klaren Formulierungen annehmen.

Matthias Brünnimann

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Matthias Brünnimann

28th ESF/EUCHEM Conference on Stereochemistry, Bürgenstock

Bürgenstock, Switzerland, May 2–May 8, 1993

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