

TOSolution
TOSOLUTION

A Tosoh Bioscience Newsletter for users of liquid chromatography.

Feb 2005 | Issue 1

A New Year – A New Look!

In response to the many requests we have received for continued publication of our "PEAK" newsletter, we have decided to reinstate a "new look" for the Tosoh Bioscience Newsletter in 2005.



We proudly present, here, our newsletter with a fresh appearance and a new title -TOSOLUTION.

The Tosoh Bioscience GmbH team together with Mr. Hiroyuki Uchida, Director, Senior General Manager, SID and Hiroyuki Suzuki, President of Tosoh Bioscience.

With this newsletter we will continue to inform you about news and events at Tosoh Bioscience and, with technical articles and applications, we hope to provide solutions for your daily work, TO...SOLUTIONS.

We invite you to send us your feedback on this new edition. Do you like the new layout? Are the contents useful? Would you be interested in contributing your own applications?

Please send your comments by completing the Reader's Response on page 8 or by e-mail to anja.herrmann@tosohbioscience.com

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Train your Brain / Reader's Response



Separation of Hydrophilic Compounds with TSKgel Amide-80 Columns and Detection by ESI-MS.

Reversed phase chromatography (RPC), using octadecyl silica (ODS) supports, has become an indispensable method for the separation and purification of compounds from complex mixtures. However, hydrophilic compounds in mixtures are either weakly retained or eluted in the void volume of a RPC separation, and cannot be analyzed correctly by this method.

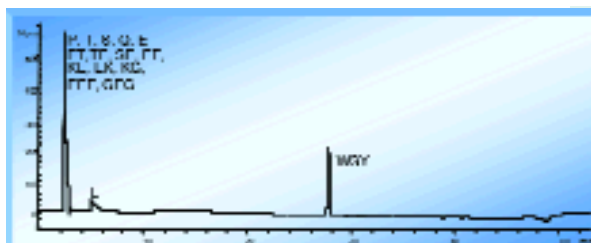
Rapid separation and analysis of hydrophilic compounds can be accomplished by normal phase liquid chromatography (NPLC) or hydrophilic interaction chromatography (HILIC). In NPLC, the elution of molecules occurs by introduction a more polar or aqueous mobile phase. HILIC is a variant of NPLC in which the stationary phase is polar and the mobile phase is mostly organic. Water present in the mobile phase forms a stagnant aqueous layer on the surface of the stationary phase, enabling partition between the mobile phase and this aqueous layer.



TSKgel Amide-80

The TSKgel Amide-80 from Tosoh Bioscience is an excellent choice for separating hydrophilic compounds such as peptides, carbohydrates and polar natural products.

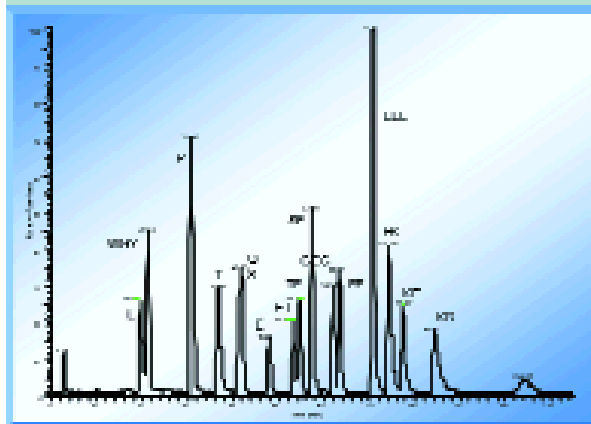
Figure 1 shows the comparison of selectivity between a mix of free amino acids and peptides separated by RPC and HILIC with detection by ESI-MS.



RP-HPLC of a mixture of free amino acids, di- tripeptides

Columns: Grom Sil 120 ODS 4 HE (3 μm; 4 mm ID x 1 cm L + 4 mm ID x 25 cm L)
Eluent A: 10 mmol/L ammonium acetate (pH 6.0) in MilliQ water
Eluent B: 9 mmol/L ammonium acetate (pH 6.0) in 60% methanol
Sample: Mix of free amino acids (P,T,S,Q,E,L) and peptides (ET, TE, SE, EE, KE, EK, KG, EEE, GEG, WGY)
Flow Rate: 0.8 ml/min
Gradient: 0-7 min 0% B
 7-30 min 0-70% B
 30-35 min 70-100% B
 35-43 min 100% B
 43-45 min 100-0% B
Temp. 50°C

Figure 1: Comparison of hydrophilic peptides separated on a RPC column and a TSKgel Amide-80 column (HILIC-ESI-MS) (Ref.: Data reprinted with permission of H. Schlichtherle-Cerny and M. Affolter; Nestlé Research Center Lausanne (2001))



HILIC-ESI-MS base peak chromatogram of free amino acids, di- and tripeptides

Columns: TSKgel Amide-80, 5 μm; 2 x10 cm + 1.5 x 25 cm (ID x L)
Eluent A: 6.5 mM Ammonium acetate (pH 5.5) in 90% ACN
Eluent B: 7.2 mM Ammonium acetate (pH 5.5) in 60% ACN
Sample: Mix of free amino acids (P,T,S,Q,E,L) and peptides (ET, TE, SE, EE, KE, EK, KG, EEE, GEG, WGY)
Flow Rate: 0.1 ml/min
Gradient: 0-90min 10-100% B
 90-95 min 100% B
 95-98min 100-10% B
 98-115min 10% B

On the RP-packing no retention of the free amino acids or of the glutamyl peptides could be observed, only the more hydrophobic leucine and the tripeptide WGY were retained.

However, the separation of free amino acids and hydrophilic glutamyl di- and tripeptides was achieved in 85 min by HILIC on the TSKgel Amide-80 column without derivatization.

Another example is shown in the work of Mark Strege of Eli Lilly and Company (see Figure 2). This illustrates the use of HILIC with detection by ESI-MS for analysis of polar compounds in fermentation broth. The fermentation broth was passed through a reversed phase solid phase extraction prior to being injected on the TSKgel Amide-80 column.

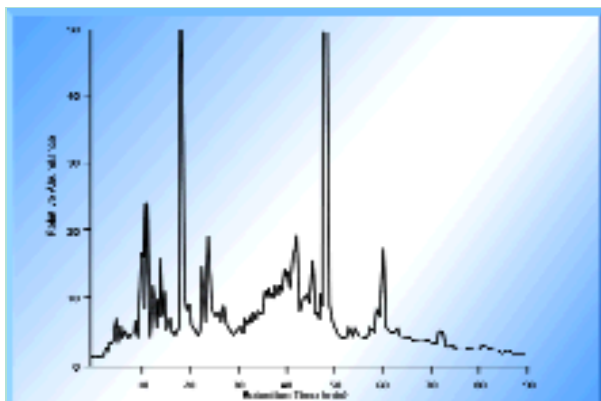


Figure 2.
HILIC-ESI-MS Total Ion Chromatogram of a fermentation broth C18 Solid Phase Extraction effluent

Column: TSKgel Amide-80, 4.6 mm ID x 25 cm L
Mobile Phase: 6.5 mM NH₄Oac, pH 5.5 buffer
90 min., 10-40% aqueous gradient
Detection: ESI-MS

Data reprinted with permission of Mark Strege, Eli Lilly and Company.

Characteristics

The TSKgel Amide-80 column is packed with 5 µm or 10 µm spherical silica particles covalently bonded with carbamoyl groups. The polar functional groups of the sample, such as hydroxy groups, form hydrogen bonds with the polar groups (amino groups) of the stationary phase. The number of hydroxy groups, conformation and solubility of the sample in the mobile phase will determine the order of elution. The retention

mechanism differs from other HILIC or Amide columns, which mainly include RP phases with embedded polar groups. On the other hand the TSKgel Amide-80 packing is superior to aminoalkyl silica gel in chemical stability and will not react with reducing sugars in carbohydrate analysis. Besides detection by ESI-MS, the stability of the TSKgel Amide-80 stationary phase also enables detection by Evaporative Light Scattering (ELS).

www.tosohbioscience.de
www.tosohbioscience.biz
www.tosohbioscience.com
www.tosohbioscience.info

In the recent years, we have significantly improved the [tosohbioscience.de](http://www.tosohbioscience.de) website. Our goal was to make the site both, user friendly and a helpful tool in assisting you, our customer, to find suitable solutions for your **separation needs**.

That is why, we for example significantly extended our **product area**.

The site offers several ways to explore Tosoh Bioscience's product portfolio – depending upon the molecule you want to separate (**Selection Guide**), based on the **Chromatographic Mode** you use, or with the fast **Column/Media search**, where you can select one or more criteria for display of our products. Last not least, we implemented a **search engine** to search the site based on keywords.

If there is any further information on the product, you will find specified buttons, indicating, that we can provide you with more **detailed documentation** (which can also be searched for in the library or our news section).

Should the site not answer your questions, you may place your question directly to one of our technical specialists – either via the contact site or in our **support forum**.

For 2005, we plan to complement the site with even more helpful information! Should you have feedback on the site – either positive or negative – please write to gesa.bartholomay@tosohbioscience.com.

TSKgel Amide-80 column: typical properties

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Pore Size (Å)	Min. number theoretical plates	Flow rate (ml/min)		Max. pressure drop (kg/cm ²)
							Range	Max.	
20009	Amide-80	1.0	5	5	80	n/a	0.15 - 0.18	0.22	250
19697	Amide-80	2.0	25	5	80	n/a	0.15 - 0.18	0.22	250
13071	Amide-80	4.6	25	5	80	8,000	0.5 - 1.0	1.2	150
14459	Amide-80	7.8	30	10	80	5,000	1.0 - 2.0	3.0	70
14460	Amide-80	21.5	30	10	80	6,000	2.0 - 4.0	8.0	30

News from our Customer Support Group and Technical Team



*Kim Scheveneels
Customer
Service/Support*

Since September 2004 there have been some changes in our Customer Service Organisation at Tosoh Bioscience.

You now have a single contact for all questions relating to product pricing, availability and delivery times. Kim Scheveneels is based at our central Logistic Platform in Tessenderlo, Belgium. Kim has handled Supply Chain Support for our Separations business for the past year and this non-technical function has now been formalised. Despite the change in location, you will still be able to reach Kim on your existing Customer Support numbers:

Customer Support Contact

(Supply chain)
Telephone +49 (0)711 13257-21
Direct Fax Line
+49 (0)711 13257-99

Christian Herkenrath, who was previously in charge of Customer Support, has moved to the new position of Quality Assurance Manager. His role will be to develop our Quality Assurance systems and maintain these on the basis of continuous improvement.



*Volker
Nödinger
Application
Specialist*

The Technical Support Team remains based at our Stuttgart office. Your well known Technical Specialists Achim Sprauer (Process Business) and Werner Conze (Laboratory Products) as well as our new Application Specialist

Volker Nödinger will be happy to assist you in all questions related to technical issues on our products or your applications.

Technical Support Contact

Telephone Hotline
+49 (0)711 13257-50, -51, -52
Fax Hotline
+49 (0)711 13257-48

Christine Wilde has moved to our Marketing group as Product Manager. She is responsible for product and marketing strategies, including promotional activities and support literature, for TSK-GEL® and TOYOPEARL® Products.

She still likes to have strong contact to our customers in workshops and conferences or at customer visits together with our Sales Team.

New Distributor in the Czech Republic

We are proud to announce that BioTech a.s. Praha/Bratislava is helping us to support our customers in the Czech Republic and Slovak Republic as our second distributor.

Eva Jedlickova (left) and Barbara Mrázová followed the intense product training in our Stuttgart office with serious interest.

You can reach our distributor in Prague under the following address:

BioTech a.s. Praha
Sluzeb 4
10852 Prague
Phone: +42 (0)800 124683
Fax: +42 (0)272 701739
www.biotech.cz



*Eva Jedlickova and
Barbara Mrázová*

*The beautiful
city of Prague*

F A Q

Question:

I was very pleased with the performance of my TSKgel SW_{XL} column. Now, with my new TSKgel SuperSW column I cannot get the enhanced separation performance as described in the Column Inspection Data Sheet.

Answer:

The characteristic of the TSKgel SuperSW column is its reduced inner column diameter and the smaller particle size of the packing material. To utilize the enhanced sensitivity and performance, it is necessary to optimize your HPLC system.

➔ Tubing

The conventional 0.1 mm tubing may be used, but length should be kept as short as possible. Void volume between the column and detector cell should be less than 20 µl.

➔ Pump

A pump capable of accurately delivering a flow rate between 0.05 ml/min and 0.35 ml/min is recommended

➔ Sample Volume

Sample volume should be 5 µl or less.

➔ Detector

For best results, use a flow cell with a maximum of 2 µl. A flow cell up to 10 µl can be used with only minor losses in plate counts.

Pore Size Effect of Resins on the Capture Ability of Proteins

New HIC resin Toyopearl PPG-600M

Besides Cation Exchange (CIEX), Hydrophobic Interaction Chromatography (HIC) has been successfully applied to the purification of biological samples such as peptides, mAbs and proteins. For initial capture steps in particular, HIC can be employed successfully because load material contains a high salt concentration and can be therefore directly applied on a HIC resin. Furthermore this mode has an additional benefit in separating aggregates when compared to the more commonly applied affinity chromatography.

Adsorption capacity of protein samples on CIEX and HIC resin depends on pore size, particle size and functionality. Until recently large pores were considered to be optimal for large molecules. But it is possible to maximize the binding capacity for each individual protein by optimizing the pore size, pore geometry, pore type and surface chemistry of a resin to the individual protein.

This approach was applied to develop a new grade of the Toyopearl HIC resin. The new **Toyopearl PPG-600M** resin (functional group: Polypropyleneglycol) is optimized for capture and intermediate stages in the pharmaceutical manufacturing process of monoclonal antibodies.

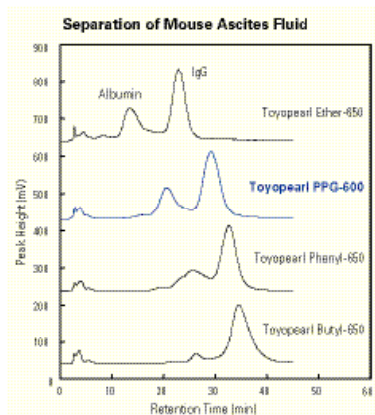


Figure 1: Chromatographic profiles of proteins on different pore resins

Separation Conditions of Toyopearl HIC Media

Column Size: 7.5 mm ID x 7.5 cm L

Eluent: (A) 0.1 M phosphate buffer containing 1.8 M ammonium sulfate (pH 7.0)
(B) 0.1 M phosphate buffer (pH 7.0)

Gradient: 0% B - 100% B in 30 min.

Flow Rate: 1 mL/min

Injection Vol.: 100 µL

Dynamic binding capacity: 38 mg/mL Mouse IgG

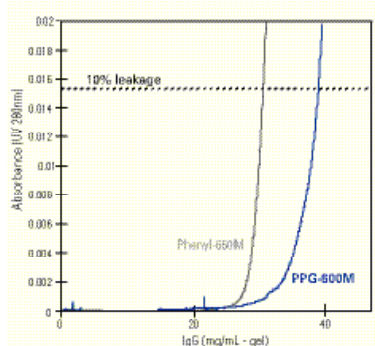


Figure 2: Breakthrough curve for monoclonal antibody

Conditions

Sample: rec. h-Mab 01

Column size: 7.8 mm x ID 20 cm L

Feed: 1 mg/mL Humanized monoclonal antibody in 31 mM Citrate, 69 mM Phosphate Buffer + 1.0 M Ammonium Sulfate (pH 6.6)

Linear velocity: 300 cm/h

Detection: UV (280 nm)

Binding Capacity (mg/mL-gel)	Recovery [%] (10% Leakage)
Phenyl-650M	34.4
Butyl-650M	42.1
PPG-600M	44.8

Toyopearl ToyoScreen Columns – for fast identification of the optimal resin.



Tosoh Bioscience recently introduced a new series of simple cartridge type pre-packed ToyoScreen columns. The columns are suitable for fast and cost effective screening of TOYOPEARL process resins in the first development step of a protein purification protocol. For closer information please click www.tosohbioscience.de

Figure 1 shows the influence of pore size on the separation, whereas the effect of optimized ligand density and functionality upon binding capacity is shown in figure 2.

Practical,
Institute of
Biochemistry,
University of
Stuttgart



Bioprocess Chromatography Workshop – March 2005

Tosoh Bioscience's 2005 International Workshop on Bioprocess Chromatography will take place in Stuttgart/Germany between March 8 and 10, 2005.

The course has been run for the last seven years and is highly recommended by previous attendees, around whose comments improvements have been made over the years.

The program
A complete day is reserved for practical hands-on use of process columns for resin packing studies and troubleshooting guidance, together with practical use of methods development systems. This is supported with presentations covering essential aspects of practical process design and operation. Modern resin design is a recently introduced section which offers an insight into how chromatography is developing in line with the demands of modern bioproduct manufacturing.

Who should attend?
The course is suited to project leaders and qualified technical process personnel, including those involved with process methods development at both practical and managerial level.

Venue
The theoretical part of the course will take place in the Millennium Hotel and Resort, Stuttgart/Germany.
The practical part will be in the Institute for Technical Biochemistry, University of Stuttgart.

Course fee
The workshop fee of EUR 995,- plus 16 % V.A.T. (incl. V.A.T. EUR 1154,20) includes full workshop documentation, all coffee breaks, three lunches, one dinner and a small excursion at the end of the workshop.

Contacts
If you are interested in participating at the course please feel free to contact one of the following persons:

Mr. John Creedy
Phone: +44 (0)1223 890098
E-mail: john.creedy@tosohbioscience.com

Mr. Denis Dehaene
Phone: +33 (0)3 2125 20 07
E-mail: denis.dehaene@tosohbioscience.com

Dr. Erich Fiedler
Phone: +49 (0)7641 915050
E-mail: erich.fiedler@tosohbioscience.com

Dr. Elke Prohaska
Phone: +49 (0)8158 997515
E-mail: elke.prohaska@tosohbioscience.com

...or visit us on
www.tosohbioscience.de.



Theory,
Millenium Hotel
and Resort,
Stuttgart

Tradeshows and Conferences

Events 2005 where Tosoh Bioscience GmbH is involved in

month	date	event	place	website
February	31.01.-03.02.	Arablab	Dubai, UAE	www.arablab.com
	22.02.-24.02.	Chromatography in Process Development and Production D1	Stuttgart, D	www.tosohbioscience.de
March	01.03.-03.03.	Chromatography in Process Development and Production D2	Stuttgart, D	www.tosohbioscience.de
	08.03.-10.03.	Chromatography in Process Development and Production INT	Stuttgart, D	www.tosohbioscience.de
	14.03.-18.03.	Biotech World 2005	Moscow, RU	www.mosbiotechworld.ru
	15.03.-17.03.	Chromatography in Process Development and Production DF	Stuttgart, D	www.tosohbioscience.de
April	11.04.-14.04.	European BioProcess International	Berlin, D	www.ibt-lifesci.com/bpi
May	24.05.-27.05.	ILMAC / R+D in Life Sciences	Basel, CH	www.ilmac.ch
June	26.06.-30.06.	29th HPLC	Stockholm, S	www.hplc2005.com
October	18.10.-20.10.	Biotechnica	Hannover, D	www.biotechnica.de
November	14.11.-15.11.	Novia Analytikertage	Mannheim, D	www.novia.de

Impressions 2004



Analytica 2004



Analytica 2004



SPICA / ISPPP 2004



Analytica 2004

Left to right:
Volker Nödinger, Dr. Elke Prohaska,
Anja Herrmann, Gerhard Kratz



Pittcon 2004

Tosoh Bioscience GmbH, member of the Tosoh Group, and our sister company Tosoh Bioscience LLC in the USA, are represented at all important exhibitions and congresses. With a worldwide network of distributors we are able to fully support our global operating customers. On a regular basis we train our customers and distributors during seminars and workshops, to support and to help them in the most effective way.



ArabLab 2004

TRAIN YOUR BRAIN

Take part in our crossword, fill in the questionnaire below and you will receive a little present.

- What is important between two peaks?
- What do we need to get Peaks?
- What is prepared before packing a column?
- What do we need to "see" the separation?
- Different to hydrophilic!
- What should always be kept smooth?
- How is peak distortion usually called?
- What should the column have before injection?
- How do we call the tubing in HPLC?

READER'S RESPONSE

Please send your response to the following fax number: +49 (0)711 13257-89

Impressum
TOSOLUTION

Editor:
Tosoh Bioscience GmbH
Zettachring 6
70567 Stuttgart
Germany
Phone: +49 (0)711 13257-0
Fax: +49 (0)711 13257-89
E-Mail: info.sep.eu@tosohbioscience.com
www.tosohbioscience.de

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Bruno Grafic-Design
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Germany

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Schelztorstraße 50
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Germany

<u>Title</u>	<u>First Name</u>	<u>Last Name</u>
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Please remove my name from the mailing list.

I like the layout of TOSOLUTION.

Yes No

I think the content is useful to my work.

Yes No

I can provide Tosoh Bioscience with applications.

Yes No

Please send me the following literature pieces:

- Laboratory Products Catalogue
- Process Products Catalogue
- TSKgel Amide-80 Literature Kit
- Toyopearl PPG Flyer

Additional comments:

Solution of the puzzle