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The Column

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Time to Hang Up the Lab Coat?

How to climb the chromatography career ladder

ADVANSTAR

Cover Story

Features

Regulars

Slipping Up the Career Ladder

Incognito investigates if chromatographers should become scientists or managers to advance their careers.

Photo Credit: Steve Taylor/Getty Images

"I'm so excited. I have just been accepted onto the 'Technical Track' — which means I can progress within the organization but remain very much hands on in the lab."

"So I told him — forget analytical chemistry, it's finished, no money in it at all. Even 'biochemistry and biopharma', which is supposed to be the emerging discipline, won't give him a good standard of living compared to a managerial job."

These are two statements that I have heard within the last month, both made in good faith. Einstein is attributed to have said: "Everybody's a genius. But if you judge a fish by its ability to climb a tree, it'll live its life believing it's stupid."

I guess that's why organizations have developed a dual (or multi-ladder) approach to career progression, to match company needs, both managerial (executive) and professional (technical), to the aspirations and strengths of the individual. Not everyone is good at people management or administrative duties, sales, and marketing. Some people are mainly fine scientists — so why should a business want to lose them to "executive" functions?

When researching this piece, a two minute internet search revealed the use of the term (or similar) *"have introduced a scientific ladder system"* in articles or blogs from the last two decades. It appears that we have been trying to get this right for a long time, but I firmly believe we have not yet succeeded.

I saw a job advert the other day which included the statement: "*Company X has a parallel career ladder structure so that a scientist can progress without any significant managerial duties……"* I don't believe it. I don't believe that in five years time the successful "manager" and successful "scientist" will be rising through the organization at the same rate and be paid the same. I think that the functions that generate the near-term worth of a company are viewed as being very important — these tend to be manufacturing, marketing, and sales functions. With perhaps the caveat that the pharmaceutical industry has, of late, come under increasing "pipeline net worth" scrutiny.

Think of the rungs on each ladder. How does one ensure each progression has a similar business impact? How can one

account for the possible differences in the cycle time for demonstrable success? A big sale or successful marketing campaign can be complete and measurable in months, while a R&D cycle may take many years. How many people do you know that could set scientific milestones for progression up the technical ladder which represent a significant impact on the business? I'm not saying it can't or shouldn't be done — I'm simply saying that in my experience the technical ladder becomes a recognition of time served, rather than contribution made. Of course, this is when that particular ladder develops a glass ceiling in terms of pay and recognition, because it's functionally meaningless to the bottom line of the business.

However, there are those with great clarity of purpose, for whom the choice between the two ladders is often straightforward — they absolutely know that their love of science will lead to them staying in the laboratory. Yet, the majority of folks that I know drift from technical to managerial or other business roles, and I've seen the following reasons behind the move (sorry — drift):

- Realizing that you aren't as smart at analytical chemistry as other people within your department and realizing the amount of study you would need to do to get there (if at all!).
- Realizing you could organize the work in your department and run things a lot better while juggling many tasks.
- Getting a girlfriend/wife/children/house/elderly parents — the amount of technical papers read outside working hours or academic conferences attended directly correlates to these factors.
- Realizing that your boss's boss's boss is paid less than the next grade up from you in marketing.
- Turning 30 years of age.

Of course, staff on the technical ladder CAN make a huge business impact. They may not be making injections into chromatographic equipment, but they still need to understand the capabilities of equipment, the analytical approach required and infer from the data produced in order to make critical business decisions on product development, quality, process efficiency, and problem-solving. A leading scientist from an analytical chemistry department with a blue chip organization once commented: "If only there was recognition for managing knowledge and IP [intellectual property], I think we would

be a lot better off." There will be people who read this piece and think "we have that here", but do you, really?

Let's look at a typical R&D Operations Manager or Chief Technical Officer (CTO). I know many of these people and I can tell you that the vast majority of their time is spent with spreadsheets and pivot tables, training requirements, annual reviews, budgets, staff allocations and rostas, productivity measurements, health and safety recording, audits, audits, and more audits. It's all vital, but it's not science. It broadens the views of individuals about corporate governance, it integrates the business, it helps to aid compliance and a safe working environment, it ensures that profitability is as it should be, but it's not science. When was the last time your CTO made an injection into a chromatograph or ran a nuclear magnetic resonance (NMR) instrument, or even set foot inside the laboratory?

The best instance I've seen where the "scientist" still gets to do proper science is when they become the CTO, recognize they do not want to "do management" and so build a management structure around themselves to take away the business administration These examples are few and far between, and if this solid structure is not already in place within your global organization, you have to pretty much own the organization to make this a reality — go ask Mark Zuckerberg.

Robert Kiyosaki, author of *Rich Dad, Poor Dad,* said: "The bigger the problem you solve, the more money people will pay you." I think we (the scientists) solve big problems for our organizations every single day — I also believe that our businesses have never figured out a way to reward this properly.

I also firmly believe that money is of secondary importance to most of our profession, and that's why the top of the tree in any "Pyramid of Motivating Factors" is always job challenge/work-life balance/career opportunities. If only I could find someone smart enough to design a ladder with rungs that reflect this!

All of this reminds me of a famous (but unattributed) tweet: "Square box. Round pizza. Triangle slices. I'm confused". It makes me ponder which aspect was designed by the food technologist, which by the marketer, and which by the logistics expert!

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News

Eberhard Gerstel Prize for Outstanding Work in Separation Sciences

The Working Group Separation Sciences of the Analytical Division of the German Chemical Society are now inviting applications for the Eberhard Gerstel Prize. The prize will be awarded to a scientist for an outstanding publication in the field of separation sciences. Gerstel sponsors the prize in honour of Eberhard Gerstel Sr., founder of the company. The Eberhard Gerstel Prize will be awarded for the third time during the Analytica 2014 Conference in Munich, Germany, in April 2014.

President of the company Eberhard G. Gerstel told *The Column*: "The world around us needs ever more chemical analysis in the fields of food safety, environmental monitoring, and production quality control. Chromatography is a key element and it is in everyone's best interest to find better technologies and materials for chromatography." He added: "The Eberhard Gerstel Prize is one way for us to motivate scientists and to promote research and development in separation techniques."

For more details about how to apply please visit **bit.ly/1fHH9l6**

Peak Scientific Receives A Royal Visit

Peak Scientific has recently opened an extension of their head office in Inchinnan, Scotland, named Cherry Tree House. The extension will accommodate the expansion of the company since it launched in 1997. The Princess Royal attended the event to officially open the new building. She toured the site and met with many of the workforce before the opening ceremony.

Robin MacGeachy, Managing Director, said: "This facility will allow us to double our capacity in manufacturing and personnel over the coming years. The new building also accommodates increased facilities for training, research and development, and service support." For more information visit **uk.peakscientific.com**

FDA and NIH Create First-of-Kind Tobacco Centers of Regulatory Science

The United States Food and Drug Administration (FDA) and the National Institutes of Health (NIH), as part of an ongoing interagency partnership, have awarded a total of up to $53 million to fund tobacco-related research in fiscal year 2013 to create 14 Tobacco Centers of Regulatory Science (TCORS). A new, first-of-its-kind regulatory science tobacco program, TCORS is designed to generate research to inform the regulation of tobacco products to protect public health. Using designated funds from the FDA, TCORS will be coordinated by the NIH's Office of Disease Prevention, directed by David M. Murray, PhD, and administered by three NIH institutes – the National Cancer Institute, the National Institute on Drug Abuse, and the National Heart, Lung, and Blood Institute.

The TCORS programme is the centerpiece of the FDA-NIH collaboration to foster research relevant to tobacco regulatory science. New research from TCORS will help inform and assess the impact of the FDA's prior, ongoing, and potential future tobacco regulatory activities implemented by the Center for Tobacco Products under the direction of Mitch Zeller, J.D. In addition, the TCORS investigators will have the flexibility and capacity to begin new research to address issues raised in today's rapidly evolving tobacco marketplace.The TCORS programme brings together investigators from across the country to aid in the development and evaluation of tobacco product regulations. Each TCORS center has identified a targeted research goal. Together, the TCORS sites aim to increase knowledge across the full spectrum of basic and applied research on tobacco and addiction. TCORS proposals were selected for funding based on their scientific and technical merit as determined by NIH scientific peer review, availability of funds, and relevance of the proposed projects to programme priorities.

Among the 14 TCORS that have been awarded are Pamela I. Clark, PhD, University of Maryland, College Park, whose various projects include exploring tobacco microbial constituents and the oral microbiome of tobacco users; Thomas E. Eissenberg. PhD, and Robert Balster, PhD, Virginia Commonwealth University, Richmond, whose projects include analytical lab methods for MRTP evaluation; Stanton A. Glantz, PhD, University of California-San Francisco, overseeing projects that include quantification and biomarkers of short-term pulmonary effects of tobacco smoke exposure and infection-related acute lung injury; and Robert Tarran, PhD, University of North Carolina at Chapel Hill, School of Medicine, whose projects include translational studies to identify epithelial biomarkers of smoke exposure. — C.D.

Photo Credit: Stella/Getty Images

Silicone Protheses for Biomonitoring POPs in Humans

Discarded silicone explants, a waste material from the cosmetic surgery industry, could be a new source of sample material for studying POP accumulation in humans.

Scientists from the Norsk Instituutt for Vannforsking (Norwegian Institute for Water Research), Oslo, Norway, have proposed the analysis of silicone explants for the monitoring of persistent organic pollutants (POPs) in humans.[1] The team suggest the formation of repositories for discarded silicone implants from the cosmetic surgery industry.

So, where did this idea come from? Lead author Ian J. Allan told *The Column* that his team has been developing passive sampling methods using silicone to measure environmental POPs for over 10 years. In a recent trial study, the team used a silicone tag in a living fish to measure the contaminants in the river.[2] Analysis of the tag showed that levels of POPs were comparable to the levels in the river water, therefore providing proof of concept.

Allan said: "Directly comparing concentrations in biota (in units of ng per gram lipids) with concentrations in abiotic compartments, for example in water, (pg/ng per litre) is difficult. The use of the same polymer for sampling different environmental compartments allows the comparison of the activity or fugacity of chemicals in these compartments more simply."

Currently, human biomonitoring of POPs is generally performed using blood, milk, and adipose tissues — the World Health Organization (WHO) and Stockholm Convention are currently working together to survey global human exposure. These samples are difficult and expensive to obtain, whereas explanted silicone from humans is readily available. The paper quotes that an estimated 5–10 million women worldwide are implanted with silicone breast protheses, with 300,000 breast augmentations performed in the USA in 2010 alone. As implants have a life-span of 10–15 years, they have to be explanted and are then disposed of.

Allan said: "*In vitro* tissue exposure with passive sampling devices and *in vivo* methods in clinical settings (for example, with solid-phase microextraction fibres) have received increased attention in recent years and following discussions with my colleagues we realized that it may be possible to use explanted silicone prostheses as passive sampling devices for human biomonitoring."

With the co-operation and help of a plastic surgeon based in Oslo, Norway, 33 explanted protheses were collected from 22 female clinics undergoing surgery at a clinic in Norway. Acetone was used to extract whole samples — according to Allan, acetone was selected because of the limited effect it has on the swelling of the silicone during extraction compared to nonpolar pentane or hexane. Gas chromatography coupled to mass spectrometry (GC–MS) was subsequently performed to detect organochlorides, polychlorinated biphenyls, and polybrominated diphenyl ethers.

The samples required concentrated sulphuric acid clean-up before GC–MS analysis for acid-resistant chlorinated and brominated substances, limiting the number of compounds that could be detected and investigated. Allan told *The Column* that the team are now currently working on sample clean-up methods that eliminate this step, and therefore increase the number of chemicals that can be sampled and quantified. The advantage of the method is that samples can be taken from different people but still be directly compared. Allan told *The Column*: "Ultimately we would like to combine this sampling with non-targeted screening analytical methods."

The team will be working on developing the study to determine the application of the method to a wider range of silicone samples produced by different manufacturers, in addition to optimizing the method to reduce the level of solvents used and to increase the number of contaminants detectable. — B.D.

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Peaks of the Week

eTopics heating up on social media

We have a rich range of informative and practival content across our print and digital portfolio. Here are some of the highlights that have been posted onFacebook, Twitter, and LinkedIn:

- **The LCGC Blog: What to Do if Zombies Infiltrate the Analytical Laboratory: A Brief Survival Guide:** "Fabulous article! Definitely something to share with my labmates!" "Love it!" — @LC_GC Twitter Follower.
- **The Role of the Signal-to-Noise Ration in Precision and Accuracy, LC Troubleshooting 2005:** Selected from the archives as part of "Throwback Thursday" on Twitter. bit.ly/1hF8pPm
- **Making Successful Connections with Capillary Tubing in LC–MS Applications: "**I agree wholeheartedly! Many people do not pay proper attention to use of the best tubing dimensions for their application and as importantly, making proper connections." — LinkedIn member. bit.ly/17C66aa

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Online Highlights

LCGC is pleased to present a new series of educational e-books covering the latest innovations in chromatography. A format for everyone – These new e-books are available for the iPad or for a standard computer web browser.

- **Five Keys to Successful LC Methods**
- **Innovations in Environmental Analysis**
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Available to download here: bit.ly/18gRjWH

LCGC E-Books

Editors' Series: Optimising Your Extraction by Removing Uncertainty
The analysis of biological fluids has been aided by the development of LC–MS. This presentation focused on the use of solid phase extraction and the benefits that using a high quality approach to method development can have on data integrity. Available to watch here: bit.ly/17O2nfq

Interview

News In Brief

US Senate Avert Impending Helium Crisis

The US Senate has passed a bill halting the planned shutdown on 7 October of the Federal programme for crude helium sale, storage, and delivery. At present, the programme supplies 40% of helium demand in the USA and 30% worldwide. The new legislation extends the operation of the reserve for a further six years, allowing alternative sources to be made available, with full closure planned in 10 years.
bit.ly/185d5vv

Statistical Significance of MS Similarities

Scientists in Japan have developed a method to assess statistical significance of MS similarities using a modified basic local alignment search tool (BLAST) for application in metabolomics studies. **DOI: 10.1021/ac401564v**

Malaria Mouse Model Metabolites

A paper published in the journal *Scientific Reports* has described a novel metabolic profiling framework for the discovery of candidate diagnostic markers of malaria in mouse models using a combination of statistical spectroscopy, multinuclear NMR, and iterative LC–MS.
DOI: 10.1038/srep02769

PHARMACEUTICAL/DRUG DISCOVERY

Sophisticated Antibody Analysis by GPC/SEC with RALS

Monoclonal antibodies (mABs) are increasingly growing in importance for the diagnosis and therapy of various diseases, including cancer and autoimmune and inflammatory disorders. One essential parameter to define their quality is the content of aggregates (dimers, trimers, and higher aggregates). These aggregates can be formed during processing and purification or are the result of long-term storage. As a result of aggregation, antibodies lose their pharmaceutical efficacy and can facilitate an immunology response.

Antibody fragments which lack the Fc region can be used for the treatment of diseases. They can also be the result of degradation of full length antibodies. Therefore, a GPC method, which offers the opportunity to analyse antibodies and their aggregates, as well as antibody fragments simultaneously, with superior resolution and high sensitivity is invaluable.

Experimental

GPC/SEC analysis was performed on a PSS SECcurity GPC system, equipped with a PSS SECcurity SLD1000 light scattering detector, using the following conditions:

Columns:	PSS PROTEEMA, 5 µm, 2 × 300 Å (8 × 300 mm each) + precolumn
Solvent:	100 mM sodium phosphate pH 6.7 + 0.25 M NaCl
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	Refractive index (RI), ultraviolet (UV) at λ = 214 nm, PSS SLD1000 (right-angle light scattering [RALS]) at λ = 488 nm
Calibration:	Light scattering
Injected mass:	60–80 µg
Data acquisition, calibration, and evaluation:	PSS WinGPC UniChrom 8.1

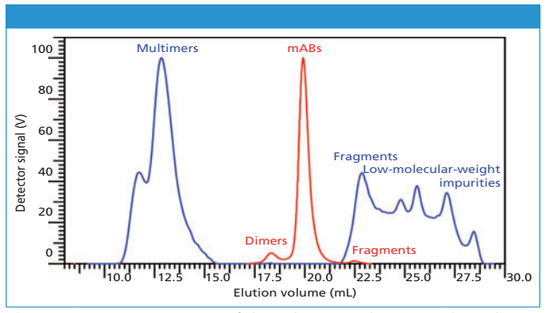

Figure 1: Separation range of the column combination. The red curve shows the UV signal of a full length antibody and its dimers plotted against the elution volume. The blue curve is the elugram of antibody fragments and their high level aggregates.

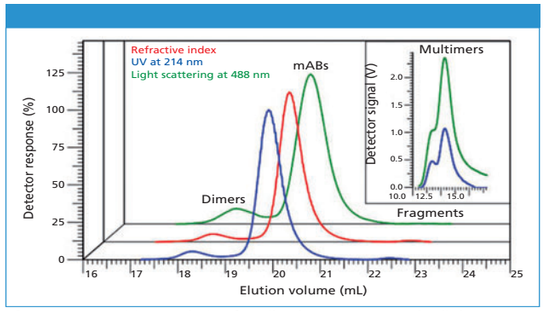

Figure 2: Sensitive analysis of antibody aggregates. The light scattering signal for the dimer is relatively high compared to that of the mABs because of molar mass dependency and provides improved sensitivity for the detection of high aggregates (inset).

Results

Figure 1 shows an overlay of elugrams obtained for a full length antibody and antibody fragments analysed on a single set of columns.

All three detector signals for the analysis of a monoclonal antibody are shown in Figure 2. The light scattering signal shows improved sensitivity for high aggregates compared to the other signals.

Conclusion

The GPC/SEC method including UV, RI, and RALS can be used for the simultaneous determination of aggregate content of monoclonal antibodies as well as antibody fragments. The column combination covers the separation range for all three types and provides a high resolution for the determination of the dimer content. Because of its molecular weight dependency, the PSS SLD1000 RALS detector offers high sensitivity for very small quantities of high aggregates and also allows the determination of the absolute molecular weight of the antibodies. In addition, it has a unique feature for a light scattering detector as the wavelength can be altered to increase the sensitivity.

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Characterization of Styrene-Butadiene Rubbers by SEC–MALS and AF4–MALS

Stepan Podzimek, SYNPO, Pardubice, Czech Republic, and University of Pardubice, Institute of Chemistry and Technology of Macromolecular Materials, Pardubice, Czech Republic.

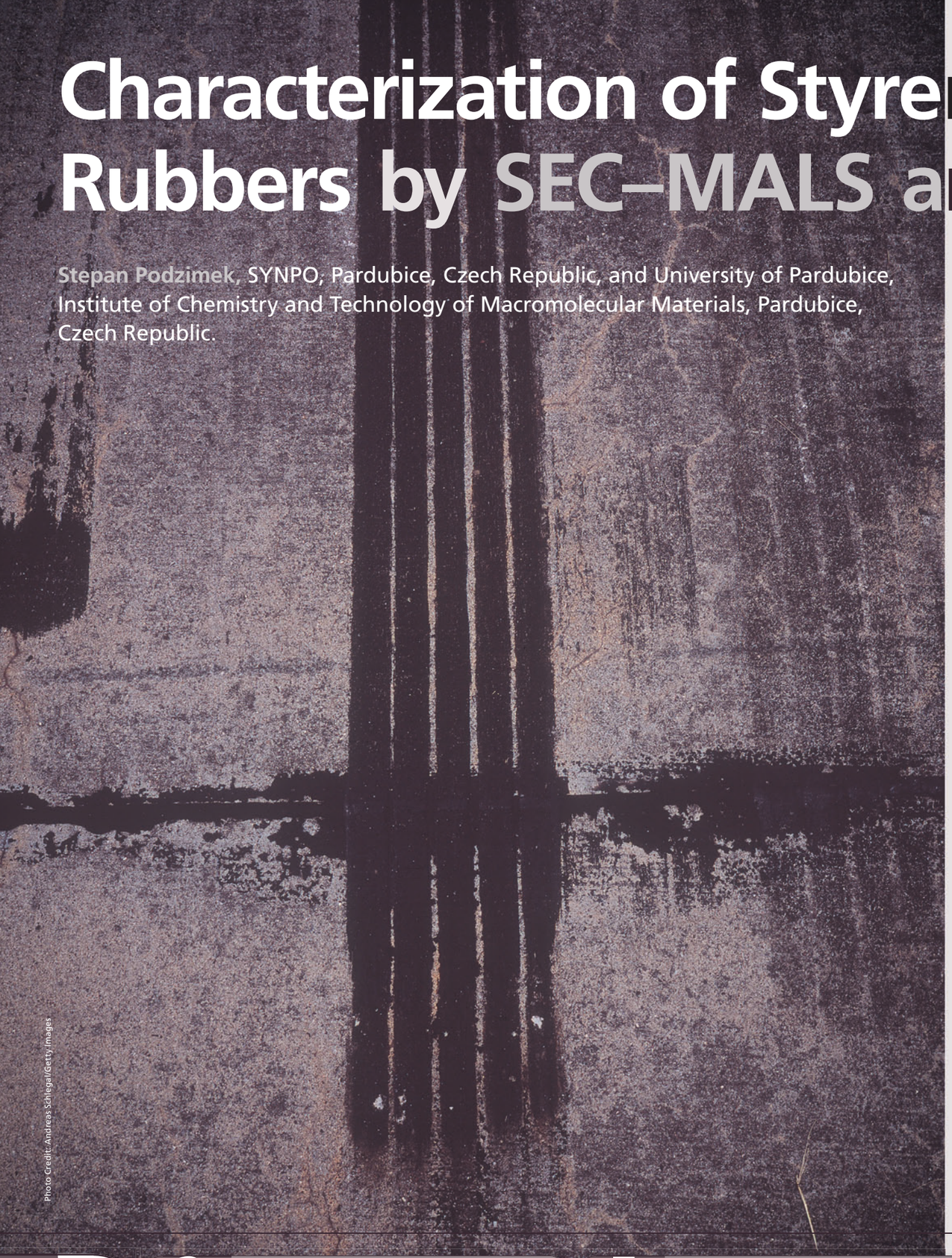


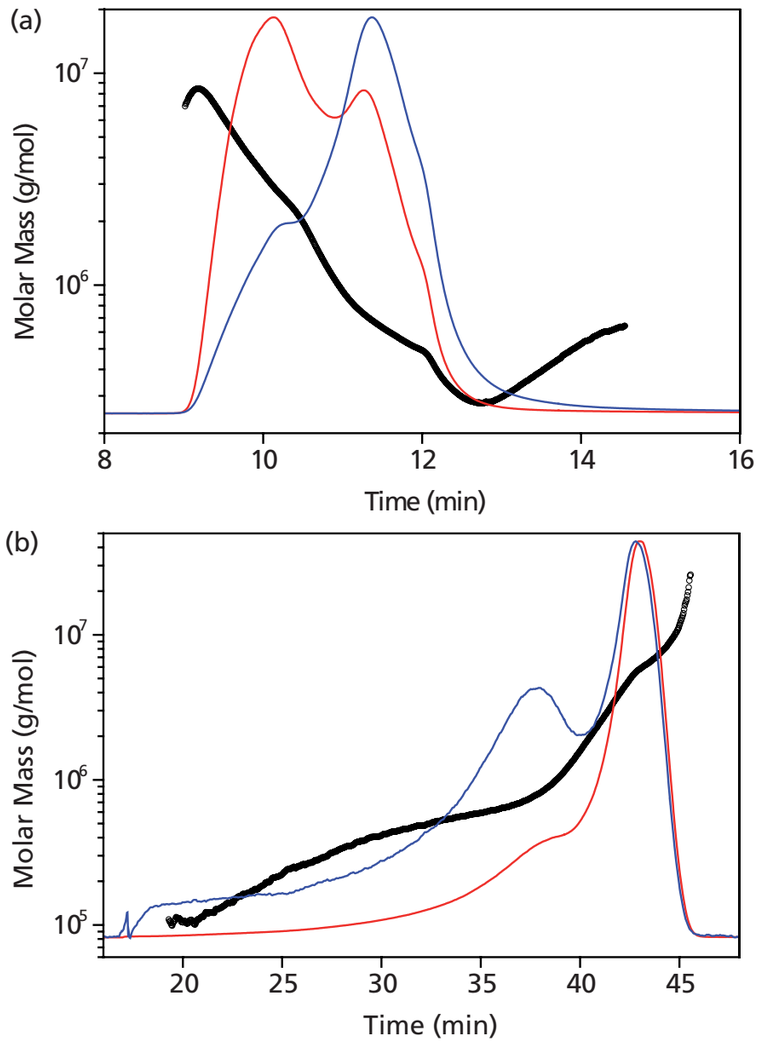
Photo Credit: Andreas Schlegel/Getty Images

Two samples of styrene-butadiene rubbers (SBR) were analyzed by size-exclusion chromatography (SEC) and asymmetric flow field-flow fractionation (AF4) coupled with a multi-angle light scattering (MALS) detector. The results were compared from the viewpoint of the molar mass distribution and the separation performance of SEC and AF4.

Styrene-butadiene rubbers (SBR) represent an important group of synthetic elastomers that are used in a variety of applications, generally as an abrasion-resistant replacement for natural rubber. The viscoelastic and mechanical properties of this material are affected by the molar mass distribution and by the topology of macromolecular chains. Traditionally, the molar mass distribution is characterized by conventional size-exclusion chromatography (SEC) with column calibration based on polystyrene standards. Polystyrene calibration results in incorrect molar mass distribution because of different hydrodynamic volumes of polystyrene and the polymer under analysis. Although various procedures for transforming the polystyrene calibration to the calibration valid for the polymers undergoing analysis have been developed,[1–3] some of them specifically for SBR rubbers,[4] the most effective way of solving the calibration problem is using a multi-angle light scattering (MALS) detector with SEC. The theory of light scattering and MALS detection has been described in detail in several papers and books.[5–8]

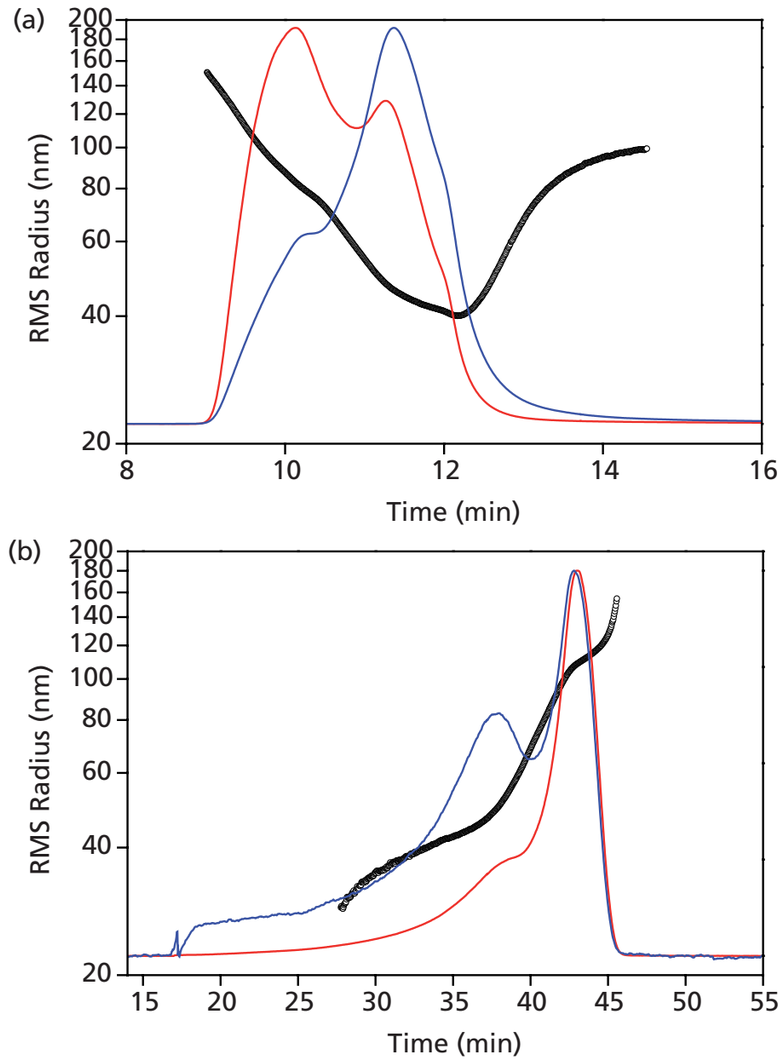
Although a MALS detector converts a relative and calibration dependent SEC method into an absolute method of molar mass determination, there are still several potential issues when polymers of very high molar mass are characterized by SEC–MALS. These include possible shearing degradation and incomplete separation as a result of various non-SEC separation mechanisms.[9] Branched macromolecules in particular show non-SEC separation behaviour that may strongly affect the results obtained by SEC.[10] Asymmetric flow field-flow fractionation (AF4) represents a powerful alternative to traditional SEC, with several advantages compared to SEC.[5]

Figure 1: Molar mass versus retention time plots from (a) SEC–MALS and (b) AF4–MALS analysis of styrene-butadiene (SBR). Signals from MALS at 90° (red) and RI (blue) detectors are overlaid here.



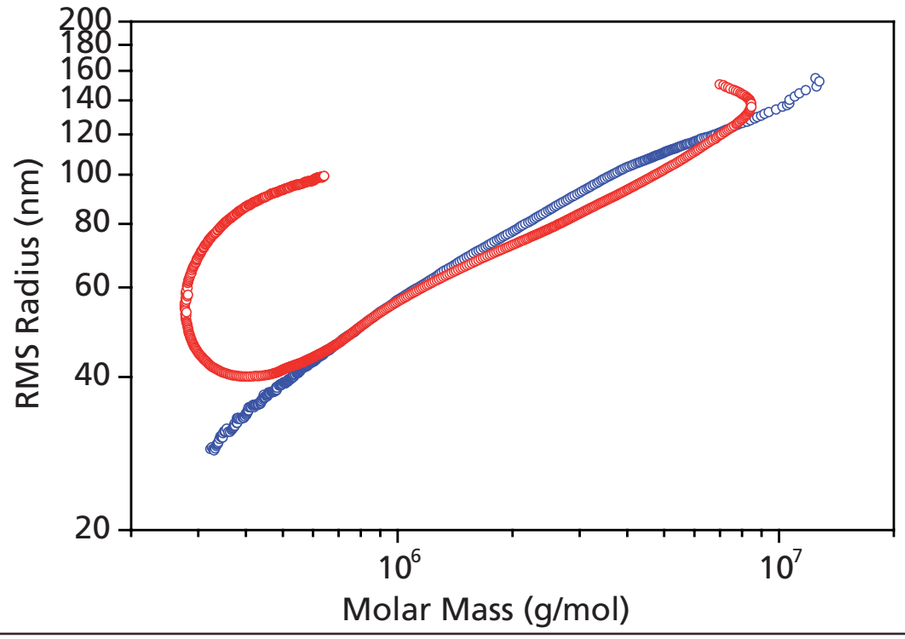
These include the possibility to separate molecules with ultra-high molar mass with a significantly reduced possibility of shearing degradation, elimination of entlapic interactions with SEC column packing, and elimination of specific elution behaviour of branched macromolecules in SEC.

Figure 2: RMS radius versus retention time plots from (a) SEC–MALS and (b) AF4–MALS analysis of SBR. Signals from MALS at 90° (red) and RI (blue) detectors are overlaid here.



SBR are a good example of polymers that can benefit from AF4 separation as they typically contain high molar mass fractions with a molar mass over 10^6 g/mol and branched macromolecules. Since the introduction of AF4 by K.G. Wahlund,[11] the AF4 method has undergone a substantial development

Figure 3: Conformation plots of SBR acquired by SEC–MALS (red) and AF4–MALS (blue).



that has established it as a reliable analytical technique suitable for routine applications.

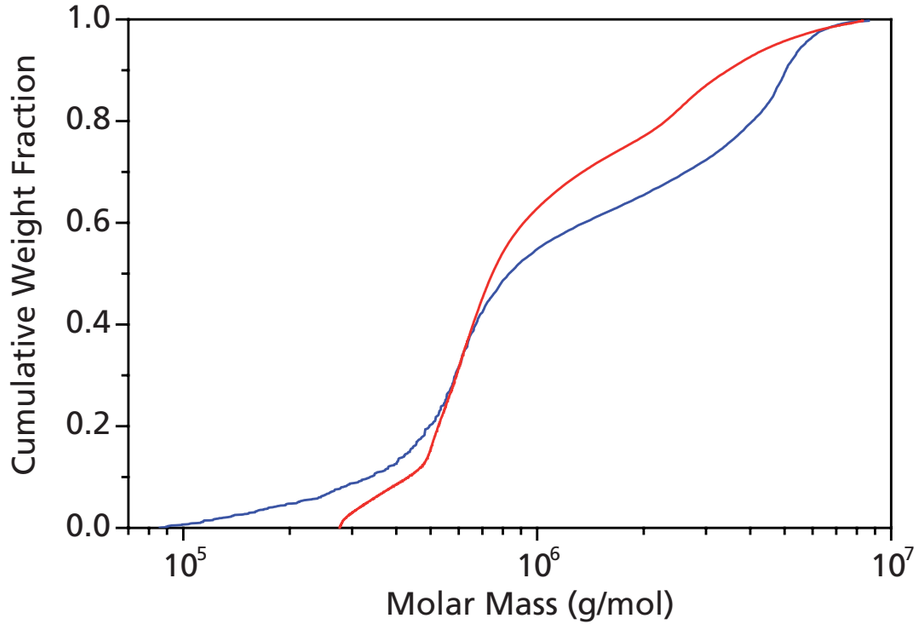
Experimental

SEC and AF4 set-ups consisted of an Agilent 1100 Series HPLC pump and a Waters 717 autosampler. The SEC separation was achieved using two 300 mm × 7.5 mm, PLgel Mixed-C columns (Agilent). The solvent was tetrahydrofuran (THF) at a flow rate of 1 mL/min (SEC) or detector flow rate of 1.8 mL/min (AF4). An AF4 system Eclipse 3+ (Wyatt Technology Europe) was used for AF4–MALS. A cross flow gradient from 2.4 mL/min to 0 mL/min was used for AF4 separation using a 350 µm spacer and a 5 kDa regenerated cellulose membrane. The detectors used were MALS photometer Dawn Heleos and refractive index (RI) detector Optilab T-rEX (Wyatt Technology Corporation). The samples were prepared as solutions in THF at a concentration of approximately 2 mg/mL, the injected volume was 100 µL. The data were acquired and processed using light scattering software Astra 6 (Wyatt Technology Corporation).

Results and Discussion

Molar mass versus retention time plots

Figure 4: Cumulative molar mass distribution plots from SEC–MALS (red) and AF4–MALS (blue).



obtained for an SBR sample by both SEC–MALS and AF4–MALS are contrasted in Figure 1. Corresponding plots of the root mean square (RMS) radius are depicted in Figure 2. The upswings on the molar mass and RMS radius plots at the end of the SEC chromatogram (see Figure 1[a] and Figure 2[a]) are typical for branched polymers and are caused by the specific elution behaviour of branched macromolecules in the pores of SEC column packing.[5] The anchoring effect of SEC packing results in the increased polydispersity of the elution volume slices at the end of the SEC chromatogram. For polydisperse slices the MALS detector measures the weight-average molar masses and the *z*-average RMS radii, which count mainly the high molar mass fractions. As a consequence, both plots show the curve-up trend. As the *z*-average is more sensitive to polydispersity than the weight-average, the corresponding conformation plot (log-log relation between the RMS radius and molar mass) becomes up-turned (Figure 3).

In AF4, the separation takes place in an empty channel filled in solely by the mobile phase and the anchoring effect is completely missing. This is evident from

Table 1: Molar mass averages determined by SEC–MALS and AF4–MALS.

Sample	M_n (10^3 g/mol)		M_w (10^3 g/mol)		M_z (10^3 g/mol)	
	SEC–MALS	AF4–MALS	SEC–MALS	AF4–MALS	SEC–MALS	AF4–MALS
1	780	650	1610	2310	3960	5310
2	660	620	1270	2010	3280	6750

Figures 1–3 which show no upswings on the plots yielded by AF4–MALS.

Information about branching can be obtained from the conformation plot. Unfortunately, the curved conformation plots obtained by SEC–MALS make the characterization of branching difficult or even impossible. The plots obtained by AF4–MALS are not curved and such accurate branching characterization can be achieved over the entire molar mass range. For example, the conformation plot from AF4–MALS shown in Figure 3 has a decreasing slope with an increasing molar mass — this is a typical pattern for polymer materials consisting of a mixture of linear macromolecules and branched macromolecules with a branching degree increasing towards high molar masses. The slope at the region of molar masses up to $\approx 800 \times 10^3$ g/mol is 0.57 (a typical value for linear random coils in thermodynamically good solvents); the slope from $\approx 800 \times 10^3$ g/mol – 3×10^6 g/mol is 0.46 (a value typical for branched macromolecules); and the slope over $\approx 3 \times 10^6$ g/mol is 0.30 (a value typical for highly compact structures).

The anchoring of the large branched macromolecules in the column packing also affects the determination of the molar mass distribution at the region of lower molar masses, which results in the overestimation of the number-average molar mass (M_n) (Table 1) and the shift of the molar mass distribution curve towards higher molar masses as seen from Figure 4. In addition, shearing degradation in SEC columns may affect the high molar mass part of the distribution and the weight-average molar mass (M_W) and in particular the *z*-average molar mass (M_Z). Comparison of the data in Table 1 reveals not only the overestimation of M_n as a result of the anchoring effect, but also the underestimation of M_W and M_Z because of shearing degradation in SEC packing. Both anchoring and shearing degradation effects make the molar mass distribution narrower, as evidenced in Figure 4.

Conclusions

AF4–MALS provides better separation than SEC–MALS for high molar mass branched SBR and other similar polymers. As a result of better separation not only can more accurate information about branching be obtained, but also correct molar mass averages are obtained. The results from AF4–MALS are unaffected by the anchoring of the branched molecules in the column packing and/or by shearing degradation of molecules with very high molar mass.

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Optimizing Gel Permeation Chromatography for Analyzing Polymers used as Drug Excipients

Michael McGinley, Matt Trass, and Sky Countryman, Phenomenex, California, USA.

Although gel permeation chromatography (GPC) is not as widely used as other chromatography methods, it continues to be a useful technique for analyzing the size and solution characteristics of organic polymers. In this study, GPC was applied to analyze different polar polymers that are commonly used in drug excipients. As a result of their polarity, some specialized operating conditions were required. Optimizing the separation brought to light many of the common parameters involved in optimizing GPC separations, and those are discussed in detail here.

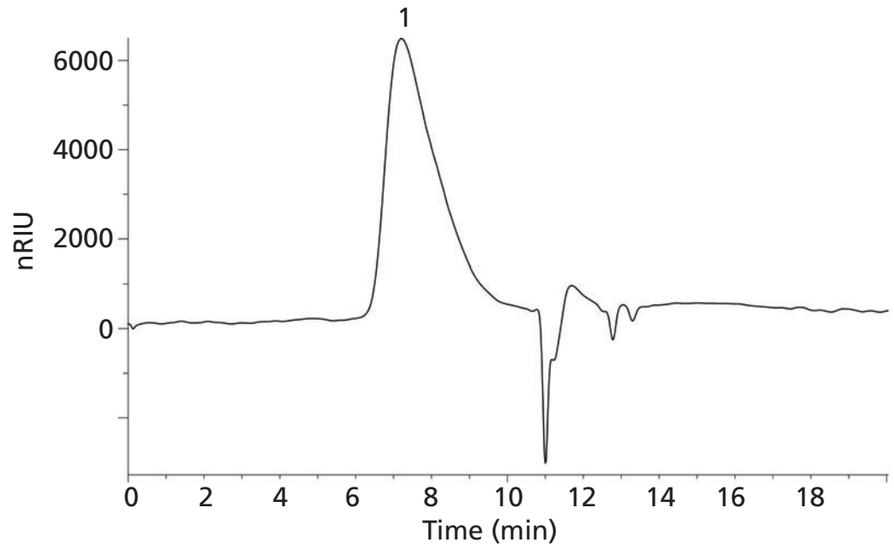
Photo Credit: Radius Images/Getty Images

For a long time, therapeutic drug analysis was primarily focused on the active pharmaceutical ingredient (API), and the breakdown products generated during manufacturing, storage, or pharmacokinetics *in vivo*. In the past, the only analytical analysis performed for tablets was dissolution testing with minimal analysis, despite the fact that excipient components typically represent 80% or more of the total mass of a tablet. The use of alternative chromatography methods for identifying excipient compounds has recently increased as a result of an increased focus on drug delivery of specific APIs with "time-release" formulations to improve "druggability". Furthermore, analysis of excipients in formulations has arisen as a method for detecting counterfeit drugs.

Excipient formulations typically use polymers (organic or otherwise) as fillers, dispersants, binders, and flavouring agents. These polymers are often polydisperse and do not contain a chromaphore — such components are therefore not amenable to reversed-phase chromatography and are not visible to ultraviolet (UV) detection. To characterize such polymers — by size, degree of polymerization, and aggregation state — other chromatographic methods are used such as: Ion exclusion, ion exchange, gel filtration, and gel permeation chromatography. Determination of the average degree of polymerization and dispersity is often sufficient to elucidate pharmacokinetic properties of certain polymers or determine the source of a formulation.

Size-exclusion chromatography (SEC) (gel filtration with aqueous mobile phase

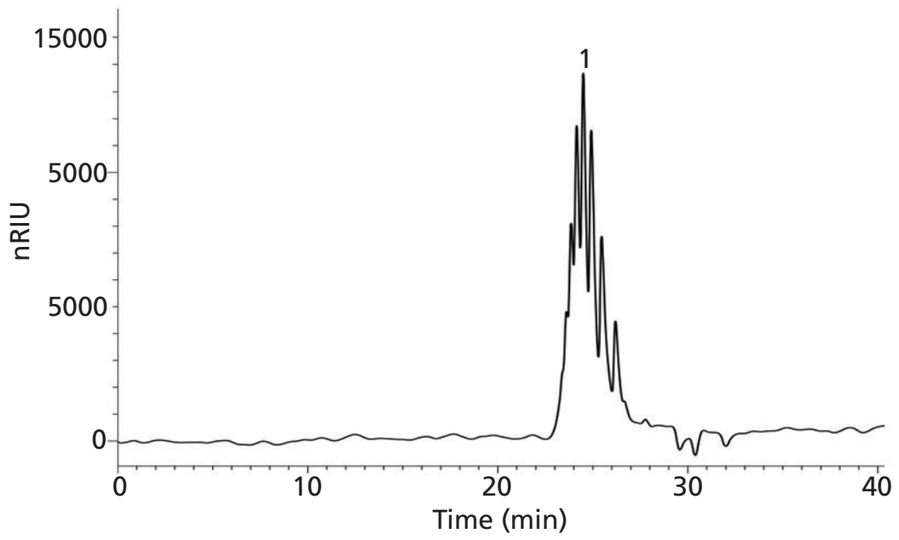
Figure 1: Polyvinyl pyrollidone (PVP) sample injected on a series of two mixed bed gel permeation chromatography (GPC) columns. A sample of 10 µL of a 10 mg/mL PVP solution was injected and detected by refractive index (RI) with a flow rate of 2 mL/min. Note the broad molecular weight distribution of PVP where individual degrees of polymerization cannot be seen. However, the average molecular weight of the polymer can be calculated and used as an identity measure.



or gel permeation in organic mobile phase, [GFC and GPC]) is the principal method for determining solution size of a polymer. Excipient polymers are often very polar, meaning that the two separation modes can sometimes be used in parallel when determining size and dispersity (as well as aggregation state). However, differences in the viscosity of the diluent containing the polymer and the mobile phase solvent mean that compatibilities of GPC and GFC phases will usually favour one separation mode over the other.

In this article two excipient polymers, polyethylene glycol (PEG) and polyvinyl pyrollidone (PVP), were analyzed by GPC as well as tablet formulations containing such polymers. The different separation requirements for GPC, as well as sample preparation strategies relevant to GPC, will be discussed.

Figure 2: Polyethylene glycol (PEG) standard injected on three fixed pore GPC columns in series. A sample of 10 µL of a 1% PEG 300 solution was dissolved in tetrahydrofuran (THF) and was injected and detected by RI with a flow rate of 1 mL/min. Note that different length PEG can be partially resolved by GPC.

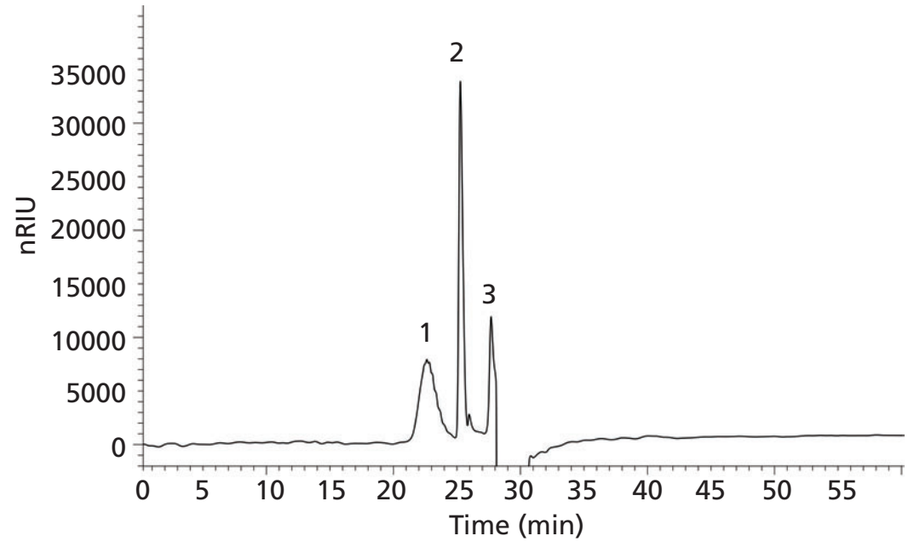


Materials and Methods

All solvents and reagents were purchased from either Sigma Chemical or EMD Chemicals. Over the counter ibuprofen capsules were purchased from a local pharmacy.

All analyses were performed on an Agilent 1200 HPLC system (Agilent Technologies) with an autosampler, degasser, column heater, and refractive index (RI) detector. Data was collected using ChemStation software (Agilent Technologies). The columns used for GPC were 300 mm × 7.8 mm, 5 µm Phenogel in series using either fixed pore (Phenogel 50 Å, 100 Å, and 500 Å) columns or mixed bed Phenogel Linear(2) columns (Phenomenex). Tetrahydrofuran (THF) with 10 mM lithium bromide was used as the mobile phase in all separations. Lithium bromide was added to reduce secondary interactions between the analyte and the stationary phase as is typical with all GPC analysis. The LC was run in isocratic mode; see Figures 1–3 for specific flow rates. The column

Figure 3: Advil cold and sinus capsule was injected on the same series of columns and conditions as Figure 2. The capsule was first dissolved in water then diluted with THF prior to injection. Note the presence of PEG (the large peak at 25.5 min) in the tablet along with other components.



temperature was maintained at 40 °C for all chromatographic runs and the injection volume used was 10 µL for all samples.

Results and Discussion

Chromatographers new to polymer analysis and GPC are often perplexed by the peak shape and low resolution that GPC provides for seemingly pure polymer samples compared to the results typically seen for reversed-phase chromatography of small molecule APIs. Polymers are polydisperse mixtures of recurring small organic subunits (typically 30–300 daltons) and so obtaining any resolution between different degrees of polymerization is often difficult. Instead, GPC provides a general value for the average degree of polymerization of a polymer. Figure 1 shows the separation of PVP, a common tablet binder agent, on a pair of mixed bed GPC columns in series. Note the broad molecular weight distribution of the PVP sample. Peak retention time and peak width can be used in conjunction with PVP standards to determine the average molecular weight as well as the degree of dispersity. Such information can be used to identify polymers in a mixture, but in many cases does not give exact polymer length composition.

Figure 2 shows the separation of a PEG sample, where some resolution between similarly sized polymers was seen. Three fixed pore columns were used to gain better resolution in the low-molecular-weight range versus mixed bed GPC columns which provide moderate resolution across a wide molecular weight range. A good general rule for GPC is to use fixed pore columns for applications that require maximum resolution. For samples with a wide molecular range or unknown analyte, a combination of mixed bed (linear) columns provides the widest applicable mass range with moderate resolution. In Figure 2 the use of three small pore GPC columns (50 Å, 100 Å, and 500 Å) allows a focus on a lower molecular weight range. By using adjoining pore sized columns the molecular weight range can be expanded slightly without compromising the resolution offered by fixed pore columns.

The choice of mobile phase and diluent used for a particular sample are critical points in GPC. The solvent used as a mobile phase must be compatible with the sample being analyzed. While nonpolar polymers may require strong solvents to be solubilized, in the case of excipient analysis the challenge is the exact opposite; to find an organic solvent that is polar enough to maintain solubility of the analytes being separated. Choice of diluent is equally important; the diluent must solubilize an excipient sample and be miscible with the mobile phase solvent. Viscosity differences between the mobile phase and diluent can negatively impact a chromatographic separation, and thus should be minimized. When comparing Figure 1 and Figure 2 it can be seen that Figure 2 has a much more stable baseline with no negative peaks because of solvent difference between the diluent and mobile phase. In Figure 1 some baseline interferences are seen as a result of the dimethylformamide (DMF) used in the diluent. A more extreme example is shown in Figure 3 where an Advil gel cap was dissolved in water and then diluted with THF before running on the same series of columns shown in Figure 2. Note the large negative peak in the chromatogram as a result of the diluent.

Figure 3 shows the use of GPC for looking at excipients in a gel cap

formulation. The peak for PEG is clearly resolved in the excipient mixture and both the concentration and molecular weight can be elucidated based on comparison to a PEG standard, as shown in Figure 2. A resolved peak for PVP can be seen that could be quantitated with the use of PVP standards under the same running conditions. Such analyses demonstrate the power of using GPC in the analysis of excipients seen in this formulation sample.

Conclusions

Although its use is not as widespread as reversed-phase chromatography or other methods, GPC demonstrates good utility in analyzing polar polymers commonly used in the formulation of common APIs. An important part of getting useful data from GPC columns is having suitable standards of the polymer of interest to quantitate, as well as to determine its average molecular weight. When developing a method for analyzing polar polymers by GPC, the mobile phase and the diluent used should be considered to minimize any sample or matrix interferences that could impact accuracy. Finally, the choice of the column series used for such analysis (either mixed bed or fixed pore media) has the most impact on the resolution and molecular weight range being analyzed.

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Investigating the Molecular Weight and Structure of Linear and Cross-Linked Hyaluronic Acid by SEC–MALS and SEC–Triple Detection

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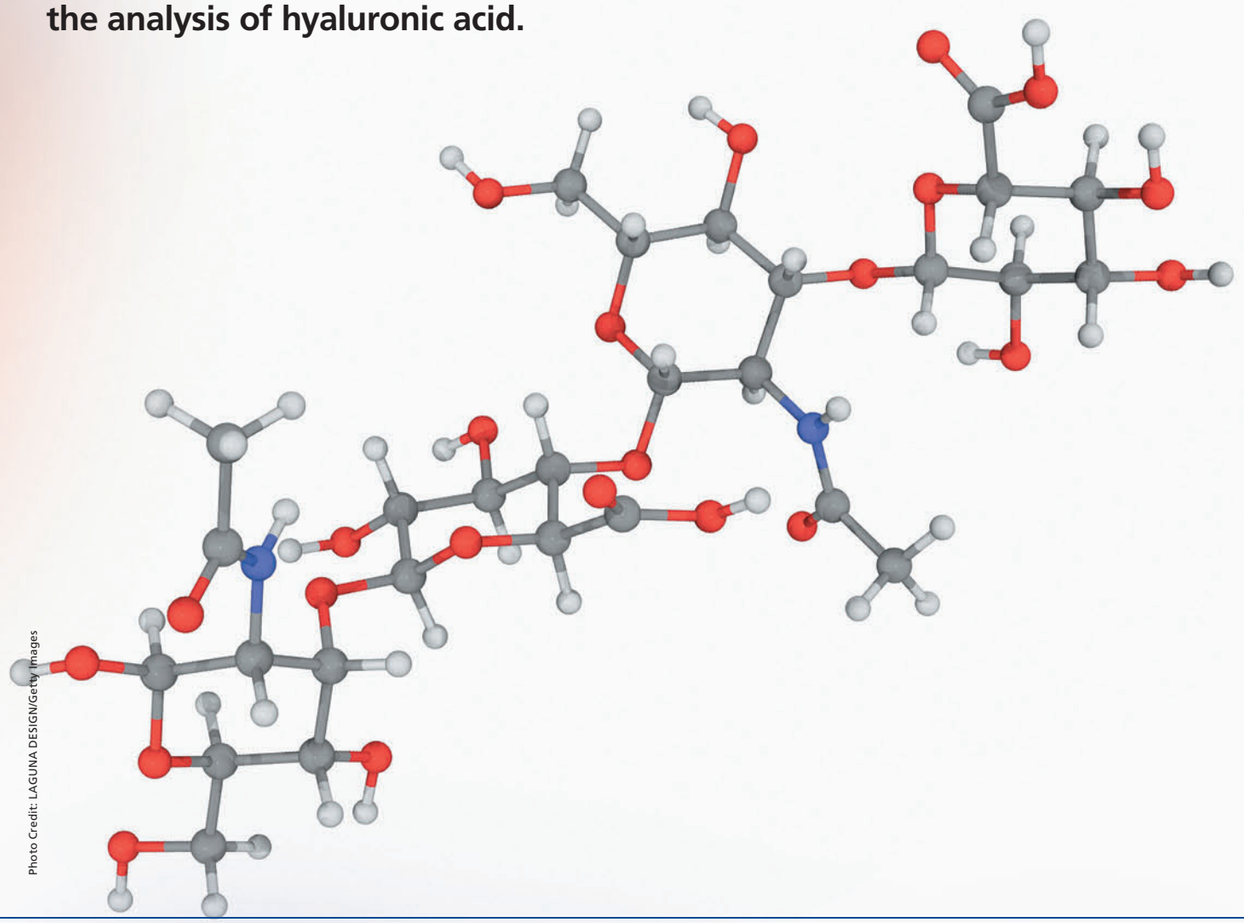
This article demonstrates the application of a multi-angle light scattering (MALS) detector within a triple detector array to measure absolute molecular weight distribution and gain insight into molecular structure for an application involving the analysis of hyaluronic acid.



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Size-exclusion chromatography (SEC) is widely used for polymer molecular weight characterization for many applications. Basic SEC systems use a single refractive index (RI) detector and require suitable calibration standards. Such systems provide relative molecular weight unless the calibration standards used have the same structure and chemical composition as the polymer being analyzed and are therefore not suitable for research applications. Light scattering detectors such as MALS (multi-angle light scattering) and LALS (low-angle light scattering) are now commonly used with SEC because of their ability to measure molecular weight directly.

With a light scattering detector specific calibration standards are no longer required because the molecular weight measured is independent of polymer type or structure. Light scattering detectors directly measure the absolute, rather than relative, molecular weight and when coupled with a refractometer as a concentration detector and a viscometer for measuring intrinsic viscosity, SEC with "triple detection" becomes a powerful technique to characterize biomacromolecules and polymers.

Hyaluronic acid (Hyaluronan or HA) is a natural heteropolysaccharide consisting of alternating residues of D-glucuronic acid and N-acetyl-D-glucosamine. HA forms the capsule of some bacterial species and is also widely distributed throughout the human body. It plays an important role as the structural and

Figure 1: Triple detection overlay chromatogram for linear hyaluronic acid (RI in red, SEC–MALS [90°] in amber, and viscometer in blue).

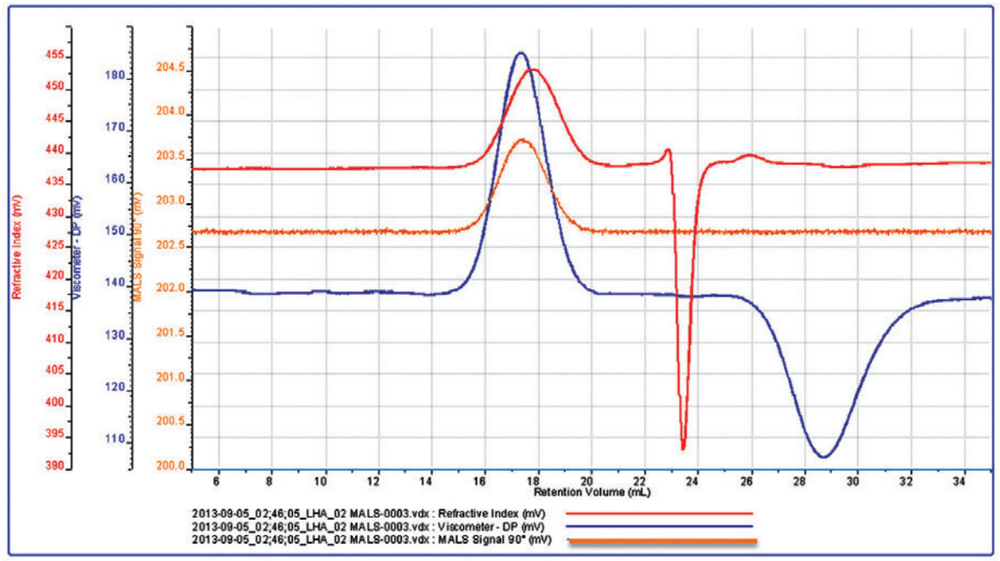


Table 1: Quantitative results from the two sample measurements.

Sample Id	Mw	Mn	IV	Rh	Rg	M-H a
LHA	265,000	192,000	6.22	28.8	44.6	0.84
XHA	384,000	217,000	6.97	33.1	49.4	0.68

Mw (Da) is the weight-average molecular weight; Mn (Da) is the number-average molecular weight; IV (dL/g) is the intrinsic viscosity; Rh (nm) is the hydrodynamic radius; Rg (nm) is the radius of gyration; and MH-a is the Mark-Houwink slope.

mechanical support for tissues, such as skin, tendons, muscles, and cartilage. The unique physico-chemical properties of HA have led to a wide range of applications in the cosmetic, pharmaceutical, and medical industries. In some applications HA has to be crosslinked or derivatized to delay its degradation and to improve its mechanical performance. An example of the use of this biodegradable material is in the controlled delivery of bioactive agents.

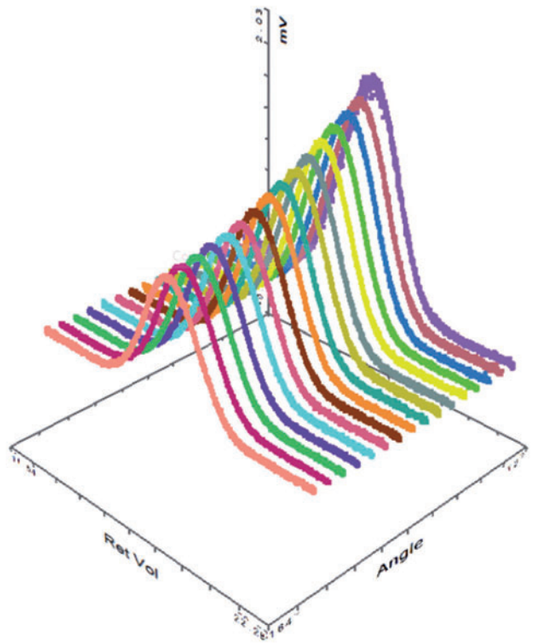
In this article, we discuss the multi-detection SEC analysis of a linear and a modified HA sample, highlighting the differences between the samples studied and underlining the information the technique can provide.

Experimental

Two samples of hyaluronic acid were kindly supplied by the Second University of Naples (Italy). The samples submitted were identified as linear hyaluronic acid (LHA) and cross-linked hyaluronic acid (XHA). The cross-linked HA was produced from the linear HA by auto-crosslinking via ester bonds using carbodiimide chemistry,[1] opportunely modified. In particular, 1% (molar percentage) of carboxyl groups of HA were activated. The resulting product, XHA, was still soluble in an aqueous medium.

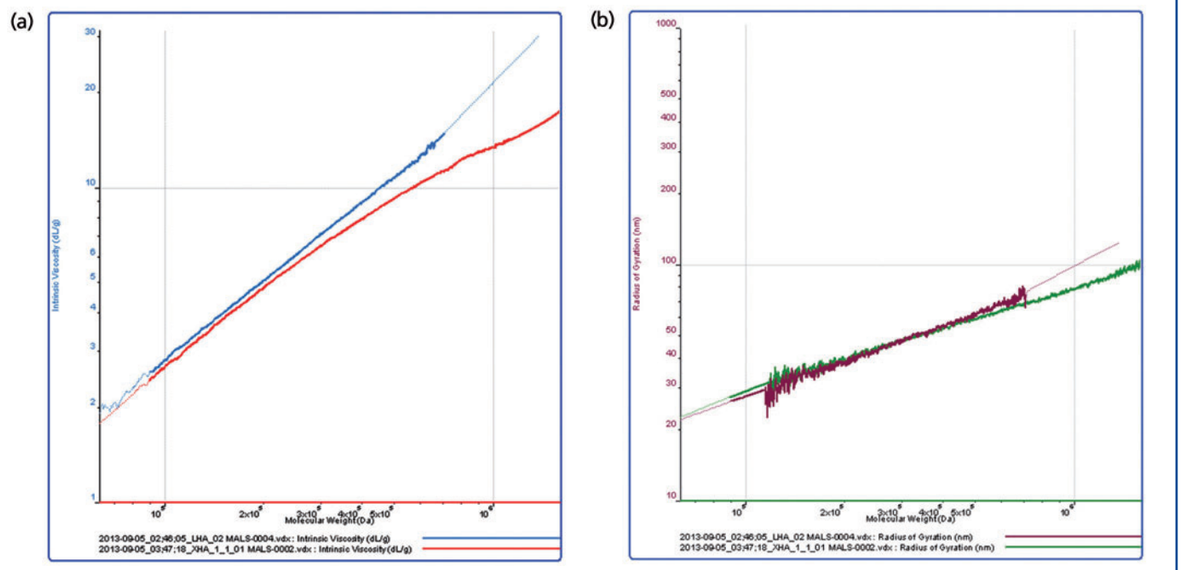
Figure 1 shows the triple detection chromatogram of LHA. Analysis was performed on a Viscotek SEC–MALS 20 multi-angle light scattering detector (Malvern Instruments) coupled with a Viscotek TDAmax triple detector SEC system (Malvern Instruments). Each sample was injected twice at a volume of 100 µL and separated in 0.1 M $NaNO_3$ at 0.6 mL/min using two ViscoGEL aqueous

Figure 2: Retention volume versus measurement angle versus signal for cross-linked hyaluronic acid.



columns (Malvern Instruments) in series. The triple detector SEC system, including columns, detectors, and interdetector tubing, was maintained at a constant temperature of 40 °C. The interdetector volumes, band broadening, and instrument constants were determined using a narrow-dispersity pullulan standard. The value of dn/dc used for both samples in these measurements was 0.155 mL/g.

Figure 3: (a) The Mark-Houwink plots and (b) conformation plots for the samples measured.



Results

The molecular weight calculation uses the signal observed by the light scattering detector. The responses from the MALS detector are shown in Figure 2. Taking a closer look, we can observe the different responses from the light scattering detectors at each angle. These highlight angular dependence where intensity at the lower angles of measurement increases compared with higher measurement angles because of the large HA molecules scattering light anisotropically. This angular dissymmetry enables a molecular size (Rg) to be obtained from this data.

Table 1 summarizes the quantitative results yielded from the measurement of the samples submitted. The Mw and Mn for the cross-linked HA are, as expected, higher than the linear starting material. It is also worth noting that the values from the SEC–MALS detector are close to the reference values obtained by SEC–LALS but still remain a little low because of the use of extrapolation to determine molecular weight by MALS compared to the direct measurement by LALS.[2]

The structural differences between the samples can be explored through the

conformation and Mark-Houwink plots

A Mark-Houwink plot is a plot of intrinsic viscosity (IV), obtained directly from the viscometer detector, as a function of molecular weight. Since it relates directly to molecular density, very fine changes in structure are visible between samples on this type of plot. The Mark-Houwink plot for the two HA samples is shown in Figure 3(a). Overall, the XHA sample exhibits lower intrinsic viscosity values compared with LHA over the entire molecular weight distribution. This provides confirmation of a more dense structure expected from a cross-linked sample compared to its linear substrate. Additionally, the slope of LHA is uniform as would be expected from a linear molecule, where the constant structure will give a linear relationship between the intrinsic viscosity and the molecular weight. In contrast, XHA shows curvature of the Mark-Houwink plot indicating the molecule's intrinsic viscosity (and therefore its density) has a changing relationship with the molecular weight. This is explained by the increased cross-linking sites on the higher molecular weight (longer) molecules.

In comparison to many polymers and most biopolymers, HA is a large molecule and therefore the SEC–MALS data can provide the molecular size, Rg. By plotting Rg as a function of molecular weight, we can obtain the conformation plot, see Figure 3(b). Here we can also see the structural change from LHA to XHA but the differences observed are small compared to the density changes observed in the Mark-Houwink plot.

Conclusion

Cross-linked hyaluronic acid was successfully analyzed using triple detection with SEC–MALS. Comparison with linear HA revealed that the cross-linked HA presents a higher molecular weight than linear HA. The viscometry data and Rg data conclusively demonstrate that the polymer structure is also different, which confirms the cross-linking of the linear substrate.

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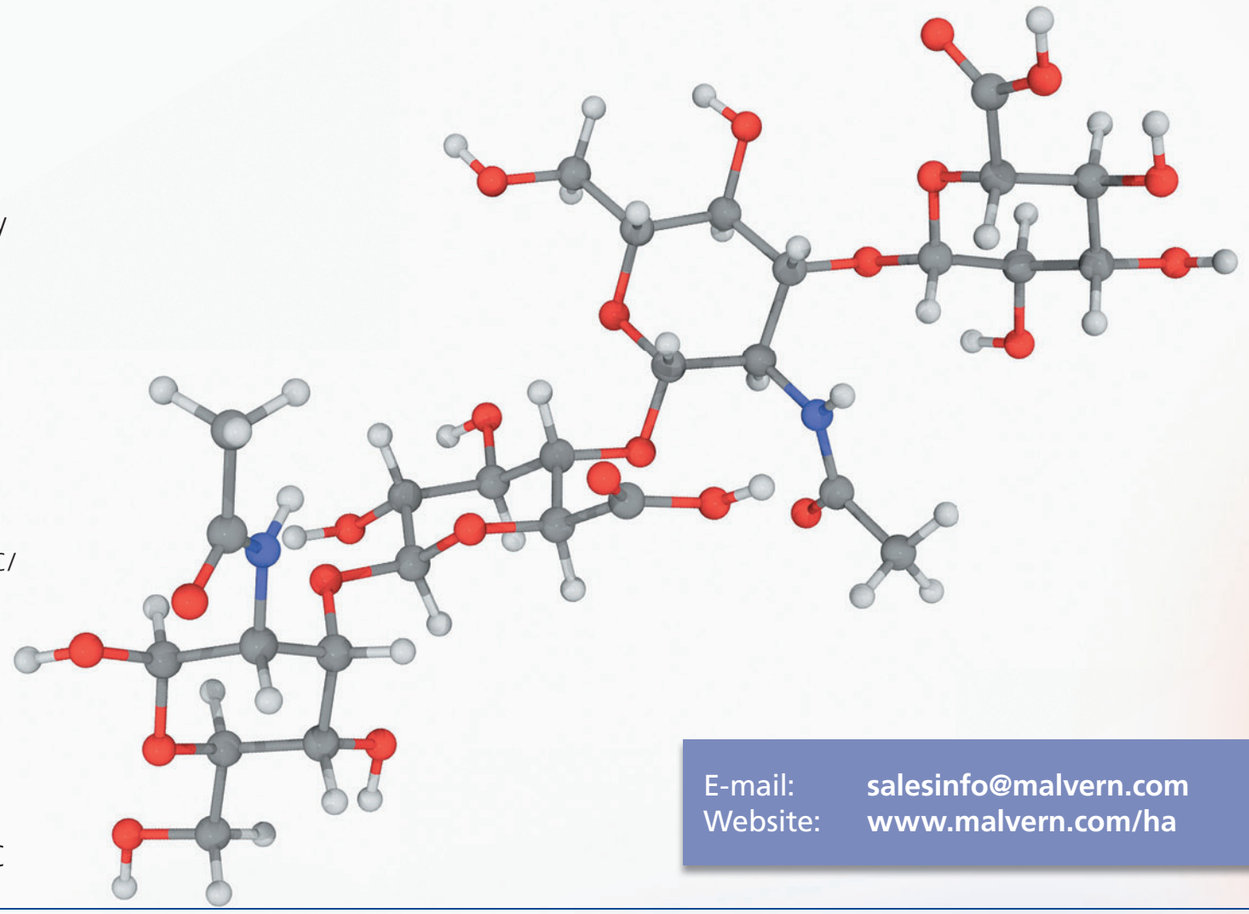
Agata Papa is currently working towards a PhD in biomedical science and biotechnologies at the Second University of Naples, Italy. She has completed a master's degree in medical biotechnologies and a bachelor's degree in biotechnologies, also at the Second University of Naples. Her PhD project concerns the development of different chemical crosslinking strategies of polysaccharides, as well as physico-chemical and biological characterization of the resulting biomaterials to obtain new medical devices that can be used in the biomedical field.



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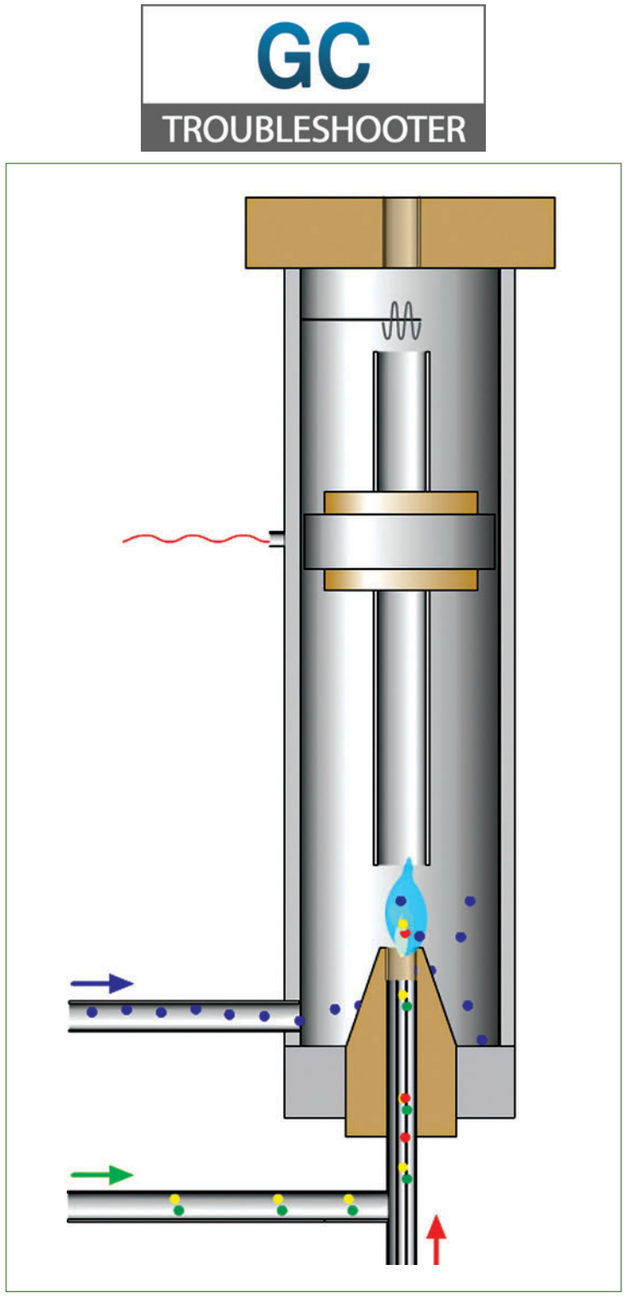

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