ACS Macro Letters

Letter

pubs.acs.org/macroletters

Further Insight into the Detailed Characterization of a Polydisperse Cyclodextrin-Based Polyrotaxane Sample by Electrospray Ionization Mass Spectrometry

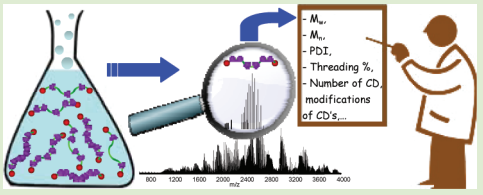
Cédric Przybylski,*[,†] Véronique Bonnet,[‡] and Nathalie Jarroux[†]

[†]Laboratoire Analyse et Modélisation pour la Biologie et l'Environnement, Université d'Evry-Val-d'Essonne, CNRS UMR 8587, Bat. Maupertuis, Bd François Mitterrand, 91025 Evry, France

[‡]Laboratoire des Glucides, Université de Picardie Jules Verne, CNRS, UMR 6219, Institut de Chimie de Picardie, 80039 Amiens, France

Supporting Information

ABSTRACT: An easy and fast approach based on electrospray mass spectrometry (ESI-MS) was developed to provide a detailed characterization of a mixture containing polydisperse cyclodextrin-based polyrotaxane (CD-based PR). Here, method gave access to usual data such the weight-average molecular weight, the number-average molecular weight and the polydispersity index, but also to more specific features as the average number of CDs threaded and the average threading degree. Moreover, the nature and the average number of groups grafted per CD, such as sulfate or silyl groups, can be accurately determinate. This ESI-MS approach advantageously complements the widely used NMR and SEC methods and, thereby, constituting a milestone in the actual MS bottleneck regarding the analysis of polydisperse supramolecular assemblies.



Poly pseudo/polyrotaxanes (PPRs/PRs) are supramolecular assemblies of growing interest.[1−5] They are constituted of inclusion complexes in which macrocyclic rings (host) are threaded onto polymeric chain (guest) forming a PPR, which is finally blocked at the two extremities by bulky groups yielding a PR. Cyclodextrins (CD) are the most used host molecules due to a hydrophobic cavity which can encapsulate a guest molecule, and thus allows a host−guest relationship with well controlled properties.[6−8] In addition, CD are commercially available and can be functionalized in well-defined ways. CD-based PPRs/PRs have demonstrated interest as molecular semiconductors[9] and promising biomedical applications such as gene delivery,[10] trypsin inhibition,[11] support for multivalent interactions,[12] or also as inhibitors of cell agglutination.[13] The control of the physicochemical properties involves the modification of host and guest as well as to estimate the threading degree, that is, the percentage of CD threaded along the polymeric chain.

Except for the emerging static and dynamic light scattering techniques (SLS and DLS),[14] NMR spectroscopy and size exclusion chromatography (SEC) are actually the most widely used techniques for molecular weight estimation and structural characterization of CD-based PRs.[15] Nevertheless, these two tools suffer from serious limitations. NMR requires often amounts at mg/μg scale of samples, which can be very difficult to obtain from low yield multisteps synthesis of some PPRs/PRs. Moreover, NMR gives access to only an average picture of the sample content. SEC calibration requires appropriate molecular weight standards, which is a difficult task because highly threaded PRs behave unlike any commercially available standard due to their semirigid rod-like morphology. Consequently, use of conventional standards gives rise to an overestimation of the molecular mass.

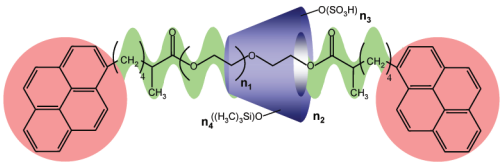
Mass spectrometry (MS) is an underestimated tool in the supramolecular chemistry field, which can favorably complement aforementioned techniques by providing absolute, direct determinations of molecular weights and achieves a fine structural deciphering. Matrix-assisted laser desorption/ionization (MALDI), which is widely used for polymer analysis because it mainly yields monocharged species, has been reported to be very useful for the analysis of high molecular weight CD-based PRs.[16−22] However, accurate values require absence of mass discrimination. In addition, MALDI was often connected to a time of flight (TOF) analyzer offering poor resolution and low accuracy for such compounds. Electrospray ionization (ESI) is another method well-suited to study large noncovalent assemblies,[23−28] although its advantage comes at the expense of producing multiply charged ions that can clutter the spectra.

We chose as model compound, a PR constituted of α-CDs threaded on poly(ethylene oxide) (PEO; Chart 1). Its synthesis was described elsewhere.[29] Briefly, four samples were analyzed: sample 1 contains PEO α,ω-dipyrenyl chains together with α-CD (1:2 mol equiv); samples 2 and 3 contain PR with the reaction performed in a water/DMSO mixture equal to 33/66 (v/v) and 50/50 (v/v), respectively; and sample 4 is sample 3 submitted to a silylation reaction. ESI mass spectra were

Received: February 3, 2012
Accepted: April 2, 2012
Published: April 10, 2012

   dx.doi.org/10.1021/mz3000607 | *ACS Macro Lett.* 2012, 1, 533−536

Chart 1. Structure of Studied Polyrotaxane Constituted of α-CDs (blue) Threaded along PEO Chains (green) and Blocked by Pyrenyl Groups (red)[a]



[a] n_1 is the number of EO units, n_2 is the number of CD threaded, and n_3 and n_4 are the number of sulfate and silyl groups, respectively, grafted on CDs.

recorded in positive mode using a LTQ-Orbitrap mass spectrometer with a mass to charge (m/z) up to 4000 in the high range setting. Main details of the sample preparation and mass analysis are given in Supporting Information.

The positive ESI spectrum of sample 1 (Figure 1a) displays two distinct charge state envelopes having approximately

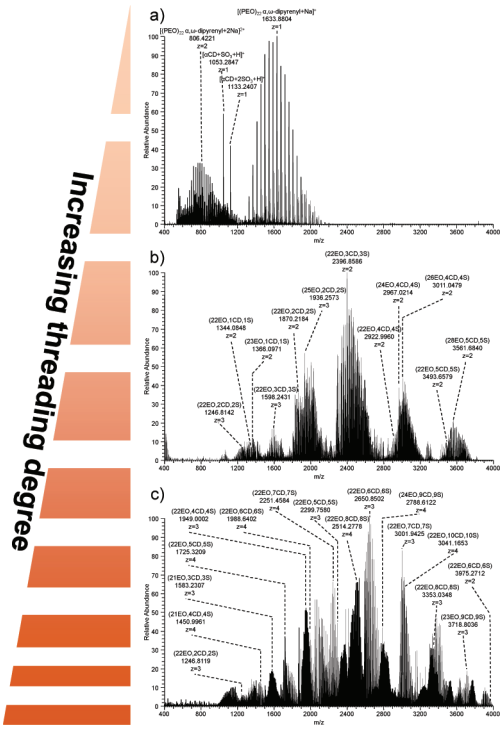


Figure 1. Positive ESI spectra of a mixture of α-CD and PEO α,ω-dipyrenyl (sample 1; a) and polyrotaxane with average threading degree of 28% (sample 2; b) and 58% (sample 3; c). S is the total number of sulfates grafted. Samples were at 0.5 mg/mL in water/methanol 1:1 (v/v).

Gaussian distributions. The first is centered around m/z 1633.8804 and the second around m/z 806.4221 for 1^+ and 2^+ forms, respectively, both bearing 22 ethylene oxide units (EO). Polymeric molecules were mainly ionized by binding to Na^+ and to a less extent to K^+. Protonated CDs were mainly detected by two intense ions at m/z 1053.2847 and m/z 1133.2407 for the monosulfated and the disulfated forms, respectively. These two forms represent more than 90% of the all α-CD. Simultaneous analysis of PEO α,ω-dipyrenyl with α-CD constituted the reference of the threading efficiency, that is, 0% of threading degree ($n_2/2 \times n_1 = 0$, Chart 1). Table 1 shows the comparison of results obtained by SEC and MS for the calculation of weight-average molecular weight (M_w), the number-average molecular weight (M_n), the polydispersity index (PDI), the average number of CDs threaded (nCDs), and the average threading degree (%TD). MS results of sample 1 were in good agreement with values obtained by SEC (Table 1).

Threading percentages can be controlled by varying the solvent conditions used for the rotaxanation reaction. Furthermore, the solvent used for either the threading or the rotaxanation reactions must not form a better complex with the macrocycle than the threaded polymers; otherwise, the solvent will dethread the macrocycle. In the case of CD-based PRs formation, polar solvents, such as water, DMSO, or also DMF, are most typically used. When such a reaction was performed in water/DMSO 33/66 mixture (sample 2), the equilibrium was slightly moved toward the formation of PRs. The resulting spectrum (Figure 1b) exhibits at least two unambiguously identified charge states 2^+ and 3^+, while 4^+ was rarely observed. Thorough examination of the spectrum highlights two different kinds of distribution representing two additional levels of sample content. The first set could be seen as a global snapshot of the average threading efficiency. Indeed, considering the increasing number of CDs, we have a relatively Gaussian repartition of the 2^+ charge state centered on PR forms containing 3 CDs. In detail, we estimated 4, 23, 52, 12, and 7% for PR bearing 1, 2, 3, 4, and 5 CDs, respectively, of the whole identified 2^+ ions. From these observations, we can easily conclude that only partial threading has been obtained. Additionally, the second set is constituted by the distribution within a given both charge state and number of threaded CDs. By combination of 2^+ and 3^+ distributions, the average number of CDs threaded was 3.1. Considering that no more than 1 α-CD per 2 EO units can be threaded,[30] we deduced an average threading degree of 28%. This result is well correlated with SEC data (Table 1). Water content at 50% during synthesis (sample 3) clearly shifted the equilibrium in favor of the self-assembly.

The corresponding spectrum (Figure 1c) shows multiple-charge states mainly constituted of 3^+ and 4^+, with some minor ions under 2^+ and 5^+. Surprisingly, the apparent charge states are not excessively high, as expected from a significant increasing size of molecular assemblies. A hypothesis can be

Table 1. Detailed Features of Samples 1−3 Obtained by SEC and MS

	M_n (kDa)		M_w (kDa)		PDI		nCDs			%TD		
sample	SEC[a]	MS	SEC[a]	MS	SEC	MS	SEC		MS	SEC[a]		MS
1	1.50	1.60 ± 0.04	1.64	1.62 ± 0.04	1.01	1.01			0	0		0
2	4.90	4.80 ± 0.12	22.05	5.10 ± 0.13	4.46	1.06	2.0[b]	3.0[c]	3.1 ± 0.2	18.5[b]	27[c]	28 ± 6
3	7.30	7.71 ± 0.20	25.55	8.07 ± 0.21	3.47	1.05	4.7[b]	8.3[c]	6.4 ± 0.3	43[b]	75[c]	58 ± 5

[a]Estimated in PEO equivalents. [b]Direct peak area by refractive detection. [c]Free CDs titration by refractive detection.

dx.doi.org/10.1021/mz3000607 | ACS Macro Lett. 2012, 1, 533−536

the elongated conformation of PRs resulting from a high number of CDs threaded, and numerous hydrogen bonding interactions between close neighboring CDs. This semirigid rod-like morphology leads to a higher packed structure impairing access for the protonation/cationization processes. The poly[8]rotaxanes constituted of 22 EO units and 7 monosulfated CDs (m/z 2251.4584) is the most intense form. Among all 3^+ species sample content was estimated to 1, 4, 11, 12, 24, 17, 17, and 4% for PRs with 2−9 CDs. Similarly for 4^+ species, 4, 12, 17, 18, 23, 18, 13, and 6% were detected according to 3−10 CDs threaded. Taking into account these results, 6.4 CDs were threaded with an average threading degree of 58%. This value was again close to those obtained by SEC (Table 1). Higher molecular weight assemblies can be masked during MS analysis due to their compact structure. Hydrogen bonding between CDs can be limited by modification of hydroxyl functions, for example by silylation. To check this hypothesis, sample 3 was submitted to a silylation of CDs (sample 4). The MS spectrum obtained after the reaction exhibits more ions than in sample 3 without modification (Figure 2). This is presumably due to increasing

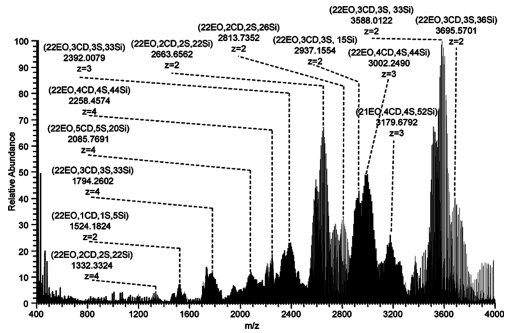


Figure 2. Positive ESI spectrum of sample 4 at 0.5 mg/mL in water/methanol 1:1 4% DMF. Si corresponds to the number of silyl groups.

of modified CDs containing sulfation silylation or both. The spectrum reveals that beyond the higher masses obtained, absolute signal was overall enhanced by 2.5−3-fold. The significant diminishment of interactions involving hydrogen bonds between close CDs involve an improvement of the PRs solubilization and consequently better ionization efficiency, as proven by detection of 4^+ species. This is particularly true for structures with a high number of CDs threaded. Nevertheless, due to the mass cutoff of the Orbitrap, we cannot monitor the presence of 2^+ forms with more than 3 CDs as well as species with m/z > 4000, i.e., with molecular weight >12000 g·mol^{-1} (3^+) or >16000 g·mol^{-1} (4^+). From the obtained data and taking into account that the main compound is the poly[4]-rotaxane with 22 EO units and 3 α-CDs threaded, we deduced that the most intense ion at m/z 3588.0122 includes 3 α-CDs with 3 sulfations and 33 silyl moieties. The corresponding experimental isotopic pattern matches very well with the theoretical one within 0.4 ppm (see Supporting Information). Furthermore, the MS/MS experiment allowed us to determine that each α-CDs contains both 1 sulfation and 11 silylations (see Supporting Information). Considering the overall MS of sample 4, the average number of silylations per CD (Si/CD) was estimated to be 9−10, which is equivalent to 1.8−2 persilylated α-CDs (CD-Si; Table 2). These results were slightly different from the molecular weight provided by SEC and the three persilylated CDs obtained by ^{1}H NMR analysis (see Supporting Information).

In conclusion, we have demonstrated that ESI associated with a high resolutive analyzer such as Orbitrap can be a useful tool to probe the average threading degree of polydisperse CD based PRs. In addition, the usual data such as M_w, M_n, and PDI and specific features as the average number of CDs threaded and the average threading degree can be easily reached. Furthermore, the combination of MS and MS/MS offers the possibility to unambiguously decipher both the nature and the number of substituting groups grafted on CDs. This study constitutes a milestone in the actual MS bottleneck regarding the analysis of polydisperse supramolecular assemblies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details; ESI-MS/MS spectrum of m/z 3587.0208 and theoretical isotopic distribution; SEC and ^{1}H NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: cedric.przybylski@univ-evry.fr. Fax: +33-1-69-47-76-55.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge Génopole-France and the Conseil Régional d'Île-de-France for the generous financial support and Dr Marie-Jeanne Clement for ^{1}H NMR of sample 4.

■ REFERENCES

(1) Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; Wiley-VCH: New York, 1995.
(2) Harada, A.; Hashidzume, A.; Yamaguchi, H.; Takashima, Y. *Chem. Rev.* **2009**, *109*, 5974−6023.
(3) Huang, F.; Gibson, H. W. *Prog. Polym. Sci.* **2005**, *30*, 982−1018.
(4) Jiang, W.; Schalley, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10425−10429.
(5) Sauvage, J.-P. *Molecular Catenanes, Rotaxanes and Knots. AJourney Through the World of Molecular Topology*; Wiley-VCH: Weinheim, 1999.
(6) Wenz, G.; Han, B.-H.; Müller, A. *Chem. Rev.* **2006**, *106*, 782−817.
(7) Araki, J.; Ito, K. *Soft Matter* **2007**, *3*, 1456−1473.
(8) Nepogodiev, S. A.; Stoddart, J. F. *Chem. Rev.* **1998**, *98*, 1959−1976.

Table 2. Detailed Features of Sample 4 Obtained by MS

ions[a]	M_n (kDa)	M_w (kDa)	PDI	nCDs	%TD	nSi	Si/CD	CD-Si
2^+ to 4^+	7.69 ± 1.34	8.46 ± 1.36	1.03	3.3 ± 0.7	30.2	32.9 ± 6.3	10.0 ± 2.1	1.8 ± 0.7
3^+, 4^+	7.93 ± 0.22	8.71 ± 0.16	1.03	3.7 ± 0.1	33.8	36.3 ± 3.4	9.1 ± 0.8	2.0 ± 0.2

[a]Ions taken into account to calculate the average values.

dx.doi.org/10.1021/mz3000607 | *ACS Macro Lett.* 2012, 1, 533−536

(9) Cacialli, F.; Wilson, J. S.; Michels, J. J.; Daniel, C.; Silva, C.; Friend, R. H.; Severin, N.; Samori, P.; Rabe, J. P.; O'Connell, M. J.; Taylor, P. N.; Anderson, H. L. *Nat. Mater.* **2002**, *1*, 160−164.
(10) Ooya, T.; Choi, H. S.; Yamashita, A.; Yui, N.; Sugaya, Y.; Kano, A.; Maruyama, A.; Akita, H.; Ito, R.; Kogure, K.; Harashima, H. *J. Am. Chem. Soc.* **2006**, *128*, 3852−3853.
(11) Eguchi, M.; Ooya, T.; Yui, N. *J. Controlled Release* **2004**, *96*, 301−307.
(12) Ooya, T.; Eguchi, M.; Yui, N. *J. Am. Chem. Soc.* **2003**, *125*, 13016−13017.
(13) Nelson, A.; Belitsky, J. M.; Vidal, S.; Joiner, C. S.; Baum, L. G.; Stoddart, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 11914−11922.
(14) Kume, T.; Araki, J.; Sakai, Y.; Mayumi, K.; Kidowaki, M.; Yokoyama, H.; Ito, K. *J. Phys.: Conf. Ser.* **2009**, *184*, 12−18.
(15) Loethen, S.; Kim, J.-M.; Thompson, D. H. *Polym. Rev.* **2007**, *47*, 383−418.
(16) Przybylski, C.; Blin, F.; Jarroux, N. *Macromolecules* **2011**, *44*, 1821−1830.
(17) Takahashi, A.; Katoono, R.; Yui, N. *Macromolecules* **2009**, *42*, 8587−8589.
(18) Terao, J.; Tsuda, S.; Tanaka, Y.; Okoshi, K.; Fujihara, T.; Tsuji, Y.; Kambe, N. *J. Am. Chem. Soc.* **2009**, *131*, 16004−16005.
(19) Miyawaki, A.; Miyauchi, M.; Takashima, Y.; Yamaguchi, H.; Harada, A. *Chem. Commun.* **2008**, 456−458.
(20) Michels, J. J.; O'Connell, M. J.; Taylor, P. N.; Wilson, J. S.; Cacialli, F.; Anderson, H. L. *Chem.—Eur. J.* **2003**, *9*, 6167−6176.
(21) Takashima, Y.; Sakamoto, K.; Oizumi, Y.; Yamaguchi, H.; Kamitori, S.; Harada, A. *J. Inclusion Phenom. Macrocyclic Chem.* **2006**, *56*, 45−53.
(22) Taylor, P. N.; O'Connell, M. J.; McNeill, L. A.; Hall, M. J.; Aplin, R. T.; Anderson, H. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 3456−3460.
(23) Jiang, W.; Schäfer, A.; Mohr, P. C.; Schalley, C. A. *J. Am. Chem. Soc.* **2010**, *132*, 2309−2320.
(24) Jiang, W.; Schalley, C. A. *J. Mass Spectrom.* **2010**, *45*, 788−798.
(25) Jiang, W.; Winkler, H. D. F.; Schalley, C. A. *J. Am. Chem. Soc.* **2008**, *130*, 13852−13853.
(26) Przybylski, C.; Jarroux, N. *Anal. Chem.* **2011**, *83*, 8460−8467.
(27) Anderson, S.; Aplin, R. T.; Claridge, T. D. W.; Goodson, T., III; Maciel, A. C.; Rumbles, G.; Ryan, J. F.; Anderson, H. L. *J. Chem. Soc., Perkin Trans.* **1998**, 2383−2398.
(28) Schalley, C. A.; Rivera, J. M.; Martín, T.; Santamaría, J.; Siuzdak, G.; Rebek, J. *Eur. J. Org. Chem.* **1999**, *1999*, 1325−1331.
(29) Jarroux, N.; Guegan, P.; Cheradame, H.; Auvray, L. *J. Phys. Chem. B* **2005**, *109*, 23816−23822.
(30) Harada, A.; Kamachi, M. *Macromolecules* **1990**, *23*, 2821−2823.