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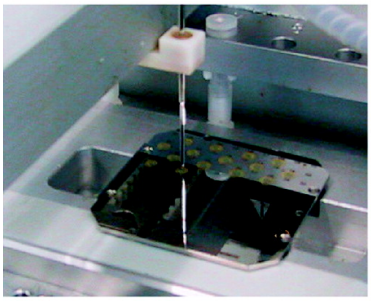

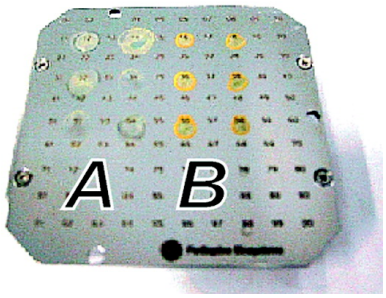

Automated MALDI-TOF-MS Sample Preparation in Combinatorial Polymer Research

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A new automated matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) sample spotting technique that allows the integration of MALDI sample preparation in the workflow of combinatorial polymer research is described. The technique is performed utilizing a commercially available synthetic robot and was first evaluated with polymer standards of known composition and later on used for the monitoring of the living cationic ring-opening polymerization of 2-ethyl-2-oxazoline. The spotting was carried out as a multiple layer approach, which offers the ability of complex sample preparation without the requirement of premixing the different components. The described technique reduces the time required for sample preparation and offers the possibility of automated sample spotting during polymerization reactions performed in a synthetic robot. This allows the integration of molecular weight screening and polymer end/group determination utilizing MALDI-TOF-MS as a high-throughput tool in combinatorial polymer research.

1. Introduction

Combinatorial material research is based on the synthesis of new compounds and the optimization of existing materials. To evaluate the produced new materials, fast screening methods are required to avoid bottlenecks in the workflow. Combinatorial and high-throughput methods in pharmaceuticals research were very successful, which stimulated an increased attention in parallel and combinatorial approaches for the synthesis and discovery of new inorganic materials, catalysts, and organic polymers.[1] The success of combinatorial methods in pharmaceutical research is closely related to the fact that rapid screening of new libraries of compounds on purity (LC/MS) and identifying bioactive materials by standard binding assays is relatively easy.[2] In materials research, parallel and combinatorial techniques started being used intensively during the past decade, since only then have the first high-throughput screening techniques become available.[2]

The concept of high-throughput screening (HTS) dates back to the 1950s and was developed because of the need for fast and automated analysis in clinical testing and medicine.[3] Nowadays, HTS is mainly a field of research in parallel drug discovery[4,5] and catalyst design.[6,7] However, there are a few examples known in which HTS is applied to synthetic polymers in order to obtain information about molecular weight, optical properties, morphology, or other specific properties.[1]

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a very powerful analytical tool for the investigation of properties of synthetic polymers, such as molecular weight, molar mass distribution, and end group analysis.[8,9] Furthermore, mass spectrometry in principle is a highly selective and high-throughput analytical technique that is ideally suited for the identification of a large number of compounds,[10] even in the form of mixtures.[11] During the past few years, several studies have been performed to develop analytical MALDI methods for the fast analysis of a large number of samples,[12,13] and MALDI-TOF-MS has been utilized for the automated identification of proteins[13] as well as for the screening of peptide libraries.[14] Unfortunately, to the best of our knowledge, until now, no studies have been available concerning the screening of polymerization reactions with MALDI-TOF-MS. Therefore, we developed a new automated MALDI sample preparation method that allows fast and easy spotting without the need for matrix-analyte mixing. The technique was evaluated with polystyrene standards and was implemented as a screening tool for the living polymerization of 2-ethyl-2-oxazoline (see, e.g., refs 15 and 16 for that type of polymerization technique).

2. Experimental Details

2.1 Chemicals and Reagents. 1,8,9-Anthracenetriol (dithranol), sodium iodide, and silver hexafluorophosphate were purchased from Sigma Aldrich (Oakville, ON, Canada). Analytical grade chloroform and acetone were purchased from Biosolve LTD (Valkenswaard, The Netherlands). The polystyrene standards were obtained from Polymer Labora-

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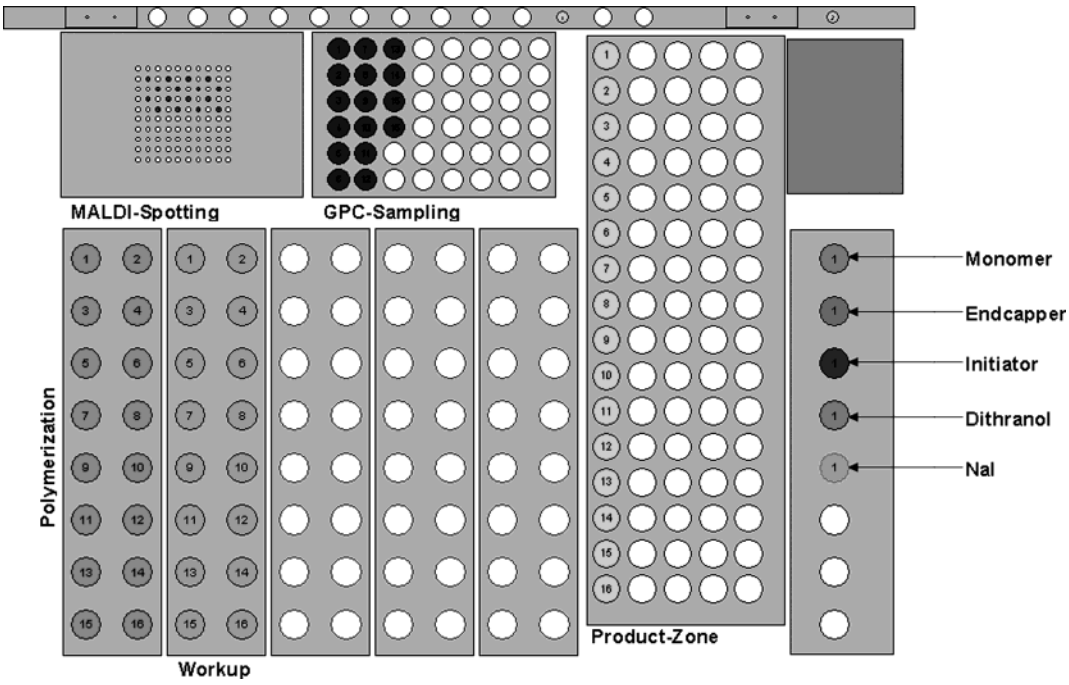
Scheme 1. Layout of the ASW2000 Synthesizer for 16 2-Ethyl-2-oxazoline Polymerizations with Automated Product Workup, GPC Sample Preparation, and MALDI Spotting



tories (Polymer Laboratories Ltd., Church Stretton, Shropshire, U.K.).

2.2 Sample Preparation. Dithranol was utilized as a 20 mg/mL solution in chloroform. Fresh matrix solution was prepared prior to every measurement, because dithranol is known to decompose rapidly[17,18] and, therefore, its effectiveness for the ionization process is reduced. The spotting was performed as a multiple layer approach: First, 1 μL of the analyte solution was dropcast onto the MALDI sample target. Subsequently, 1 μL of salt additives (NaI or $AgPF_6$) and, finally, 1 μL of the matrix were dropcast on top of the analyte spots. The time required by the robotic system to start with the spotting of the next layer was sufficiently long enough to ensure complete drying of the previous spot (at least 90 s).

2.3 Instrumentation. All MALDI experiments were carried out on a Voyager-DE PRO Biospectrometry Workstation (Applied Biosystems, Foster City, CA) time-of-flight mass spectrometer using linear mode for operation. All spectra were obtained in the positive ion mode. Ionization was performed with a 337-nm pulsed nitrogen laser. All data were processed using the Data Explorer software package (Applied Biosystems, Foster City, CA). The automated spotting was carried out on a Chemspeed ASW2000 (Chemspeed Ltd., Augst, Switzerland) automated synthesizer. For the spotting, a needle with 0.8-mm diameter was utilized in combination with a custom-made MALDI target holder (now commercially available from Chemspeed). The sample positions of the MALDI target within the ASW2000 were programmed in the synthesizer software.

3. Results and Discussion

3.1 Spotting Procedure. To integrate molecular weight screening and polymer end group determination by MALDI-TOF-MS as a high-throughput tool in combinatorial polymer research, a new automated spotting technique utilizing a synthetic robot has been developed. This technique makes it possible to obtain and spot samples during polymerization reactions in the synthetic robot and, therefore, allows monitoring of polymerization reactions with MALDI. For example, samples can be spotted directly from the polymerization vessels onto the MALDI target. The spotting of the sample onto the MALDI target was accomplished as a multilayer approach. This is also described in the literature for DNA[19] and peptide analysis,[20] in which the matrix solution was spotted prior to the spotting of analyte solutions onto the target. The spotting was carried out using the liquid handling system of the robotic synthesizer. The solution is aspirated and subsequently spotted onto a defined position on the MALDI target. These positions were programmed into the software of the automated synthesizer on an *xyz* basis. First, 1 μL of the sample solution was spotted onto the target. The time required by the robotic system to finish all sample spots (e.g., from 16 parallel polymerization reactions; compare with Scheme 1) was long enough ($\sim$90 s/spot) to ensure complete drying of the spots before the next layer was applied. Figure 1 (left) shows the needle, which is attached to the robotic arm of the synthetic robot, spotting the matrix solutions onto the MALDI target in the custom-made rack. For the polystyrene standards, the sample solutions were prepared as 5 mg/mL solutions in chloroform, whereas the poly(ethyl oxazolines) were spotted after they were automatically purified in the ASW2000 synthetic robot.[21] In contrast to our recently described sampling method, premixing of analyte, additive, and matrix is no longer necessary, which saves time ($\sim$90 s/sample). As a second step, 1 μL of the salt additive solution was dropcast on top of the analyte. Therefore, readily prepared solutions of NaI and $AgPF_6$ (both in acetone) for polystyrene and poly-

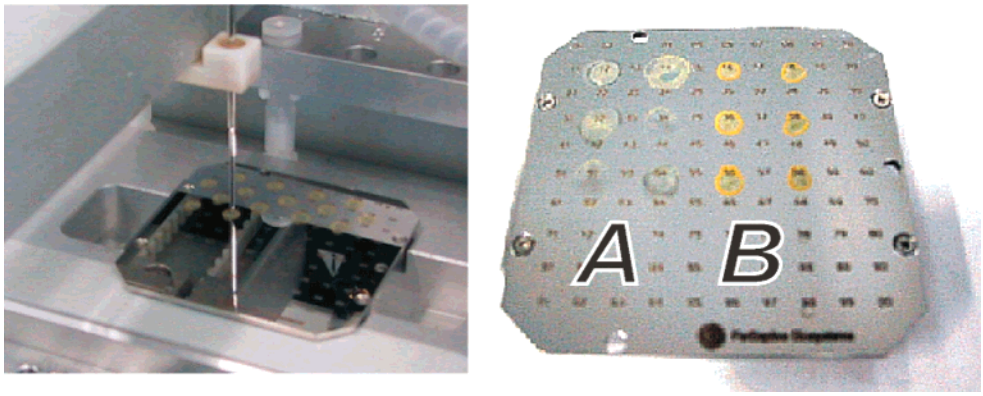


Figure 1. Left: Spotting of matrix solution in the automated synthesizer. The rack is custom-made, and the sample positions were programmed in the synthesizer software. Right: Comparison of automatically (A) and manually (B) spotted samples.

Table 1. Molecular Data of Polystyrene Standards[a]

	data given by manufacturer[b,c]				measured data[d]		
standard	M LS	M_n GPC	M_w GPC	PDI GPC	M_n MALDI	M_w MALDI	PDI MALDI
1	1300	1180	1270	1.08	1491	1585	1.06
2	2050	2010	2100	1.05	2021	2134	1.06
3	3080	2840	2950	1.04	3035	3138	1.03
4	5200	4840	4970	1.03	5140	5413	1.05
5	7500	6930	7150	1.04	7120	7220	1.01

[a] Provided by the manufacturer and obtained by own measurements. [b] LS, light-scattering,. [c] GPC, gel-permeation chromatography. [d] MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

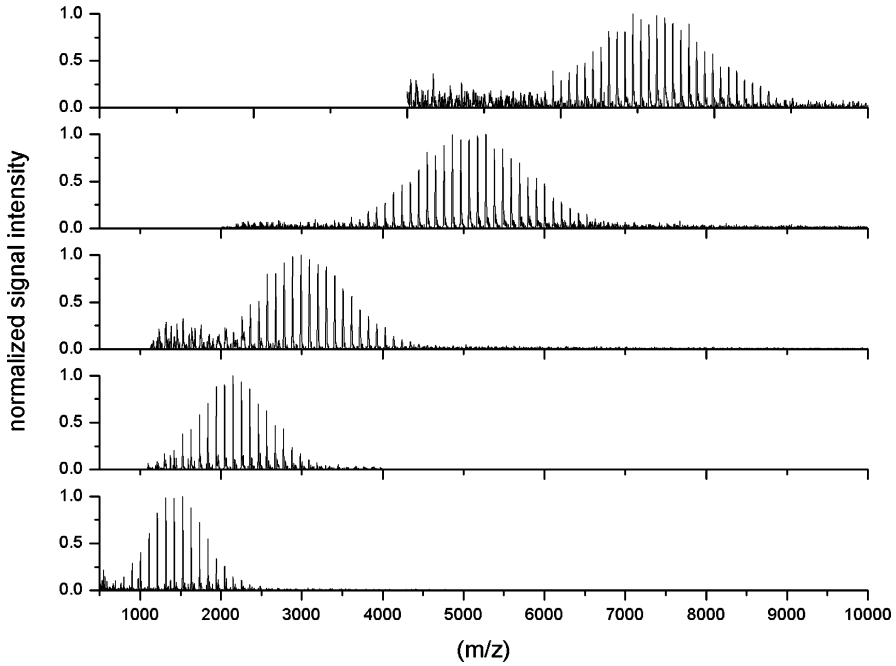


Figure 2. MALDI mass spectra obtained from the automated spotting of polystyrene standards. The high noise in the low molecular mass regions of the spectra is cut off.

(ethyl oxazoline), respectively, were stored within the synthetic robot. As the last step during this sample preparation technique, 1 μL of the matrix solution was spotted as a third layer on top of the other layers. The time required for spotting additive and matrix solutions (~45 s/spot) is shorter than the time required for sample spotting, because no additional rinsing steps of the robot needle are required between the drop-casting of individual spots. This technique allows fast (~3 min/sample), easy, and automated sample preparation within a synthetic robot and, therefore, improves the workflow of combinatorial polymer research and offers high-throughput screening possibilities.

3.2 Evaluation of Polystyrene Standards. Five polystyrene standards with known molar mass and polydispersity were used in order to demonstrate the feasibility of the above-described automated spotting technique. Their molecular weight (M, light-scattering), number average (M_n, GPC) and weight average molecular weight (M_w, GPC), as well as their polydispersity index (PDI, GPC), are provided in Table 1. The samples were measured after the automated spotting

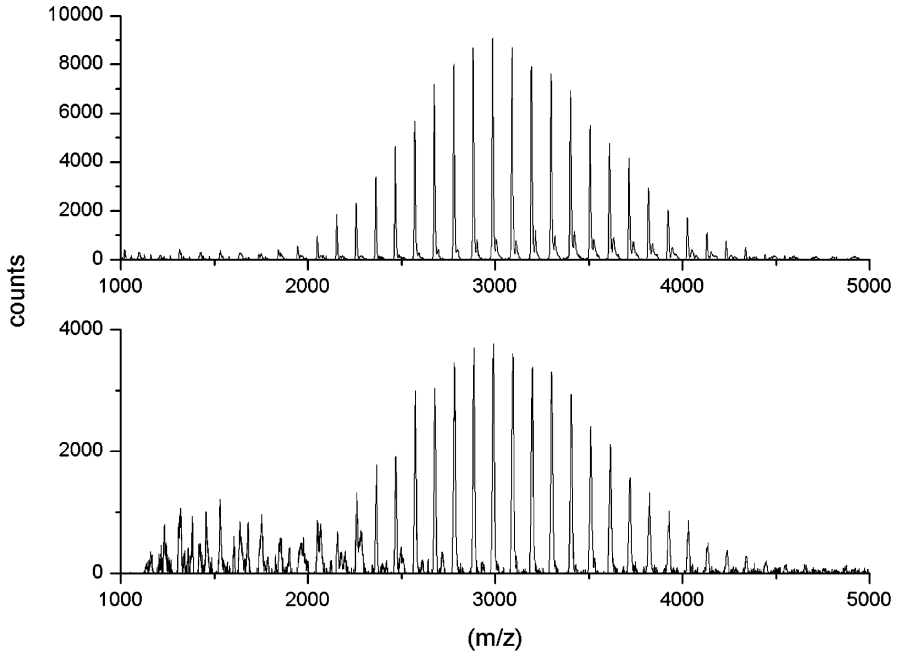
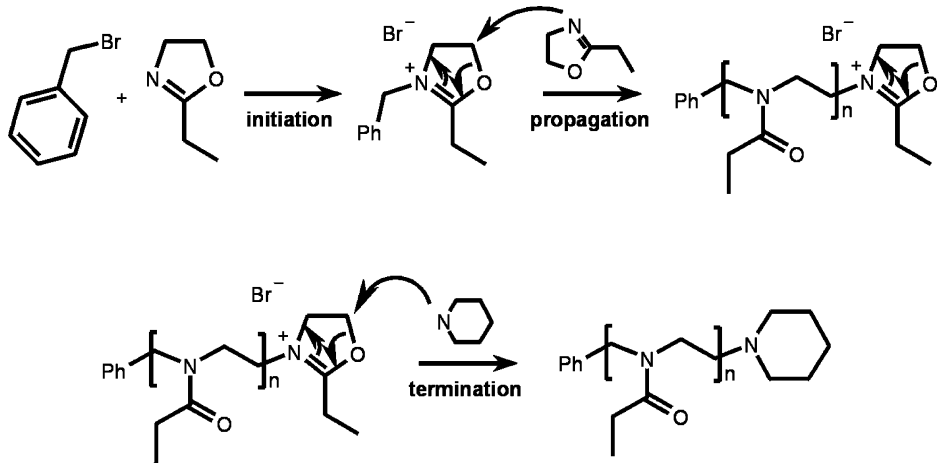


Figure 3. Comparison of manually (top) and automatically (down) spotted polystyrene standard number three. The manually spotted standard reveals a better signal/noise ratio.

Scheme 2. Reaction Mechanism of the Living Cationic Ring-Opening Polymerization of 2-Ethyl-2-oxazoline



resulting in good resolved spectra of the polystyrene standards (see Figure 2). The difference between two peaks was found to be 104 mass units, which corresponds to the mass of polystyrene monomer units. M_n and M_w were calculated with the Data Explorer software and were found to be in good correlation with the data supplied by the manufacturer of the standards (compare Table 1). The light-scattering data reveals a better correlation, whereas the GPC data is always too low, but this is understandable, since light scattering is an absolute analytical technique, such as MALDI, and GPC is a relative one. The GPC data are required for the comparison of the PDIs, which also revealed good correlation between measured and provided data. In addition, the multiple layer approach was carried out manually in the same way and the same amount of solutions as described earlier. Figure 3 displays polystyrene standard number 3 from manually (top) and automatically (down) spotted samples. These results show clearly that the molecular weight distribution is not changed as a result of sample preparation. Nevertheless, the manually spotted samples showed better signal-to-noise ratios than the automatically spotted ones (compare Figure 3). This could be an effect of the more accurate manual spotting, which resulted in more defined spots (compare Figure 1, right). This could be overcome in the future by using thinner needles or other solvents. Samples were also prepared manually by premixing all components and subsequently spotting 1μL of this mixture onto the MALDI target. This resulted in poorly crystalline spots, which were measurable only for the lower molecular weight samples with very high laser intensities. This outlines the advantage of the multiple layer approach. Therefore, the described automated MALDI sample preparation technique can be utilized for the evaluation of the molecular weight and its distribution for synthetic polymers. In general, it should be mentioned that the most difficult part in MALDI analysis is the sample preparation, since this step is crucial for the success of the MALDI experiment.[22] Therefore, this spotting technique can be integrated into the workflow for

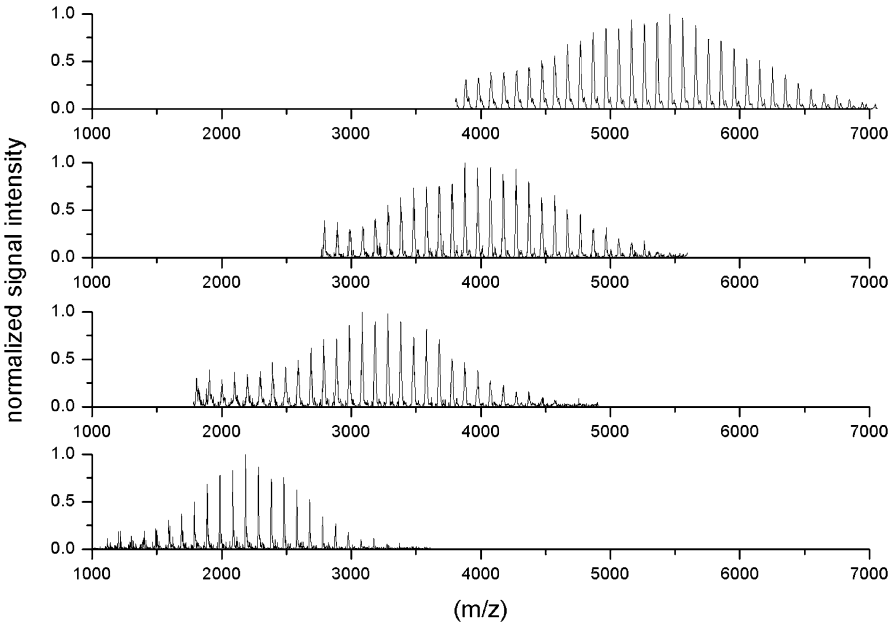


Figure 4. MALDI mass spectra from automatically spotted poly(ethyl oxazoline) samples. The results reveal a good correlation of [monomer]/[initiator] ratio and obtained molar mass, which outlines the livingness of this polymerization.

combinatorial polymer research, offering faster and easier sample preparation as well as the possibility of high-throughput screening of polymerization reactions.

3.3 Screening of a Living Polymerization. The cationic ring-opening polymerization of 2-ethyl-2-oxazoline (see Scheme 2) was screened by the described high-throughput automated spotting technique.[21] The polymers were prepared utilizing the ASW2000 automated synthesizer, and the samples were prepared from the automatically purified products (precipitation at −20 °C in diethyl ether). Scheme 1 provides an overview of the combinatorial workflow within the synthetic robot. In this example, 16 polymerizations can be carried out in parallel with automated workup and sample preparation for GPC and MALDI. Molar mass characterization of the poly(ethyl oxazolines) with MALDI was possible up to 7000 Dalton, which represents an important improvement, as compared to sample preparation without NaI as additive. Furthermore, the sample preparation technique was simplified, as compared to previously described methods,[21] as a result of the multilayer spotting approach. This procedure saves time (∼90 s/sample) and valuable space within the synthetic robot and does not waste product for sample preparation, because premixing of analyte, additive, and matrix solutions is not required. The obtained spectra from the automated spotting procedure are shown in Figure 4. A linear relationship between the obtained M_n and the [monomer]/[initiator] ratios was obtained as it is also described in the literature.[21] The difference between the peaks was found to be 99 mass units, which corresponds to the mass of an ethyl oxazoline monomer unit. End group analysis confirmed that the polymers were initiated with benzyl bromide and terminated with piperidine (compare with Scheme 1). The results from the MALDI-TOF-MS experiments support a controlled polymerization mechanism for the polymerization of 2-ethyl-2-oxazoline in the automated synthesizer.

4. Conclusion

An automated sample preparation technique for MALDI-TOF-MS that offers the possibility to integrate MALDI as a high-throughput technique for combinatorial polymer research has been developed. This automated spotting technique was first evaluated with polymeric standards of known molecular weight and polydispersity and later on applied to the screening of the living polymerization of 2-ethyl-2-oxazoline in the automated synthesizer. Compared to automatically spotted samples, manually spotted samples revealed better signal-to-noise ratios, but the results obtained from both spotting techniques for the molecular weight and its distribution were in good agreement. Therefore, automated MALDI sample preparation within a synthetic robot that performs polymerization reactions, as it is described here, is a valuable tool for combinatorial polymer research, which optimizes the combinatorial workflow, allows high-throughput screening of polymerization reactions, and saves time and manual work. Moreover, multiple-layer spotting for MALDI-TOF-MS sample preparation is fast, easily applicable, reproducible (compare to ref 23), and offers more flexibility (if compared to conventional sample preparation) for the integration into the combinatorial workflow.

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References and Notes

(1) Hoogenboom, R.; Meier, M. A. R.; Schubert, U. S. *Macromol. Rapid Commun.* **2003**, *24*, 15−32.
(2) Tuinstra, H. E.; Cummins, C. H. *Adv. Mater.* **2000**, *12*, 1819−1822.
(3) McFarland, E. W.; Weinberg, W. H. *Trends Biotechnol.* **1999**, *17*, 107−111.
(4) Moore, J. M. *Curr. Opin. Biotechnol.* **1999**, *10*, 54−58.
(5) Cheng, X.; Hochlowski, J. *Anal. Chem.* **2002**, *74*, 2679−2690.

(6) Reetz, M. T. *Angew. Chem.* **2002**, *114*, 1391−1394; *Angew. Chem., Int. Ed.* **2002**, *41*, 1335−1338.
(7) Hagemeyer, A.; Jandeleit, B.; Liu, Y.; Poorjary, D. M.; Turner, H. W.; Volpe, A. F., Jr.; Weinberg, W. H. *Appl. Catal., A* **2001**, *221*, 23−43.
(8) Nielen, M. W. F. *Mass Spectrom. Rev.* **1999**, *18*, 309−344.
(9) Weidner, S.; Kühn, G.; Friedrich, J. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1373−1381.
(10) Shin, Y. G.; van Breemen, R. B. *Biopharm. Drug Dispos.* **2001**, *22*, 353−372.
(11) Chelius, D.; Bondarenko, P. V. *J. Proteome Res.* **2002**, *1*, 317−323.
(12) Keil, O.; LeRiche, T.; Deppe, H.; Volmer, D. A. *Rapid. Commun. Mass Spectrom.* **2002**, *16*, 814−820.
(13) Ekström, S.; Önnerfjord, P.; Nilsson, J.; Bengtsson, M.; Laurel, T.; Marko-Varga, G. *Anal. Chem.* **2000**, *72*, 286−293.
(14) Graven, A.; St. Hilaire, P. M.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 441−452.
(15) Aoi, K.; Okada, M. *Prog. Polym. Sci.* **1996**, *21*, 151−208.
(16) Schubert, U. S.; Heller, M. *Chem. Eur. J.* **2001**, *7*, 5252−5229.
(17) Taskinen, J.; Haarlara, J.; Wartiovaara, E.; Halmekoski, J. *Arch. Pharm. (Weinheim, Ger.)* **1988**, *321*, 103−106.
(18) Elsabbagh, H. M.; Whitworth, C. W.; Schramm, L. C. *J. Pharm. Sci.* **1979**, *68*, 388−390.
(19) Garcia, B. A.; Heaney, P. J.; Tang, K. *Anal. Chem.* **2002**, *74*, 2083−2091.
(20) Vorm, O.; Roepstorff, P.; Mann, M. *Anal. Chem.* **1994**, *66*, 3281−3287.
(21) Hoogenboom, R.; Fijten, M. W. M.; Meier, M. A. R.; Schubert, U. S. *Macromol. Rapid Commun.* **2003**, *24*, 92−97.
(22) Hanton, S. D. *Chem. Rev.* **2001**, *28*, 527−569.
(23) Meier, M. A. R.; Schubert, U. S. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 713−716.

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