Capillary electrophoresis for the characterization of synthetic polymers
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Chapter 5

Characterization of metallo *bis*(terpyridine) diblock polymers by non-aqueous capillary zone electrophoresis

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Abstract

A non-aqueous capillary zone electrophoresis (CZE) method has been developed to characterize block (co)polymers of poly(ethylene oxide) and poly(styrene) containing metallo *bis*(terpyridine) complexes as bridging units. Specific CZE separation conditions had to be applied, with barium perchlorate dissolved in *N*-methylformamide (NMF) as background electrolyte and OV-1701-OH deactivated capillaries. For detection UV absorption was measured at a wavelength of 316 nm. Metallo diblock polymers with molecular weights up to 30,000 Da could be analysed by the proposed non-aqueous CZE method. Experiments performed with polymeric compounds containing Fe, Ni or Ru as central metal ions showed that their electrophoretic mobilities were independent of the type of metal ion. Therefore, the data on the size of the polymeric compounds could be obtained using just one set of calibration standards. Polydispersities of the samples calculated from the experimental results could be correlated with the polydispersities of the polymers used in the synthesis of the metallo diblock polymers.

Several polymeric samples contained metallo *mono*(terpyridine) complexes as impurities. These by-products could be separated from the main product. With symmetrical diblock polymers only one by-product was detected, while with an asymmetrical diblock polymers two types of mono complexes were observed. The amount of the mono complexes present as impurities was dependent on the type of central metal ion (Ni > Fe >> Ru).
Chapter 5

Introduction

A new type of block polymers, bearing metallo bis(terpyridine) complexes as bridging units, was introduced in the field of macromolecular chemistry several years ago. A schematic representation of the polymeric structure of the new type of polymers is shown in Figure 5.1. An important feature of the metallo block polymers is the reversibility of the metal-to-ligand coordination, due to the relatively weak non-covalent interaction between the central metal ion and the terpyridine ligands. This property may be interesting for the design and application of smart and switchable materials [1].

![Diagram of metallo block polymer structure]

Figure 5.1 Structure of the metallo bis(terpyridine) complexed diblock polymers e.g. PEO-[M]-PEO, PS-[M]-PS and PEO-[M]-PS with different chain lengths.

The strategy applied for producing this new type of polymers is claimed to make it feasible to synthesize materials in a highly reproducible way and with a perfect control of the molecular architecture [2, 3]. In principle, block polymers of all traditional polymeric compounds can be produced using the metallo terpyridine complex as a linker. The degree of polymerisation and the chemical composition of the polymeric blocks affect the properties of the polymers such as their polarity, solubility and micelle formation.

So far, mostly linear poly(ethylene oxide) (PEO) or poly(styrene) (PS) with different chain lengths have been utilized as constituting polymers. A variety of transition-metal ions (Fe, Co, Ni, Cu, Zn, Ru, Cd, Hg) in their low oxidation state form complexes with terpyridine ligands. The main effects of the type of transition-metal ion in the polymeric complexes are on the sensitivity to oxidation and
Characterization of metallo bis(terpyridine) diblock polymers by nonaqueous CZE

on the stability of the compound, which is related to the strength of the metal-to-ligand interaction. By changing the type of polymeric chains and central metal ions, it is possible to produce polymeric compounds suitable for specific applications.

When working with new polymeric materials, detailed information on the molecular architecture and properties, such as the molar-mass distribution (MMD), chemical composition and polymer stability, is essential. Size-exclusion chromatography (SEC), often in combination with refractive-index detection, is by far the most often applied technique for the determination of the MMD of (synthetic) polymers. However, SEC measurements of the metallo block polymers are quite complicated, because of the interaction of the charged metal ions with the stationary phase. With most SEC systems studied, the repeatability and reproducibility of the separations were unsatisfactory. A SEC system with a specific column has been used to characterize diblock polymers containing ruthenium as the metal ion [4]. However, the data on the polydispersity obtained did not correspond with the expected values, which indicated that secondary interaction effects between the ruthenium complexes and stationary phase of the column might have occurred.

Another technique for the analysis of synthetic polymers is matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Mono complexes were detected by MALDI-TOF-MS, due to fragmentation of the metallo bis(terpyridine) diblock polymers [4, 5]. Although MALDI is a soft ionisation technique, the observed ratio of free ligands to metallo di-complexes increased with increasing the intensity of the laser used for spectrum acquisition. Even at low laser intensities, free ligands were detected. Therefore, with this technique it was impossible to determine impurities in the original polymeric products and the MMD of the metallo polymeric compounds could not be accurately measured. On the other hand, this behaviour made it possible to investigate the relative binding strengths of the metal-to-ligand coordination [5].

Since SEC and MALDI-TOF-MS appear to be problematic for the determination of the MMD of the metallo diblock polymers, another characterization technique is required. It has already been demonstrated that capillary zone electrophoresis (CZE) is an efficient separation technique for the analysis of various synthetic macromolecules [6]. Since most of the diblock polymers containing terpyridine-metal-complexes are hydrophobic and only soluble in organic solvents, it is necessary to apply a CZE system with an organic solvent as the background solution.

Walbroehl and Jorgenson [7] first demonstrated the CZE separation of non-polar analytes carried out with pure acetonitrile. They introduced the keyword non-aqueous CE for this type of system.
Other organic solvents suitable for electrophoretic separation systems are, for example, methanol, propylene carbonate, dimethyl sulfoxide (DMSO), formamide, N-methylformamide (NMF), and N,N-dimethylformamide (DMF) [8]. The type of organic solvent can affect all relevant parameters of electromigration separations. This offers an additional way to optimize the resolution and efficiency of the separation of specific analytes.

We present the development of a non-aqueous CZE system for the characterization of the new type of block polymers containing metallo *bis* (terpyridine) complexes as linkers. Especially for this type of polymers, the choice of organic solvent, background electrolyte, and type of capillary appeared to be critical for a successful separation of the polymeric compounds. Data on the MMD and information on the purity of the polymeric samples could be obtained with the proposed non-aqueous CZE method.

**Experimental**

**Chemicals**

Acetonitrile (HPLC grade) was obtained from Rathburn Chemicals Limited (Walkerburn, Scotland) and *N*-methylformamide 99% (NMF) came from Aldrich (Steinheim, Germany). Barium perchlorate and mesityl oxide were from Fluka (Buchs, Switzerland). Anhydrous lithium chloride was obtained from Across Organics (Geel, Belgium).

The diblock polymers containing metallo *bis* (terpyridine) complexes as bridging units were synthesized at the Technical University of Eindhoven (Eindhoven, The Netherlands). The polymers provided included homo- and hetero structures of PEO and PS with different degrees of polymerization, and with different central metal ions (Fe, Ni, Ru) in their low oxidation state (2+). Details on procedures for the synthesis of the block PEO and PS (co)polymers with various metal ions have been published [9, 10].

The diblock polymers were dissolved either in acetonitrile or in NMF at concentrations of 1-5 g l⁻¹ depending on the molar mass (MM) of the compounds. To each sample solution the monomeric compound (EO-[M]-EO) at a final concentration of 0.1 g l⁻¹ was added. The sample solutions contained 0.2% (v/v) mesityl oxide, which was the marker for the velocity of the electro-osmotic flow (EOF).
Apparatus and procedures

All CE experiments were carried out on an Agilent CE instrument equipped with a diode-array detector (Waldbronn, Germany). The Chemstation CE software (Agilent) was used for instrument control and data acquisition. UV detection was performed at a wavelength of 316 nm with a bandwidth of 16 nm. Absorption spectra in the range of 220 – 350 nm were acquired.

Solutions of LiCl or Ba(ClO₄)₂ in acetonitrile or NMF (ionic strengths of 5 mM) were applied as the background electrolyte. Most experiments were performed with fused-silica capillaries deactivated with OV-1701-OH, which were obtained from BGB Analytik (Adliswil, Switzerland). The capillary dimensions were 75 μm I.D. × 375 μm O.D. with a total length of 38.8 cm and a UV detection window at 30 cm. The polyimide coating was removed from both the capillary ends to avoid undesired effects on the repeatability of injection. New capillaries were pretreated by flushing the background solution at 1 bar for 5 min., followed by a voltage of 20 kV for 15 min. Prior to the separation the capillary was flushed with the background solutions at 1 bar for 1 min. Injections were performed hydrodynamically, typically by a pressure of 20 mbar for 3 s. The analyses were carried out with a voltage of 20 kV at a temperature of 25°C.

Results and discussion

Non-aqueous CZE

It was required to select non-aqueous CZE conditions for the analysis of the metallo bis(terpyridine) diblock polymers, since the polymers containing PS chains are not water-soluble. Organic solvents suitable to dissolve the polymeric compounds are acetonitrile and NMF. Both solvents have been previously applied as separation media in CZE to analyze non-polar compounds [7, 11]. Solvent properties are given in Table 5.1. The ratios of dielectric constant over viscosity (κ/η) of acetonitrile and NMF are similar. Therefore, ionic analytes will migrate with mobilities of the same order of magnitude in acetonitrile or NMF. The κ/η-ratio is also an important parameter for the magnitude of the EOF. However, for the latter the degree of dissociation of the silanol groups is also a key factor. The effect of the choice of solvent on this factor is unfortunately difficult to predict. Still, both acetonitrile and NMF can be regarded as promising solvents for non-aqueous CE of synthetic polymers. The main disadvantage of acetonitrile is its low boiling point, whereas the difficulty with NMF could be its high UV- background absorbance at wavelengths below 240 nm. In this work,
acetonitrile and NMF were investigated as separation media to analyse the metallo-*bis*(terpyridine)-containing macromolecules.

In principle, CE separations can be performed in pure NMF since its dielectric constant is high (ε ~182.4). However, such a system will be easily overloaded. Therefore, it is necessary to add a background electrolyte to the separation solvent. The salts mostly applied in aqueous buffer solutions are not soluble in acetonitrile or NMF. Of the many (inorganic) salts tested, only with LiCl and Ba(ClO₄)₂ as background-electrolyte salts did we succeed in preparing ionic solutions in both organic solvents.

Mesityloxide was used as a marker compound to study the velocity and direction of the EOF in acetonitrile and NMF. Results obtained are given in Table 5.2. In general, for the separation of positively charged analytes an EOF with a low velocity is preferred, and LiCl in acetonitrile is therefore not the first choice. With Ba(ClO₄)₂ in acetonitrile an EOF with a reversed direction and a reduced velocity was found in a fused-silica capillary. In acetonitrile barium ions apparently interact strongly with the deprotonated silanol groups on the capillary inner wall, which causes a positive surface charge and a reversed direction of the EOF. This charge reversal can be advantageous for the separation of the positively charged metal ion complexes, since the polymeric compounds will be repelled from the capillary inner wall and, moreover, they will migrate against the EOF. However, with acetonitrile the UV-baseline stability and the run-to-run repeatability of the EOF were poor. Replacing in-and outlet vials before each run did not improve the repeatability of the EOF velocity. Because of this, NMF was used as background solvent in further work. In NMF similar EOF velocities were found with LiCl and Ba(ClO₄)₂.

### Table 5.1 Solvent properties of acetonitrile and NMF [8].

<table>
<thead>
<tr>
<th>Solvent</th>
<th>η (mPa·s)</th>
<th>ε</th>
<th>ε/η</th>
<th>T_b (°C)</th>
<th>UV cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>0.341</td>
<td>35.94</td>
<td>105.4</td>
<td>81.6</td>
<td>190</td>
</tr>
<tr>
<td>NMF</td>
<td>1.65</td>
<td>182.4</td>
<td>110.5</td>
<td>~199.5</td>
<td>240</td>
</tr>
</tbody>
</table>

### Table 5.2 The mobility of the EOF (10⁻⁹ m² V⁻¹ s⁻¹) in various non-aqueous systems.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Fused-silica capillary</th>
<th>OV-1701-OH deactivated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LiCl</td>
<td>Ba(ClO₄)₂</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>88.5</td>
<td>-22.5</td>
</tr>
<tr>
<td>NMF</td>
<td>38.9</td>
<td>39.4</td>
</tr>
</tbody>
</table>

*not measured*
In preliminary experiments it was found that several types of metal complexes interacted strongly with the silanol groups of the bare fused-silica capillary inner wall using either LiCl or Ba(ClO$_4$)$_2$ in NMF. When a capillary with an OV-1701-OH deactivated inner surface was installed, such interactions could be avoided. With this deactivated capillary the EOF mobility was significantly lower than with a bare fused-silica capillary. The reduced EOF mobility provided somewhat higher separation efficiencies.

All diblock polymer samples were analysed with a deactivated capillary and a background electrolyte of Ba(ClO$_4$)$_2$ in NMF at an ionic strength of 5 mM. Figure 5.2 shows an electropherogram typical for the separation of the polymers. The symmetrical diblock PS$_{20}$ polymer depicted contained Ru$^{II}$ as the central metal ion. The corresponding monomeric compound (EO-[Ru]-EO) was used as a reference. Plate numbers were in the order of 260,000 for the monodisperse standard. With this CZE system separations of the metallo polymers with molar masses up to 30,000 Da were achieved within 10 min. UV-absorption detection was performed at a wavelength of 316 nm, which was specific for the metallo bis(terpyridine) complex. The UV signal at 245 nm could be used to specifically detect the PS containing polymers.

Samples of PEO$_{70}$-[M]-PEO$_{70}$ containing Fe, Ni and Ru in the 2+ oxidation state were compared. The electropherograms obtained experimentally showed similar peak top mobilities for the

![Electropherogram](image-url)

**Figure 5.2** Non-aqueous CZE separation of PS$_{20}$-[Ru]-PS$_{20}$ with EO-[Ru]-EO as internal standard (IS). Background electrolyte: Ba(ClO$_4$)$_2$ in NMF at an ionic strength of 5 mM. Voltage: 20 kV.
investigated compounds. This demonstrates that the type of transition metal ion has no significant influence on the charge-to-size ratio of the metallo polymeric compounds. It makes the separation of a mixture of polymers with different metal ions impossible. On the other hand, it allows for a simple MMD characterization of the polymer samples using just one set of reference materials.

**Determination of the MMD**

The average molar mass and the polydispersity of (synthetic) polymers are important parameters for product performance. Previously, it has been demonstrated that CZE can be a useful tool to determine the MMD of linear synthetic polymers with a fixed charge [12]. Since all metal ions in the diblock polymers have the same oxidation state (+2), for this kind of polymers it can also be expected that their electrophoretic mobility reflects the effective size of the polymeric compound. Analyses were performed with symmetrical ruthenium bis(terpyridine) complexes with PEO chain lengths between 1 and 375 monomeric units. It was found that the inverse of the electrophoretic mobility increased linearly with the degree of polymerization of the symmetrical diblock PEO polymers. This observed relationship was used for the determination of the polydispersity of the polymer samples.

Electropherograms obtained experimentally were translated into MMDs using a home-written Excel program. As an example, Figure 5.3 shows the translation of the electropherogram of PEO$_{70}$-[Ru]-PEO$_{70}$ into its MMD. By integration of the transformed data, the values of peak-molar mass ($M_p$), weight-average molar mass ($M_w$) and the polydispersity of the polymers were obtained. In some separations of the metallo polymers additional peaks showed up in the electropherograms that were clearly related to an impurity in the polymeric sample. For the calculation of the polydispersity of the main polymer the minor peak of the by-product was not taken into account. In Table 5.3 the data on the average molar mass and polydispersity of the investigated metallo diblock polymers as determined by the proposed non-aqueous CZE method are summarised. All synthesized polymers had polydispersity values in the range of 1.02-1.06. These values were similar to the polydispersities of the individual PEO and PS standards utilized to prepare the diblock polymers. The calculated $M_p$ values of the mono- and diblock PS polymers were close to their nominal molar-mass values. This demonstrated that these types of polymers behaved very similarly in the CZE system as the symmetrical PEO diblock polymers.
Figure 5.3  Calculated MMD of PEO70-[Ru]-PEO70 as obtained from a CZE separation.

<table>
<thead>
<tr>
<th>Metallo polymer</th>
<th>nominal MM</th>
<th>( M_p ) (Da)</th>
<th>( M_w ) (Da)</th>
<th>polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEO70-[Ru]-PEO70</td>
<td>6,700</td>
<td>7,100</td>
<td>7,200</td>
<td>1.02</td>
</tr>
<tr>
<td>PEO125-[Ru]-PEO125</td>
<td>11,600</td>
<td>11,200</td>
<td>12,700</td>
<td>1.03</td>
</tr>
<tr>
<td>PEO225-[Ru]-PEO225</td>
<td>20,400</td>
<td>21,600</td>
<td>27,000</td>
<td>1.06</td>
</tr>
<tr>
<td>PS20-[Ru]-PS20</td>
<td>4,800</td>
<td>3,900</td>
<td>4,400</td>
<td>1.03</td>
</tr>
<tr>
<td>PEO70-[Ru]-PS20</td>
<td>5,750</td>
<td>5,400</td>
<td>5,900</td>
<td>1.02</td>
</tr>
<tr>
<td>PEO70-[Fe]-PEO70</td>
<td>6,700</td>
<td>7,400</td>
<td>8,000</td>
<td>1.02</td>
</tr>
<tr>
<td>PS20-[Fe]-PS20</td>
<td>4,800</td>
<td>4,200</td>
<td>4,600</td>
<td>1.03</td>
</tr>
<tr>
<td>PEO70-[Ni]-PEO70</td>
<td>6,700</td>
<td>7,100</td>
<td>7,500</td>
<td>1.02</td>
</tr>
<tr>
<td>PS20-[Ni]-PS20</td>
<td>4,800</td>
<td>4,000</td>
<td>4,900</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Data calculated using calibration curve obtained using PEO-[Ru]-PEO standards

Table 5.3  Experimental values of the average molar mass and polydispersity of the mettallo diblock polymers.
Chapter 5

Determination of by-products

As already mentioned above, some metallo diblock polymers contained by-products that migrated with a higher mobility than the main compound in the CZE system. An example is shown in Figure 5.4, which shows the separation of a symmetrical PS$_{20}$-[Ni]-PS$_{20}$ sample. The impurities are clearly related to the original monomeric- and polymeric compounds. There are two possible explanations for the higher charge-to-size ratio of the by-products: (i) oxidation of the transition metal ion to the 3+ state, or (ii) instability of the metallo-bis-complexed polymer with formation of the metallo mono-complex. When the oxidation process is the source of the formation of the by-products, it can be expected that the quantity of the by-products would be related to the tendency of the metal ions to oxidize. Iron and ruthenium can be oxidized relatively easily from 2+ to 3+, while the nickel ions have a much higher oxidation potential. The quantities of by-products observed were related to the type of central metal ion in the order Ni > Fe >> Ru. From this, it is unlikely that the oxidation of the metal ions is the cause of the impurities in the polymeric samples; incomplete diblock formation is a more likely explanation. Results obtained by MALDI-TOF-MS showed a similar order of complex instability as found here [5].

The calibration curve used for the determination of the MMDs of the diblock polymers was also used for the determination of the average molar masses and polydispersities of the impurities. For almost all by-products somewhat higher M$_p$ values were calculated than expected. A possible reason for this is that the effective sizes of the mono- and diblock complexes are not completely comparable, since both types of compounds differ slightly in molecular architecture. The polydispersities of the by-products were equal to the values for the main diblock polymers. While in the symmetrical diblock polymers a single mono-complex was found as impurity, in the separation of the asymmetrical block polymer PEO$_{70}$-[Ru]-PS$_{20}$ three additional peaks were observed (Figure 5.5). The peaks corresponding to the metallo complexes containing single PS$_{20}$ or PEO$_{70}$ chains were clearly determined. Similar fragmentation behaviour as seen in MALDI-TOF-MS measurements of the same kind of polymer were observed [4]. For the additional peak that would correspond to a high-MM compound no obvious explanation could be found.

It did not become clear whether the original samples contained impurities or that these were formed during dissolution in NMF. In any case, the polymeric compounds were stable on the time-scale of the CZE experiments, since the by-products and main polymeric compounds were detected as individual peaks.
Characterization of metallo bis(terpyridine) diblock polymers by nonaqueous CZE

Figure 5.4  Electropherogram of a symmetrical PEO$_{70}$-[Ni]-PEO$_{70}$ polymer containing mono(terpyridine) complexes as impurities. Conditions as in Figure 5.2.

Figure 5.5  MMD of the asymmetrical diblock polymer PEO$_{70}$-[Ru]-PS$_{20}$ including by-products.

95
Conclusion

Since the determination of the size of metallo diblock polymers could not be accurately obtained with classical techniques such as SEC or MALDI-TOFMS, we developed a non-aqueous CZE method for the characterization of this new type of polymers. The proposed method allowed for the fast and efficient separation of diblock polymers with molar masses up to 30,000 Da according to their charge-to-size ratio.

Diblock polymers with different types of transition-metal ions behaved similarly in the separation system. It appeared that the determination of the MMD of the polymers is simple using a calibration curve obtained using just one series of standards. It was found that the synthesised diblock PEO and PS polymers had polydispersities in the range of 1.02 - 1.06. The observed values were similar to the data on the polydispersity of the original polymeric compounds. This demonstrated one of the advantages of the applied synthesis strategy, namely good control over the polydispersity of the polymers.

Above and beyond the determination of the degree of polymerisation of the metallo polymers, the CZE method can also be used to determine the presence of mono complexes in the polymeric samples. In the separations of the polymeric compounds containing nickel as the central metal ion high amounts of mono complexes were detected, while the ruthenium complexes were more stable.

Acknowledgement

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References
