Poly(amido-amines)s with novel molecular architecture: Synthesis and thermodynamic studies of protonation and metal [Cu(II), Zn(II)] ion complexes

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Introduction

Since the early work of Danusso and Ferruti,[1] many poly(amido-amine)s (PAAs) have been synthesized and studied for many different applications.[2-3] They show considerable potential for biomaterial application[2-4] and for the selective chelation of transition metal ions.[3,7,8]

More recently, they have attracted attention as soluble drug carriers[9,10] and membrane-active polymers with fusogenic properties.[11,12]

Linear PAAs belong to a class of water-soluble polymers containing amido and tertiary amino groups regularly arranged along the macromolecular chain; they can be obtained readily in water or alcohols, at room temperature and without catalysts, by the general Michael-type reaction based on the hydrogen transfer polyaddition of primary or secondary amines to bis-acrylamides.[11]

The ionization of tertiary amino groups results in a remarkable conformational change from a relatively coiled to a hydrophilic relaxed open structure.[13] This also causes a stiffening of the macromolecular coil which enables weak interactions between the negative charges of rod-like biological molecules. Besides complexing with transition metal ions,[13,14] some PAAs bind heparin at a physiological pH.[15] It has been found that lengthening...
of the hydrocarbon chain between two tertiary nitrogen atoms in PAA sharply decreases the ability of metal ion complexation,\(^{10}\) whereas it increases the ability to form ionic complexes with heparin.\(^{17}\) This was correlated with the high charge density at physiological pH values as a consequence of the higher nitrogen basicity.\(^{13,18}\) PAA can therefore be tailored showing a basic strength according to needs.

The thermodynamics of protonation and copper(II) complex formation for many PAAAs studied in the past showed a peculiar polyelectrolyte behaviour in aqueous solutions. The basicity constants (logK) and the enthalpy changes (\(-\Delta H^0\)) have been found to be “real”, i.e. not dependent on the pH of the solution, meaning that the monomer unit behave like separate low-molecular-weight analogues.\(^{13,19}\) The typical polyelectrolyte behaviour was attributed to the shielding effectiveness of the rigid bisamidic structure, because any attempt to its modification or replacement by other different shielding groups, caused an “apparent” instead of the “real” behaviour.\(^{20}\)

In order to gain further insight into the structural reasons for this unusual trend, we have modified the monomer unit of PAAAs by shortening the methylene chain between the amido and the amino groups. A new synthetic route was studied to obtain the new series of polymers, abbreviated as SPAAs. The SPAAs have essentially the same composition like the PAAAs, but contain only one methylene group as spacer between the amido and tertiary-amino groups of the backbone chain.

The aim of the present paper is to widen the protonation and complex formation study of PAAAs by reporting the thermodynamic results of the protonation and of the coordination towards copper(II) and zinc(II) ions of three SPAAs (Tab. 1). The study should clarify the effect of the chain length between the two tertiary basic nitrogenss (SPAAs derived from \(N,N'\)-ethylene diamine, SPAA-1, and \(N,N'\)-hexamethylenediamine, SPAA-2) and the rigidity of the monomer unit (SPAA derived from 2-methyl-piperazine, SPAA-3). The investigations were performed at 25°C in 0.1 M NaCl by means of thermodynamic (potentiometry, solution calorimetry, viscometry) and spectroscopic (UV-visible) methods.

**Experimental part**

**Materials**

Simple diamines (\(N,N'\)-dimethyl-ethylene-diamine, DMEDA; \(N,N'\)-dimethyl-hexamethylenediamine, DMEXA; 2-methyl-piperazine, 2-MePip), chloro-acetyl-chloride, bromo-acetyl-bromide, calcium hydroxide, sodium hydroxide, chloroform (stabilized with amilene), \(N\)-methyl-piperidone (NMP) and dimethylsulphoxide (DMSO) were obtained from Fluka and used as received.

**Syntheses**

**Synthesis of bisbromoacetylpiperazine (BBP):** To a chloroform solution (450 mL) of piperazine (44.6 g, 0.51 mol) 60 mL of distilled water were added at a temperature between –5 and 0°C using an external cooling bath for maintaining the temperature. To this solution 100 mL of bromoacetyl bromide (1.13 mol) and contemporaneously 113 mL of a 10 M aqueous sodium hydroxide (1.13 mol) solution were added dropwise. At the end of the addition the reaction mixture was stirred for 40 min while reaching to the room temperature. The reaction mixture was then extracted with distilled water until the pH of the extracting liquids was neutral. The part of the products that precipitated was separated by filtration and washed with methanol. The remaining chloroform solution was anhydried (sodium sulphate) and evaporated to dryness in vacuo. The residue was dried to constant weight in vacuo, yielding a further crop of product. The product was found to be sufficiently pure as obtained and was used in further polymerization steps. Yield: 72%, m.p. 159°C. It was stored at 0–5°C over anhydrous calcium chloride.

IR: 1633 (\(\nu\), C=O), 1467 (\(\delta\), CH2) cm\(^{-1}\);

\(^1\)H NMR: \(\delta\) = 3.8 (4 H, s, Br3–CH1–C=O), 3.6 (8 H, m, –CH2–pip).

TLC, using isopropanol/chloroform 1:4 as eluent: single spot with \(R_f\) = 0.67.

**Synthesis of bischloroacetylpiperazine (BCP):** The same procedure as in the previous case was followed by substituting an equivalent amount of chloroacetyl chloride instead of bromoacetyl bromide. Yield: 71%, m.p. 133°C.

IR: 1640 (\(\nu\), C=O), 1462 (\(\delta\), CH2) cm\(^{-1}\);

\(^1\)H NMR: \(\delta\) = 3.8 (4 H, s, Cl–CH2–C=O), 3.6 (8 H, m, –CH2–pip).

\(R_f\) = 0.63.

**Synthesis of SPAA hydrochlorides (stoichiometric addition).** A weighed amount (60 mmol) of the \(N,N'\)-dialkyldiamine were dissolved in anhydrous NMP (70 mL). After cooling to 0°C, calcium hydroxide (84 mmol) and BCP (60 mmol) were added while stirring. The stirring was kept for 30 min. The reaction mixture was stirred for further 24 h while temperature rising to room temperature. Distilled water (350 mL) was added and the mixture was acidified using concentrated HCl. The clear solution obtained was filtered and diluted with acetone (1.6 L) under stirring. Ethanol (600 mL) was added to facilitate the separation of the product. The crude polymer was dissolved in distilled water and ultrafiltered. The final
product was finally isolated by lyophilization. Yields and intrinsic viscosities are reported in Tab. 2.

Following the same procedure for BBP yielded predominantly crosslinked, insoluble products.

Synthesis of hydrochloride SPAs (stepwise addition). A weighed amount (60 mmol) of the N,N-diaryl diamine were dissolved in anhydrous NMP (70 mL). After cooling to 0°C, 30 mmol of BBP or BCP, respectively, were added. The mixture was kept cold and stirred for 30 min. Then calcium hydroxide (30 mmol) was added. The temperature was allowed to rise to room temperature (except in the case of DMEXA, where the temperature was kept always at 0°C) and stirred for further 10 min. The reaction mixture was then cooled again to 0°C, and the procedure was repeated by using 0.15 mmol BCP and 0.15 mmol calcium hydroxide. Then 0.075 mmol of BCP and Ca(OH)\(_2\), respectively, were added and so on, until 58.125 mmol of both reagents have been added (5 steps). In the case of BCP, 1.875 mmol BCP and 1.875 mmol calcium hydroxide were added as a final step to achieve stoichiometric balance, but this was omitted in the case of BBP. After the last step the solution was kept to room temperature for 12 h. The mixture was then processed as in the previous case. Yields and intrinsic viscosities are reported in Tab. 2.

Preparation of free-base SPAs. A sample of hydrochloride polymer derived fromDMEDA (0.75 g) was dissolved in sodium hydroxide solution (pH 9.62). The solution was ultrafiltered until chloride ions were not any longer detected in the permeate. The polymer was then washed with anhydrous ether and dried until a constant weight was reached. Yield 25%.

IR: 1640 (v, C=O); 1455 (\(\delta\), CH\(_2\)) cm\(^{-1}\).

\(^1\)H NMR: \(\delta\) = 3.65 (m, 12H, O=C–CH=–N(CH\(_2\))\(_3\)N– and –CH\(_2\)–pip), 2.75 (m, 4H, –N(CH\(_2\))\(_3\)N–), 2.3 (s, 6H, \(-\text{N}–\text{CH}_2\text{–}\)).

GPC: \(M_0\) 5982; \(M_w\) 7843; \(M_n/M_0\) 1.31; [\(\eta\)] = 0.16 dL/g (CHCl\(_3\), 30°C).

Spectroscopic measurements

The infrared spectra were recorded from films cast obtained from water using a Jasco 5300 FTIR spectrophotometer, equipped with the diffuse reflection model DR-81 attachment. Proton NMR spectra were recorded in CDCl\(_3\) on a Varian XL 300 spectrometer.

Electronic (UV-visible) spectra of Copper(II)-SPAA-1 complexes were recorded at different pH values on a Pharmacia LKB-Biocrom 4060 spectrophotometer using 1 cm silica cells. Stepwise forward and backward titrations were carried out at 25°C by the addition of standardized NaOH and HCl solutions to a 0.1 M NaCl solution containing the ligand and Cu(II) in a molar ratio ranging between 1 and 3. The \(e\) value was calculated on the basis of the species distribution obtained by the previously reported program \(Fit^\text{(v)}\).\(^{[23]}\)

Viscometric, GPC and related measurements

Intrinsic viscosities were measured at 30°C in water and Tris buffer (pH 8.09) by an Ubbelohde viscometer. Viscometric titration data were measured at 25°C with an AVS 310 Schott-Gerate viscometer. Polymer solutions (ca. 0.2 mmol in 25 mL of 0.1 M NaCl), containing a known excess of HCl, were freshly prepared and stepwise titrated using a standardized 0.1 M NaOH solution delivered by a Metrohm Multidosimat piston burette. The degree of protonation \(a\), in correspondence of the desired reduced viscosity value (\(\eta/p\), dL/g), was calculated using the program \(Fit^\text{(v)}\).\(^{[21]}\)

Gel-permeation chromatograms were obtained by using a TSK-GEL G3000 PW column, with tris buffer (pH 8.09) as the mobile phase. Conditions: sample conc., 3 mg/mL; flow rate, 1 mL/min; loop size, 20 mL; injection volume, 25 mL; detector, UV-Naumer model; wavelength, 230 nm; temperature, 25°C. The calibration was performed by using poly(amide-amine) standards obtained by polyaddition of 2-methyl-piperazine to 1,4-bis-acryloyl-piperazine, according to the literature.\(^{[20]}\)

Ultrafiltrations were performed in aqueous solution of pH 4 (adjusted with HCl) by an Amicon ultrafiltering apparatus, Danvers (MA), using a membrane with a nominal cut-off of 1000.

Potentiometric measurements

The potentiometric apparatus has been described previously.\(^{[8]}\) Titrations were performed at a constant temperature (25°C) in 0.1 M NaCl using a digital PHM-84 Radiometer potentiometer, equipped with a pHG211 High pH Glass Electrode (Radiometer) and a Ref201 Reference Electrode (Radiometer), and a Metrohm Multidosimat piston burette. The burette was connected to a computer (Olivetti M20) controlling and automatically recording potentiometric data [e.m.f. readings (mV) in relation to volume (mL) of added titrant]. Between 0.1 and 0.3 mmol of the solid polymer, present in the hydrochloride form, were dissolved in the thermostated glass cell containing approximately 100 mL of 0.1 M NaCl. A measured volume of standardized 0.1 M HCl was added to the polymer solution to gain low pH values. When the polymer was completely solubilized, the titrations with standardized 0.1 M NaOH solution started under a pre-saturated nitrogen stream flowing over the surface of the solution to avoid a contamination by atmospheric CO\(_2\). The equilibrium condition was reached within the 300 s we programmed for each step of titrant addition. At least five replicates for each polymer confirmed a good agreement at the different concentrations of the ligand. The basicity constants were evaluated by means of the \(Appark\) program\(^{[20]}\) on an Olivetti M20 computer and by the program \(Superquad\)\(^{[23]}\) running on a Macintosh LCI computer.

The complex formation behaviour with copper(II) and Zinc(II) ions of the SPAs was studied in a similar way taking care to increase the equilibrium time to 500 s for each step of titrant addition. In both cases and for the three ligand/ Me(II) molar ratios (1, 2, 3), the potentiometric titration data evidenced sharp inflection points corresponding to the splitting-off of protons exactly equivalent to the stoichiometric quantity of the metal(II) ions added in solution. The stability constants were evaluated using the \(Superquad\) program.
Calorimetric measurements

The titration calorimetry (Tronac, mod 1240) has been previously described,[21] Continuous calorimetric titrations were performed in the Isothermal mode at 25 °C in a water-bath controlled by a PTC-40 (Precision Temperature Controller, from Tronac Inc.). The 25-mL stainless-steel reaction vessel was filled with 0.1 M NaCl and containing between 0.1 and 0.2 mmol of polymer. The solution was titrated with standardized 0.1 M NaOH or HCl solutions, respectively, at a constant BDR ( burette delivery rate) of 0.1000 mL · min⁻¹ governed by the Gilmont burette. Either forward and backward titrations confirmed reliable data. The instrument was calibrated with tris/HCl and HCl/NaOH before each run. The enthalpy changes were evaluated by the program Fith previously reported[20] and the ΔS° values were calculated by combining the ΔG° values, derived from the basicity constants, and the ΔH° values obtained at 25 °C. Five or more replicates were in good agreement and averaged. For the copper(II) and zinc(II) ions complexing SPAAs and PAAcs, the heats of complex formation were obtained from the direct interaction of the metal(II) ions with the ligand in the free and/or protonated forms. Standardized 0.1 M copper(II) nitrate and 0.1 M zinc(II) chloride were used as titrants. Sharp end-points of the enthalpogram were always showed at ligand/metal(II) molar ratios of 1, confirming the Me(II)L stoichiometry (L means the repeating unit of the polymer). Titrations of polymer/metal(II) ions at molar ratios of 1 and 2 were also performed with 0.1 M NaOH. The results were found in good agreement. All the calorimetric titration data [heat (cal) in relation to volume (mL) of titrant added] were stored on a floppy disk for processing. The –ΔH° for complex formation species was computed by the program Fit running on an Olivetti M24 computer. In all cases the experimental procedure, including the chemistry and calibration heater, was controlled automatically by the Isothermal program (from Tronac Inc.) on a North Star CCP 930 computer.[21]

Results and discussion

Synthesis and characterization

The general synthetic route to SPAAs (Tab. 1) follows a polycondensation reaction of a N,N'-dialkyl-diamine to a bis-a-halogenoacetyl-piperazine, according to Scheme 1:

The above synthetic process is based on the well-known Schotten-Baumann condensation reaction between alkyl halogenides and secondary amines. It is usually considered to be unsuitable for obtaining linear polymers because it normally lacks of selectivity. With monofunctional secondary amines and monofunctional halogenides a mixture of tertiary amines and quaternary ammonium salts is yielded, whereas, with bifunctional reagents, crosslinked products of mixed structures instead of linear tertiary-amine polymers will be obtained. This is due to the fact that the nucleophilicity of the amino groups, and therefore their tendency to react with electrophilic reagents, generally increases with the degree of alkyl substitution. In the present case, however, the reverse is true owing to the strong electron-attracting effect of the carbonyl group in α position. Therefore, the tendency of the amino groups to react with the halogenide groups is reduced upon substitution, and a certain degree of selectivity is attained.

The polymerizations were performed in N-methylpyrrolidone (NMP), N,N-dimethylformamide (DMF) or dimethylsulphoxide (DMSO) as solvents and in the presence of Ca(OH)₂ to neutralize the hydrogen halogenide which is evolved as by-product. Moreover, the CaCl₂

| Tab. 1. Structure of poly(amide-amine)s |
|---|---|---|
| Polymer | Structure of the repeating unit | ref. |
| SPAA-1 | ![Structure](image1) | This paper |
| SPAA-2 | ![Structure](image2) | This paper |
| SPAA-3 | ![Structure](image3) | This paper |
| PAA-4 | ![Structure](image4) | [13] |
| PAA-5 | ![Structure](image5) | [3, 18] |
| PAA-6 | ![Structure](image6) | This paper |
| PAA-7 | ![Structure](image7) | [28] |

Scheme 1.
formed is insoluble in the reaction medium and acts as a dehydrating agent. The reaction temperature was maintained at about 0°C and two different strategies of reagent addition were studied. In the first one the bis-α-halogenoacetyl-pipperazine was simply added to a cooled and well stirred stoichiometric mixture of a N,N'-dialkyl diamine and calcium hydroxide. In the second one, the whole N,N'-dialkyl diamine dissolved in a proper solvent was added to one-half (on a molar basis) bis-α-halogenoacetyl-pipperazine. After some time, one-half calcium hydroxide was added. As a second step, one fourth of bis-α-halogenoacetyl-pipperazine and, afterwards, one-fourth calcium hydroxide were added. This procedure was repeated with one-eighth amounts, then with one-sixteenth amounts and so on until a quasi-stoichiometric balance of reagents was reached. The rationale of this procedure was to keep the secondary-amino groups always in excess with respect to the halogenoacetyl groups, in order to reduce the possibility of the latter of reacting with the tertiary-amino groups of the polymer chain and resulting in crosslinks.

The first strategy was found unsuitable for X = Br, since in most cases crosslinked products were obtained. Only with NMP as solvent and DMEDA as aminic monomer a soluble polymer was obtained. On the contrary, when X = Cl good results were obtained in all cases. The second strategy offered no clear advantages over the first in the case of X = Cl, but with X = Br it allowed to obtain soluble polymers with all the aminic monomers considered.

Typical results obtained under different conditions and some molecular characterizations are reported in Tab. 2. A rather narrow molecular weight distribution could be observed for these polymers. This probably is due to the fact that they were all ultrafiltered before isolation. The molecular weight of the polymers based on DMEXA is always higher than those obtained from the other bis-amines. This probably is due to the higher nucleophilicity of DMEXA which is the strongest base of all. At any rate, the first process, involving the one step-addition of pipperazine to a BCP solution in the presence of calcium hydroxide, was finally adopted for preparing the samples to be used for the thermodynamic studies.

The soluble polymers were found essentially free from quaternary ammonium groups. Apart for the lack of any spectroscopic indications of their presence, the polymer hydrochlorides dissolved in water were treated with moist silver oxide, which is known to transform quaternary ammonium salts (as well as those deriving from tertiary amines) into the free bases. After filtration, the polymers were treated with excess acid and then titrated with a standard base, or directly titrated with a standard acid.

Tab. 2. Characterization data of poly(amido-amine)s.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Temp. °C</th>
<th>Monomers&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Method&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Yield&lt;sup&gt;d&lt;/sup&gt; %</th>
<th>$[\eta]$&lt;sup&gt;e&lt;/sup&gt; dL/g</th>
<th>$M_n \times 10^3$</th>
<th>$M_w \times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAA-1</td>
<td>DMSO</td>
<td>20</td>
<td>BBP, DMEDA</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>DMSO</td>
<td>0</td>
<td>BBP, DMEDA</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>NMP</td>
<td>20</td>
<td>BBP, DMEDA</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>NMP</td>
<td>0</td>
<td>BBP, DMEDA</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>NMP</td>
<td>0–20</td>
<td>BBP, DMEDA</td>
<td>B</td>
<td>0.22</td>
<td>6.0</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>DMSO</td>
<td>0–20</td>
<td>BBP, DMEDA</td>
<td>B</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>NMP</td>
<td>0–20</td>
<td>BCP, DMEDA</td>
<td>B</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-1</td>
<td>NMP</td>
<td>0–20</td>
<td>BCP, DMEDA</td>
<td>B</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-2</td>
<td>NMP</td>
<td>0</td>
<td>BBP, DMEXA</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAA-2</td>
<td>NMP</td>
<td>0</td>
<td>BCP, DMEXA</td>
<td>A</td>
<td>60</td>
<td>1.22</td>
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<tr>
<td>SPAA-2</td>
<td>NMP</td>
<td>0</td>
<td>BCP, DMEXA</td>
<td>B</td>
<td>90</td>
<td>0.68</td>
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<tr>
<td>SPAA-2</td>
<td>NMP</td>
<td>0</td>
<td>BCP, DMEXA</td>
<td>B</td>
<td>68</td>
<td>0.93</td>
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<tr>
<td>SPAA-3</td>
<td>NMP</td>
<td>0</td>
<td>BCP, 2-MePip</td>
<td>A</td>
<td>75</td>
<td>0.22</td>
<td>4.5</td>
<td>8.0</td>
</tr>
<tr>
<td>SPAA-3</td>
<td>NMP</td>
<td>0–20</td>
<td>BCP, 2-MePip</td>
<td>B</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PAA-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.46</td>
<td>5.5</td>
<td>7.1</td>
<td></td>
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<tr>
<td>PAA-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.57</td>
<td>9.5</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> DMSO = dimethylsulphoxide; DMF = N,N-dimethylformamide; NMP = N-methylpyrrolidone.
<sup>b</sup> BBP = N,N'-bis(2-bromoacetyl)piperazine; BCP = N,N'-bis(2-chloroacetyl)piperazine; DMEDA = N,N'-dimethylethylenediamine; DMEXA = N,N'-dimethylhexamethylenediamine; 2-MePip = 2-methylpiperazine.
<sup>c</sup> Method A: stoichiometric addition; Method B: stepwise addition.
<sup>d</sup> Crosslinks.
<sup>e</sup> Not determined.
<sup>f</sup> The GPC system used was found unsuitable for polymers deriving from DMEXA.
<sup>g</sup> Prepared according to ref. [1–3].
Their titration curves were in all cases identical to those of the native polymers. In direct titration, the presence of quaternary ammonium groups would have resulted in three jumps instead of two, whereas in back-titration a number of equivalents different from those of the native polymers in back-titration, would have been detected. Quaternary ammonium groups cannot be detected, since the free hydroxides are as strong as the titrating base.

PAAs of a structure similar to that of the polymers described in this paper were purposely synthesized by the usual process of hydrogen-transfer polyaddition of \(N,N'\)-dialkyldiamine to bis-acrylamides in aqueous solution, \[1\] in order to compare the relevant thermodynamic properties. \[18\] These polymers are also reported in Tab. 1.

### Thermodynamics of protonation

The three polymers (SPAA-1, SPAA-2 and SPAA-3) were studied by mean of potentiometry, viscometry and solution calorimetry in a 0.1 M NaCl at 25°C.

Potentiometry always revealed curves with a well defined end-point that was found reliable in either forward and backward titrations with standard NaOH and HCl solutions, respectively. The end-point was used to evaluate the purity of the compound that ranged between 85 and 96 wt.-%. Unlike the SPAA-2, polymers SPAA-1 and SPAA-3 showed two well separate buffer regions in different pH ranges. This allowed to evaluate only two basicity constants (\(\log K\)), both relative to the protonation equilibria of the two basic nitrogens of each repeating unit. Tab. 3 summarizes the equilibrium constants of the three polymers as well as the enthalpy and entropy data and compare them with those of related PAA s. \[18, 21\] Both \(\log K\)s of SPAA-1 and SPAA-3 were evaluated by the program Appark, \[20\] thus obeying the modified Henderson-Hasselbalch equation: \[14\]

\[
\log K = \log K^0 + (n – 1)\log[(1 – a)/a].
\]

\(\Delta G^0\) was obtained at \(a = 0.5\), \(L\) is the repeating unit of the polymer, values in parentheses are standard deviations.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Reaction</th>
<th>(\log K^0) a (\times 10^{-3})</th>
<th>(n)</th>
<th>(–\Delta G^0) b (\times 10^3) kJ mol(^{-1})</th>
<th>(–\Delta H^0) (\times 10^3) kJ mol(^{-1})</th>
<th>(\Delta S^0) (\times 10^{-3}) J mol(^{-1}) K(^{-1})</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAA-1</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = -0.5)</td>
<td>7.74 (5)</td>
<td>1.11 (3)</td>
<td>44.2 (3)</td>
<td>33.5 (7)</td>
<td>36 (2)</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = 0)</td>
<td>3.69 (2)</td>
<td>1.03 (4)</td>
<td>21.0 (1)</td>
<td>25.4 (7)</td>
<td>-15 (2)</td>
<td></td>
</tr>
<tr>
<td>PAA-4</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = 0)</td>
<td>8.09</td>
<td>–</td>
<td>46.2</td>
<td>30.8</td>
<td>51</td>
<td>13, 21</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = 0)</td>
<td>4.54</td>
<td>–</td>
<td>25.9</td>
<td>25.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SPAA-3</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = 0)</td>
<td>6.70 (4)</td>
<td>1.16 (2)</td>
<td>38.3 (2)</td>
<td>27 (1)</td>
<td>38 (5)</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = 0)</td>
<td>2.34 (13)</td>
<td>0.96 (10)</td>
<td>13.4 (7)</td>
<td>16 (2)</td>
<td>-8 (7)</td>
<td></td>
</tr>
<tr>
<td>PAA-6</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = -0.5)</td>
<td>7.17 (5)</td>
<td>1.18 (2)</td>
<td>40.9 (3)</td>
<td>22.3 (2)</td>
<td>62 (1)</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = 0)</td>
<td>3.30 (7)</td>
<td>0.98 (8)</td>
<td>18.8 (4)</td>
<td>17.2 (1)</td>
<td>5 (1)</td>
<td></td>
</tr>
<tr>
<td>PAA-7</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = -0.5)</td>
<td>7.01</td>
<td>–</td>
<td>40.0</td>
<td>23.9</td>
<td>54</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = 0)</td>
<td>2.98</td>
<td>–</td>
<td>17.0</td>
<td>21.9</td>
<td>-16</td>
<td></td>
</tr>
<tr>
<td>SPAA-2</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = -0.5)</td>
<td>8.48 (1)</td>
<td>–</td>
<td>48.4 (1)</td>
<td>42.3 (7)</td>
<td>20 (2)</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = 0)</td>
<td>7.60 (1)</td>
<td>–</td>
<td>43.3 (1)</td>
<td>37.0 (8)</td>
<td>21 (3)</td>
<td></td>
</tr>
<tr>
<td>PAA-5</td>
<td>(L + H^+ \rightleftharpoons LH^+) (\Delta H^0 = 0)</td>
<td>8.39 (1)</td>
<td>–</td>
<td>47.9 (1)</td>
<td>38 (1)</td>
<td>32 (4)</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(LH^+ + H^+ \rightleftharpoons LH_{2}^{2+}) (\Delta H^0 = -0.5)</td>
<td>9.09 (1)</td>
<td>–</td>
<td>51.9 (1)</td>
<td>41.9 (2)</td>
<td>33 (1)</td>
<td></td>
</tr>
</tbody>
</table>
Compared to the homologous PAAs,\cite{3,13,18} shortening of the methylene chain between the amido C==O and tertiary basic nitrogen produced two effects: lower basicity and lower shielding effectiveness. The decreased basicity was attributed to the increased inductive effect displayed by the closer amido group. As a general trend, the decrease in $\log K$ is lower than in $\log K$, as a consequence of the increased electrostatic field created by the greater charge density of the macromolecular ligand. Unlike the case of SPAA-1 and SPAA-3, the longer hydrocarbon chain in SPAA-2 improved a remarkable lower effect of the different basicity of the tertiary amino groups. Once protonated, the positively charged ammonium ion improved higher shielding effect between the monomer units, resulting in a drop of the $n$ value. This phenomenon, already observed in other related poly(amido-amine)s and poly(ampholyte)s,\cite{26,27} can be related to the magnitude of the hydration shell surrounding the ionized group that weakens the cooperativity between the repeating units of the polymer. On the other hand, the greater hydrophobic character of polymer PAA-6, due to the methyl group inserted on the piperaazine ring, improved greater cooperativity making $n > 1$ with respect to its homologous compound PAA-7 containing no hydrophobic methyl group.\cite{28}

Calorimetric titrations revealed a two-slopes enthalpogram for each polymer considered. Two enthalpy changes ($-\Delta H^\circ$) were evaluated, the first being greater than the second. The entropy changes ($\Delta S^\circ$) were obtained by combining the $-\Delta G^\circ = RT \ln K$ with $-\Delta H^\circ$ (Tab. 3). In all cases $-\Delta H^\circ$s were found “real” even if the dependence of $\log K$ on the degree of protonation $a$ was taken into account.\cite{18,28} Compared with the homologous compound PAA-4, the polymer SPAA-1 showed higher $-\Delta H^\circ$ and close $-\Delta H^\circ$, while both values of $\Delta S^\circ$ are considerably lower. These data may be well interpreted in terms of the strong H-bond interaction between the protonated nitrogen and the neighboring amido C==O group. Like previously reported PAAs whose six-membered ring, involving the H-bonding interaction, was hypothesized and supported by calorimetric\cite{21} and spectroscopic\cite{30} data, polymer SPAA-1 strongly confirm the like hypothesis because a more favourable five-membered ring results in an higher stability with an associated greater exothermicity. The lower $\Delta S^\circ$ values are in good agreement to this fact because of the involvement of two further five-membered rings formed by protonation of both amino groups. The resulting higher rigidity of each monomer unit decreased the entropy contribution also for the decreased number of accessible conformations.\cite{51} The behaviour of SPAA-3 seems to be related to the same mechanism, even if the presence of a further methyl group on the piperaazine ring increases the hydrophobicity thus lowering the $-\Delta H^\circ$ and increasing $\Delta S^\circ$, due to the further release of water molecules upon protonation. However, comparison with other related polymers\cite{18,21,24} better clarify the data improving thus the same hypothesis reported above for polymer SPAA-1. With regard to polymer SPAA-2, both $-\Delta H^\circ$ and $\Delta S^\circ$ are in line with the same hypothesis of polymer SPAA-1, while the mechanism for the second step of protonation seems to be reversed due to a greater energetic contribution of the longer methylene chain.

In all cases, viscometric titrations only indicate a gradual and linear increase of the reduced viscosity upon protonation. This is simply related to the extension of the macromolecular chain that increases its coil dimension as a whole.

**Thermodynamics of metal(II) ion complexes**

In studying the complexing ability towards heavy metal ions we found that only polymer SPAA-1 is able to form complex species in solution. This was ascribed to the particular chelating moiety of the monomer unit, since no cooperativity among several of such units was observed. Complex species require two nitrogen donor atoms with the participation of the two amido C==O groups in a distorted octahedral geometry (Scheme 2):

![Scheme 2](image)

This structure was already hypothesized for previously studied PAAs where one or two six-membered rather than the five-membered rings chelate through the amido C==O to the Cu(II) ion covalently bonded via the two tertiary nitrogens in a stable five-membered ring.\cite{16,31}

Both thermodynamic and spectroscopic data (Tab. 4) agree with the above structure. The stability constant $(log \beta)$ for the CuL$_2$ (L is the monomer unit of the polymer) species is more than 10 times greater than the homologous polymer PAA-4. In the low pH region (2.3–5.4), only a single CuL$_2$ complex species fitted the potentiometric titration curves well, even at different copper(II)/L molar ratios. The effect of the polyelectrolyte quality of SPAA-1, showing “apparent” $log K$, was found negligible due to the low pH region and the stronger polymer binding of Cu(II) ions that causes ease splitting-off of protons, further lowering the pH of the solution.\cite{8} Moreover, the $-\Delta H^\circ$ resulted in unusually high values making $\Delta S^\circ$ very low. These data are consistent with the well ordered chelate structure formed by the three condensed five-mem-
Tab. 4. Complexes of copper(II) and zinc(II) ions: Thermodynamic and spectroscopic data of poly(amido-amine)s at 25°C in 0.1 M NaCl. a)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Reaction</th>
<th>log β</th>
<th>$ΔG^0$</th>
<th>$ΔH^0$</th>
<th>$ΔS^0$</th>
<th>$λ_{max}$</th>
<th>ε</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAA-1</td>
<td>Cu$^{2+}$ + L ↔ CuL$^{2+}$</td>
<td>10.24 (3)</td>
<td>58.4 (2)</td>
<td>56.2 (1.2)</td>
<td>8 (4)</td>
<td>14200</td>
<td>66</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>CuL$^{2+}$ + OH$^-$ ↔ Cu(OH)L$^+$</td>
<td>6.21 (4)</td>
<td>8.47 (5)</td>
<td>20.3 (6)</td>
<td>51 (2)</td>
<td>51 (2)</td>
<td>This work</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn$^{2+}$ + L ↔ ZnL$^{2+}$</td>
<td>7.92 (9)</td>
<td>45.2 (5)</td>
<td>41.2 (1.0)</td>
<td>13 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA-4</td>
<td>Cu$^{2+}$ + L ↔ CuL$^{2+}$</td>
<td>8.96</td>
<td>51.2</td>
<td>33.6</td>
<td>62</td>
<td>14800</td>
<td>174</td>
<td>16, 21</td>
</tr>
<tr>
<td></td>
<td>CuL$^{2+}$ + OH$^-$ ↔ Cu(OH)L$^+$</td>
<td>5.52</td>
<td>31.5</td>
<td>11.7</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn$^{2+}$ + L ↔ ZnL$^{2+}$</td>
<td>3.49 (5)</td>
<td>19.9 (3)</td>
<td>11.6 (4)</td>
<td>28 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) L means the repeating unit of the polymer, $\Delta G^0 = RT\ln β$, values in parentheses are standard deviations.

Thermodynamic and spectroscopic data further corroborates this hypothesis because the lower molar absorption coefficient (ε) is consistent with a complex species of a less distorted structure. As it is well known, less distorted species tend to have a lower ε value, presumably because of orbital mixing with higher symmetry fields. [8, 32]

By increasing pH above 5.4 a hydroxo-complex species of Cu(OH)L$^+$ stoichiometry was evaluated by fitting potentiometric data with the program Superquad. [21, 32] The $λ_{max}$ also showed a small shift to higher energies. This species, already found for polymer PAA-4, results from the substitution of a hydroxyl group by the water molecule belonging to the coordination position. Thermodynamic quantities are greater than those previously found for the PAA-4 and are consistent with the more compact geometric arrangement of polymer SPAA-1.

The higher geometric compactness found for copper(II) ion encouraged us to study the complexing ability of SPAA-1 towards other metal(II) ions. We investigated the complexing behaviour of zinc(II) ions by a thermodynamic point of view. The ZnL$^{2+}$ was the only species present over a wide range of pH having stability constant (log β) and enthalphy change ($ΔH^0$) values lower than those obtained for the Cu$L^{2+}$ species, following a trend usually observed for low molecular weight diamines in agreement with the Irving-Williams order. [34] For our purpose, by comparing thermodynamic data of the ZnL$^{2+}$ complex species, both log β and $ΔH^0$ are always higher for the polymer SPAA-1 than for the related PAA-4 (Tab. 4) and the difference was greater than that found for copper(II). The special geometric arrangement of SPAA-1 around the Zn(II) ion increased the stability and strongly decreased the strain in the ligand molecule when the coordinating groups bond in the x-y plane. The two adjacent amido C=O coordinating groups easily take part in the chelation process around the metal(II) ion where the enthalpy contribution is noticeable.

Conclusions

From the above data, the following conclusions may be drawn.

1. Short chain SPAA*s in which amido and tertiary amino groups are separated by a single methylene can be prepared by polycondensation of $N,N'$-bis-(2-chloroacetyl-piperazine) (BCP) with $N,N'$-dialkyldiamine. It may be reasonably expected that this synthetic process is not limited to the examples described in this paper and can be extended to a wide variety of similar monomers.

2. The use of $N,N'$-bis-(2-bromoacetyl-piperazinone) (BBP) instead of BCP leads to soluble products only if a stepwise addition procedure is adopted.

3. The new SPAA*s are apparently devoid of quaternary ammonium groups, thus indicating the selectivity of the synthetic process used.

4. Shortening of the methylene chain between the amido and the tertiary amino group sensibly increases the polyelectrolyte behaviour of SPAA*s with respect to PAA*s. In fact, the basicity constants of the former were in some cases found to be “apparent”, because of the reduced shielding effect of the bis-amide moiety.

5. In comparison with PAA*s, the increased electron-withdrawing effect of the amido groups due to their shorter distance from the basic nitrogens results in lower basicities, but the enthalpy contributions are larger owing to the formation of more stable five-membered rings as a consequence of hydrogen-bond formation between the “onium” ions and the amido groups.

6. The complex ability towards transition metal(II) [Cu(II), Zn(II)] ions is improved because of the suitable arrangement of the donor groups forming three close five-membered chelate rings of high stability.

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