AMINO ACID-DERIVING CHIRAL POLYMERS WITH POTENTIAL FOR BIOTECHNOLOGICAL APPLICATIONS

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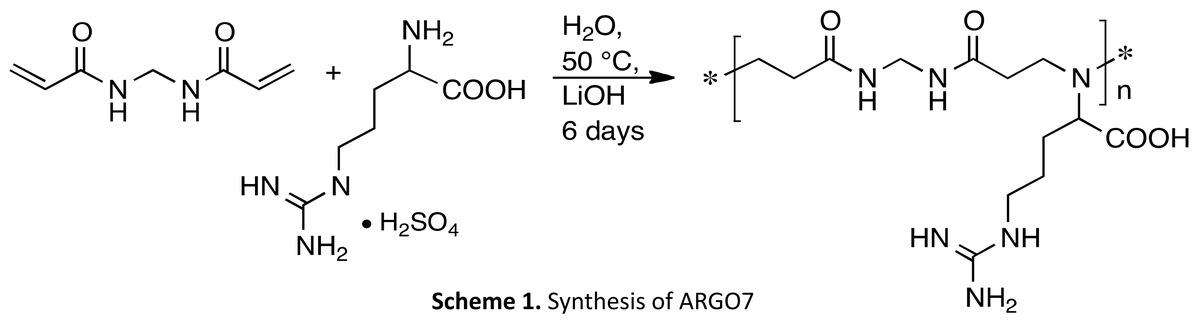
INTRODUCTION

The Michael-type polyaddition of α-amino acids with bisacrylamides in pH > 9 aqueous solutions leads to polyamidoaminoacids (PAACs) that maintain the amphoteric properties and configuration of the amino acid precursors. In particular, the PAAC obtained from the reaction of L-arginine and N,N'-methylenebisacrylamide (L-ARGO7), proved highly citycompatible with IC_{50} ≥ 8 mg/mL.[1] Cell internalization studies in Balb/3T3 cells demonstrated its preferential localization in the perinuclear region.

The interest in ARGO7 isomers is manifold:

- They retain the water solubility and acid/basic properties of arginine isomers, thus being only moderately basic and non-cytotoxic;
- They bear one guanidine pendant per repeating unit, thus mimicking, in this respect, the arginine-rich cell permeating peptides;
- They may display well-defined pH-dependent conformations associated to the configuration of the repeating units. This latter feature may, in turn, affect the biological properties of ARGO7 isomers.

SYNTHESIS



Scheme 1. Synthesis of ARGO7

Synthesis

(D)-,(L)- and (D,L)-Arginine were added to a suspension of N,N'-methylenebisacrylamide and lithium hydroxide in water under vigorous stirring. The reaction mixture was heated to 50°C for 5 days.

Work-up

- Quenching by adding diluited HCl to reach pH = 4;
- Ultrafiltration through 100 and 3kDa membranes;
- Freeze-drying.

Sample	Yield (%)	M_w	PDI	vPSD (nm)
D-ARGO7	88	7700	1.54	2.42±0.79
L-ARGO7	92	6500	1.43	3.11±0.61
D,L-ARGO7	90	6800	1.48	1.33±0.36

Table 1. Molecular weights, yield and volume particle size distribution (vPSD) of ARGO7.

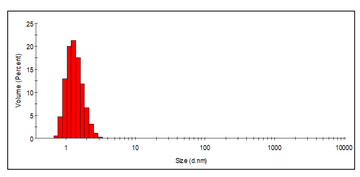


Figure 1. L-ARGO7 DLS results expressed as volume particle size distribution (vPSD).

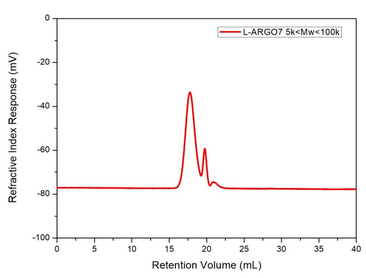


Figure 2. SEC results for L-ARGO7.

TITRATIONS

0.1 M NaCl solutions of ARGO7 isomers were potentiometrically titrated with 0.1 M NaOH and then back-titrated with 0.1 M HCl at 25°C under inert atmosphere.

pKa values for the different groups, namely pKa_1 (COOH), pKa_2 (main chain *tert*-amine) and pKa_3 (guanidine) were first obtained from the half-neutralization pHs.
β parameters for pKa_1 and pKa_2 (table 2) were then introduced in the generalized Henderson Hasselbach equation (eq. 1) to ascertain the presence of interactions between ionizable groups on adjacent monomeric units.

$$pH = pKa - \beta * Log(\frac{1-\alpha}{\alpha}) \qquad (1)$$

Sample	pKa_1	pKa_2	pKa_3	β_1	β_2	IP
L-Arginine	2.17	9.04	12.48	--	--	10.76
L-ARGO7	2.31	6.43	>12	0.60	1.14	9.7
D-ARGO7	2.24	6.41	>12	0.60	1.12	9.7
D,L-ARGO7	2.34	6.39	>12	0.57	1.25	9.7

Table 2. pKa values and β parameters of L-, D- and D,L-ARGO7.

- No significant differences were detected among the ARGO7 isomers' *pKa* values (table 2).
- Both pKa_1 and pKa_2 exhibit deviations from ideal behaviour, **(β = 1)**, more pronounced in case of the carboxyl group.

SPECIATION CURVE

Following the De Levie approach (eq. 2), a theoretical titration curve is modeled (figure 3).

$$V_T = \frac{V_0[C_0(\alpha_0 - \alpha_2 - 2\alpha_3) + C_A - \Delta] + N}{\Delta + C_T} \qquad (2)$$

From these results speciation curves, i.e. distribution diagrams of ionic species with pH (figure 4), were obtained considering β corrected pKa values.

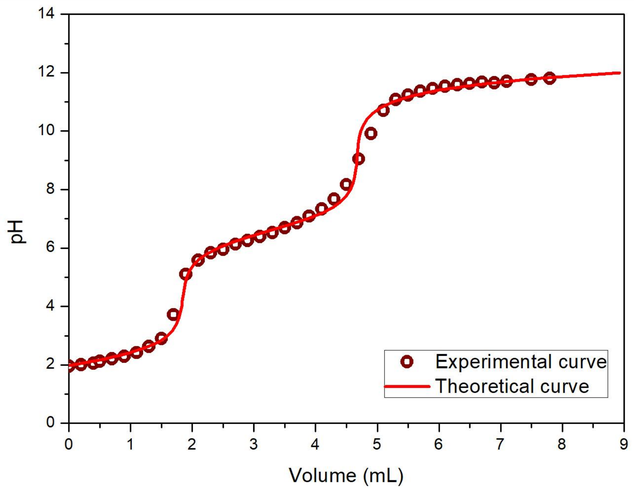


Figure 3. Theoretical curve modeled by De Levie approach

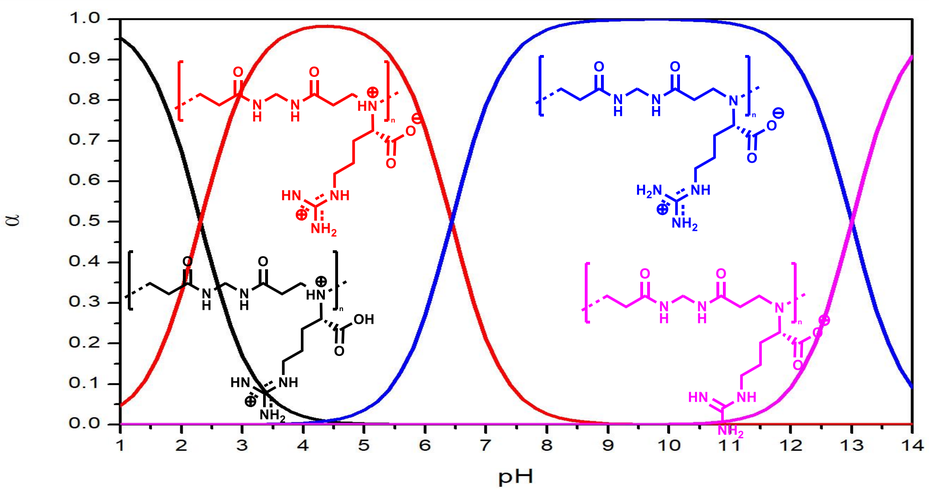


Figure 4. Speciation diagram of L-ARGO7

CIRCULAR DICHROISM

The secondary structure of D-, L- and D,L-ARGO7 was investigated in aqueous solution by circular dichroism (CD) spectroscopy at 25°C and pH values ranging from 2.1 to 12.1.
D,L-ARGO7 gave only a noisy baseline, whereas the CD spectra of L- and D-ARGO7 reflected pH-dependent conformational changes (figure 5).

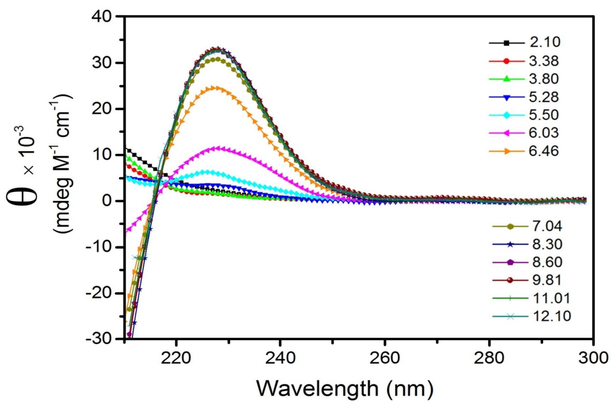


Figure 5. pH dependence of CD spectra of L-ARGO7

- At pH > 5 the L-ARGO7 spectra were characterized by a positive band at 228 nm, whose value increased by increasing pH up to a maximum at pH ~ 8.1, and then remained constant up to pH 12.1

REFERENCES

[1] P. Ferruti, N. Mauro, L. Falciola, V. Pifferi, C. Bartoli, M. Gazzarri, F. Chiellini, E. Ranucci, *Macromol. Biosci.*, 2014, *14,* 390.

CONCLUSIONS

1. Amphoteric polyamidoaminoacids were obtained by polyaddition in aqueous solution of (D)-, (L) and (D,L)-arginine with N,N'-methylenebisacrylamide.
2. Their pKa_1 (COOH) values resembled that of arginine, whereas pKa_2 (main chain *tert*-amine) decreased by two units due to the electron withdrawing effect of the acrylamide groups. Both constants exhibit deviations from ideal behaviour, more pronounced for the carboxyl group.
3. β dependent speciation curves were obtained by applying the De Levie approach, accounting for the whole titration curve without approximations.
4. D- and L-ARGO7 gave, in the pH range 3-10, CD spectra consistent with pH-dependent conformation transitions.