UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI CHIMICA

AMINO ACID-DERIVING CHIRAL POLYMERS WITH POTENTIAL FOR BIOTECHNOLOGICAL APPLICATIONS

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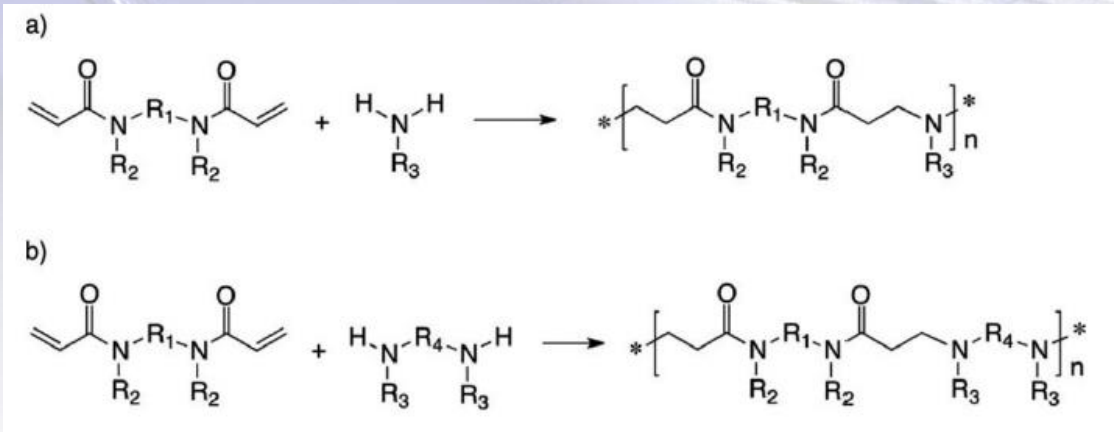
FACTS

1. Polyamidoamines (PAAs) represent a well-known family of soluble synthetic functional polymers obtained by stepwise Michael-type polyaddition of *prim*-monoamines or *sec*-diamines with bisacrylamides.



2. By using α-amino acids with bisacrylamides at pH > 9 in aqueous solutions, we obtained polyamidoaminoacids (PAACs) that maintain the amphoteric properties and chirality of the amino acid precursors.

3. By reacting L-arginine and methylene-bisacrylamide for 6 months in presence of $CaCl_2$ catalyst, a moderately basic and highly non-cytotoxic PAAC named ARGO7 is obtained.

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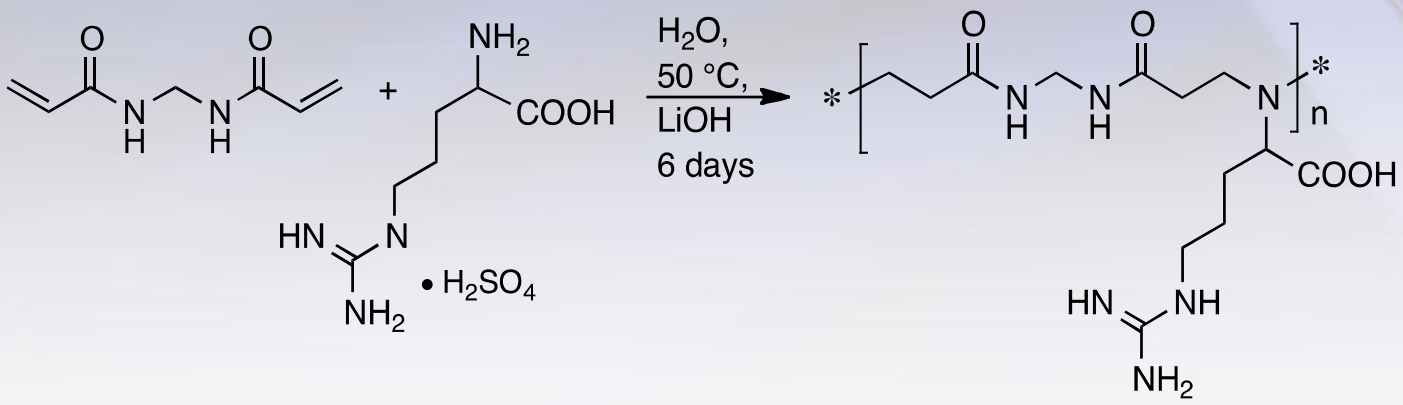
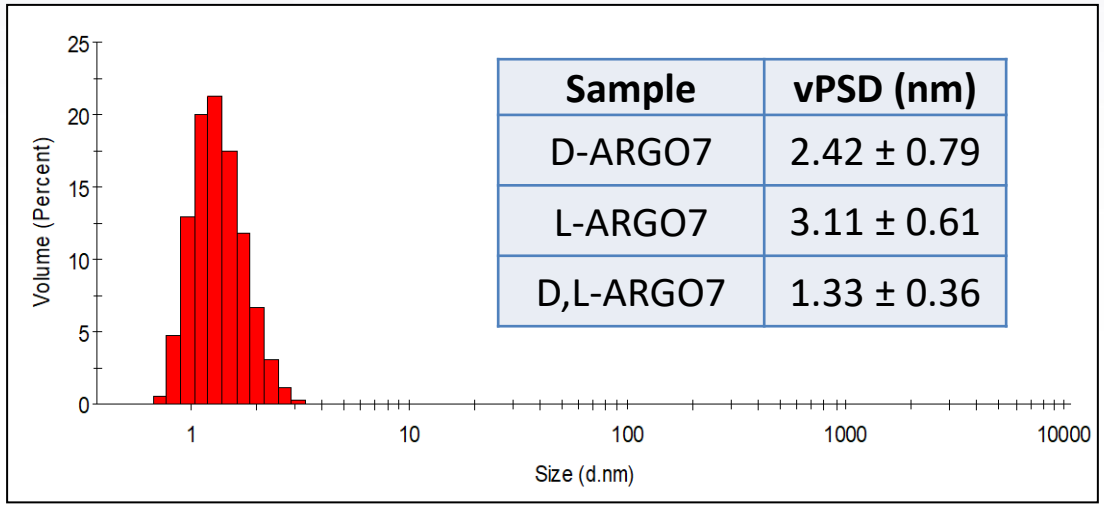
SYNTHESIS



Sample	Yield (%)	M_w	PDI
D-ARGO7	88	7700	1.54
L-ARGO7	92	6500	1.43
D,L-ARGO7	90	6800	1.48



Sample	vPSD (nm)
D-ARGO7	2.42 ± 0.79
L-ARGO7	3.11 ± 0.61
D,L-ARGO7	1.33 ± 0.36

- Reaction is run at 50°C for 6 days, reaching high molecular weight in short time;
- Reaction is extended to (D) and (D,L)-arginine;
- No catayst;
- No purification is needed;
- No traces of aggragates.

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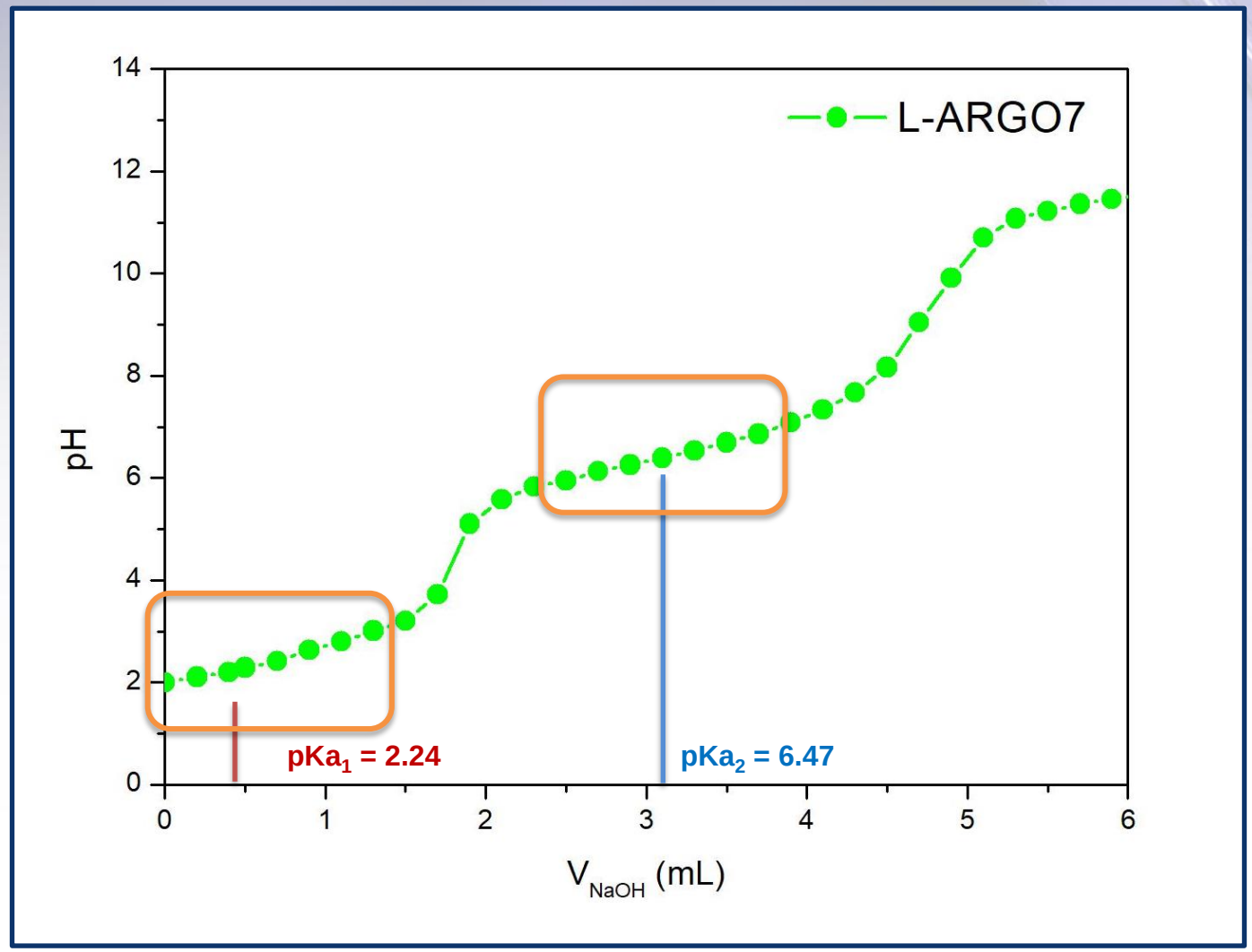
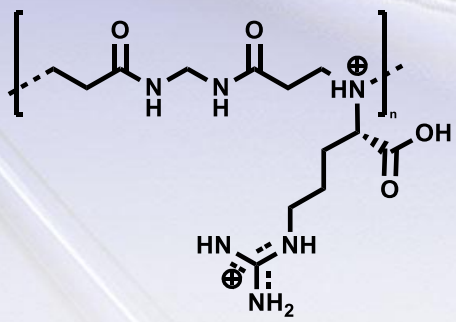
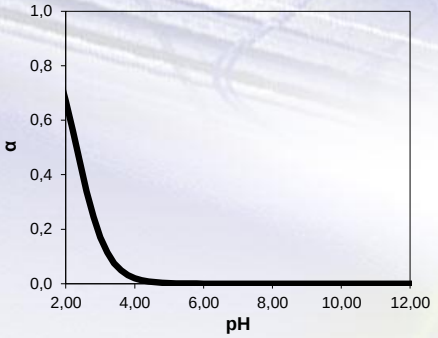
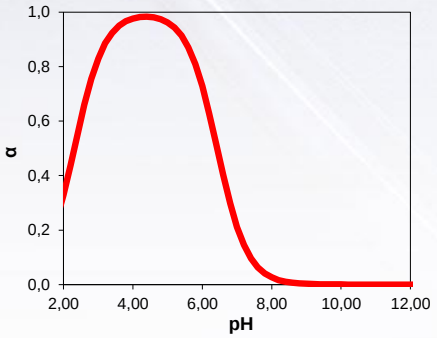
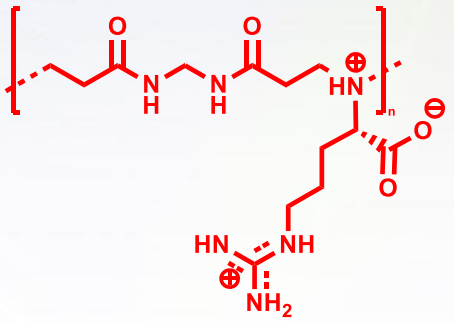
ACID-BASE PROPERTIES

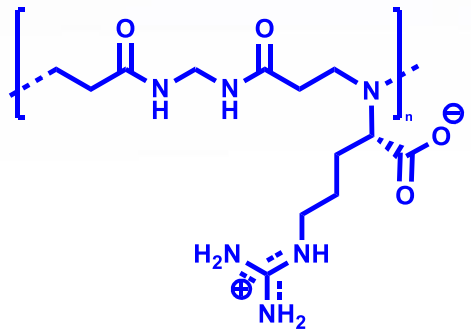


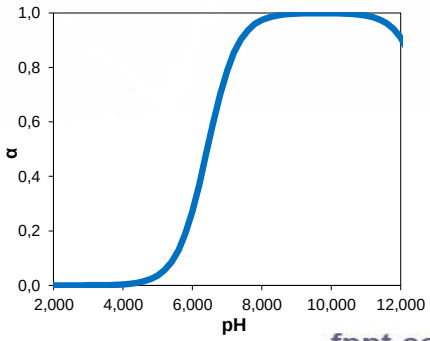












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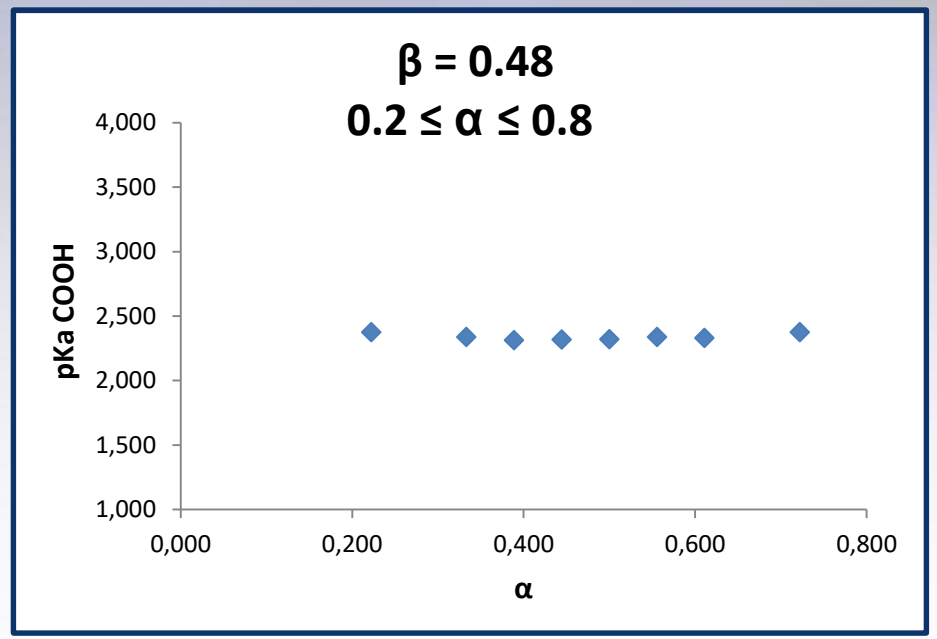
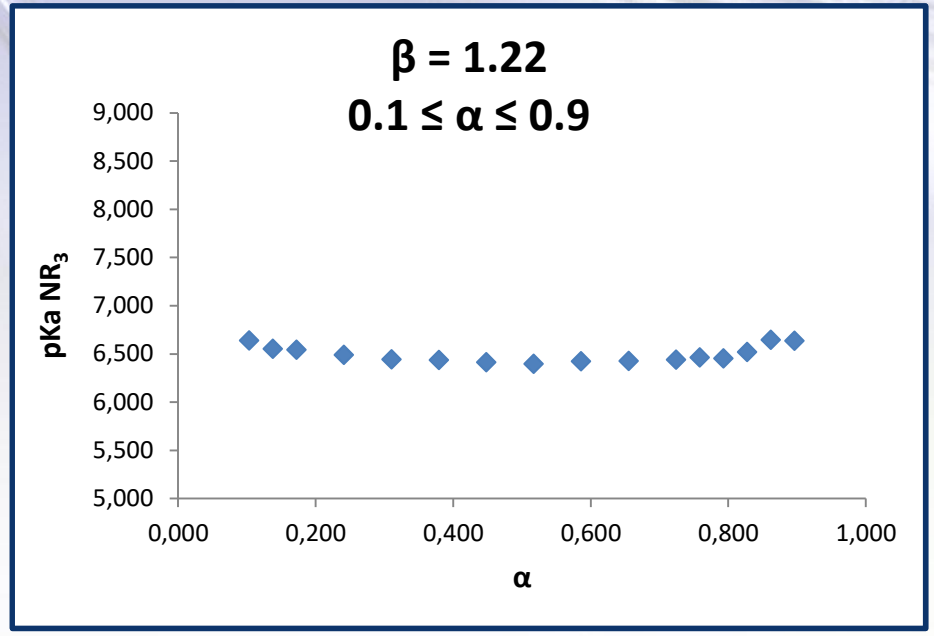
ACID-BASE PROPERTIES





β parameters for pKa_1 and pKa_2 are introduced in the generalized Henderson Hasselbach equation (Eq.1) to ascertain the presence of interactions between ionizable groups on adjacent monomeric units.

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

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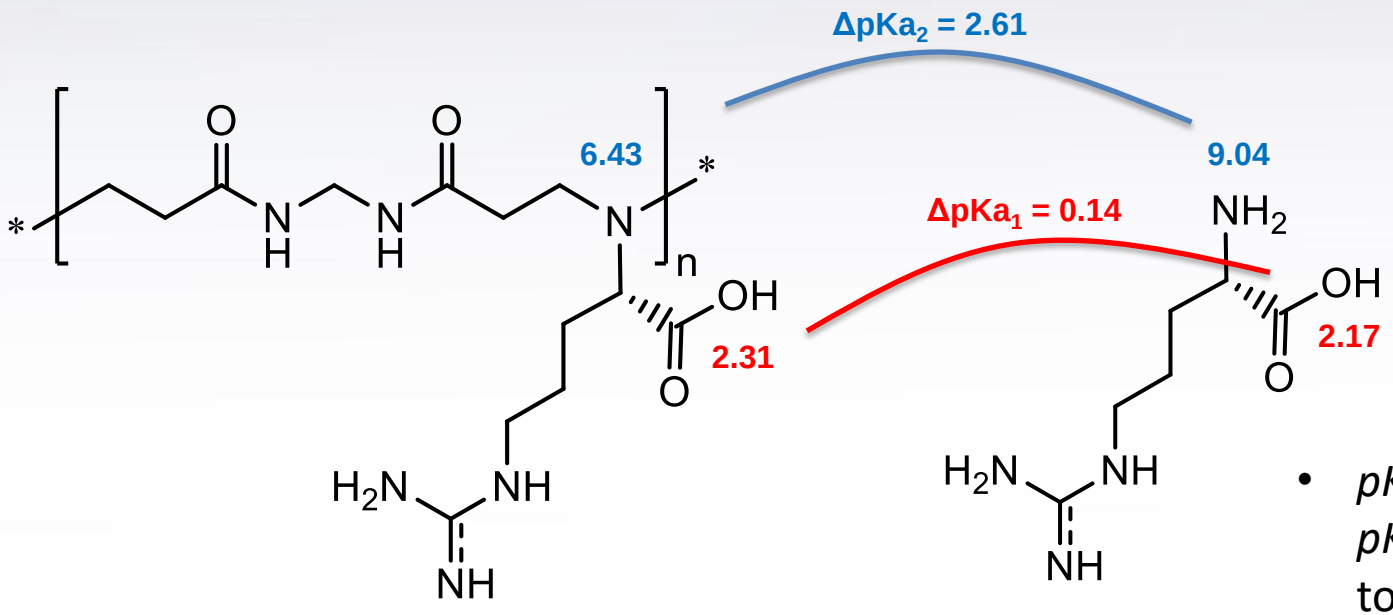
ACID-BASE PROPERTIES

- No significant differences were detected among the pKa values of the three ARGO isomers.
- Both pKa_1 and pKa_2 exhibit deviation from ideal behavior, more pronounced in case of the carboxyl groups.

Sample	pKa_1	pKa_2	pKa_3	β_1	β_2	pI
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



- 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.

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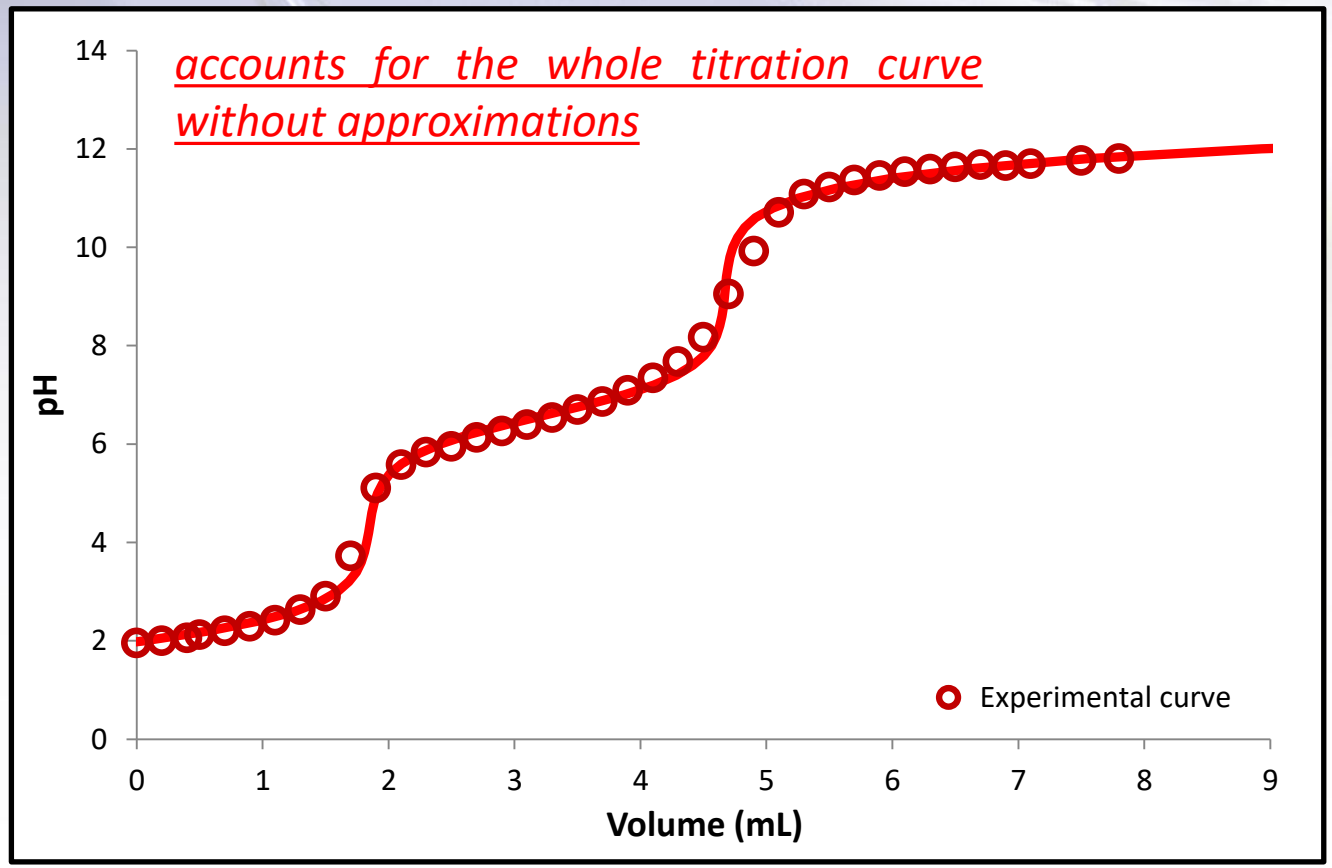
DE LEVIE APPROACH

Charge and mass balance

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

- HCl used to correct pH is taken into account in the initial balance;
- Differences are minimized by reducing the sum of square roots (SRR) between V_t data.



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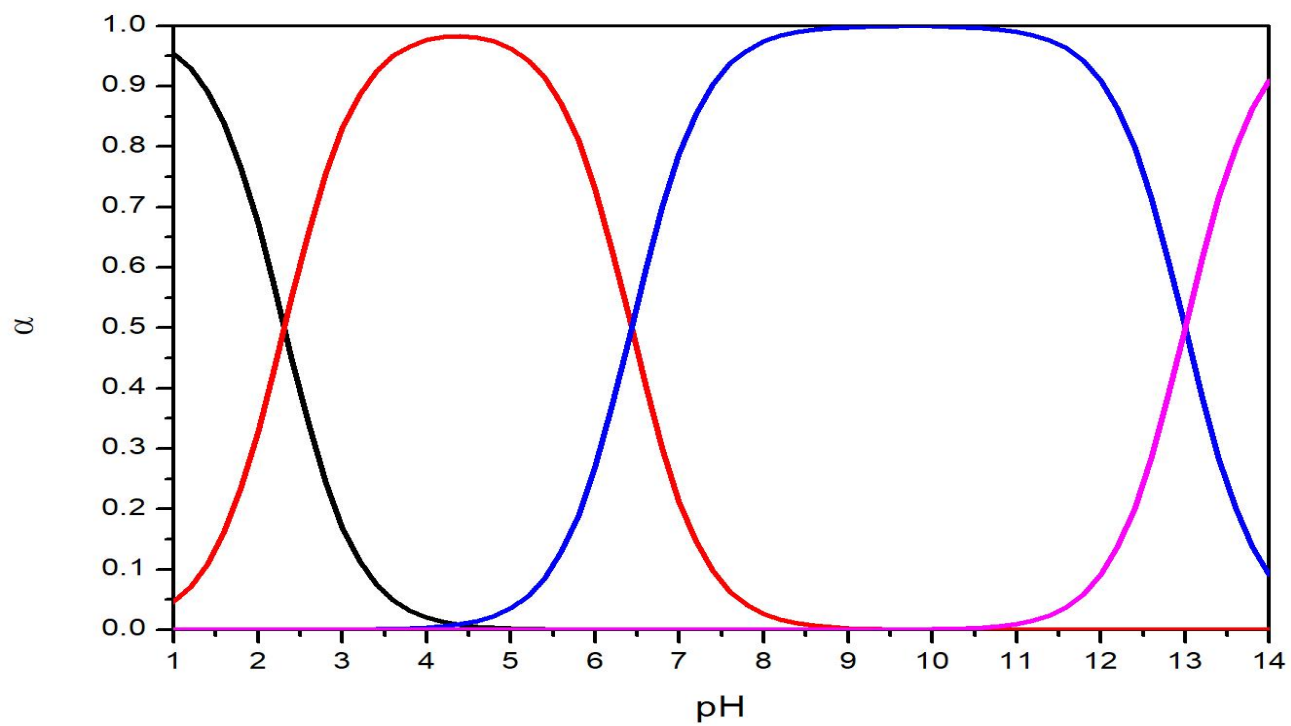
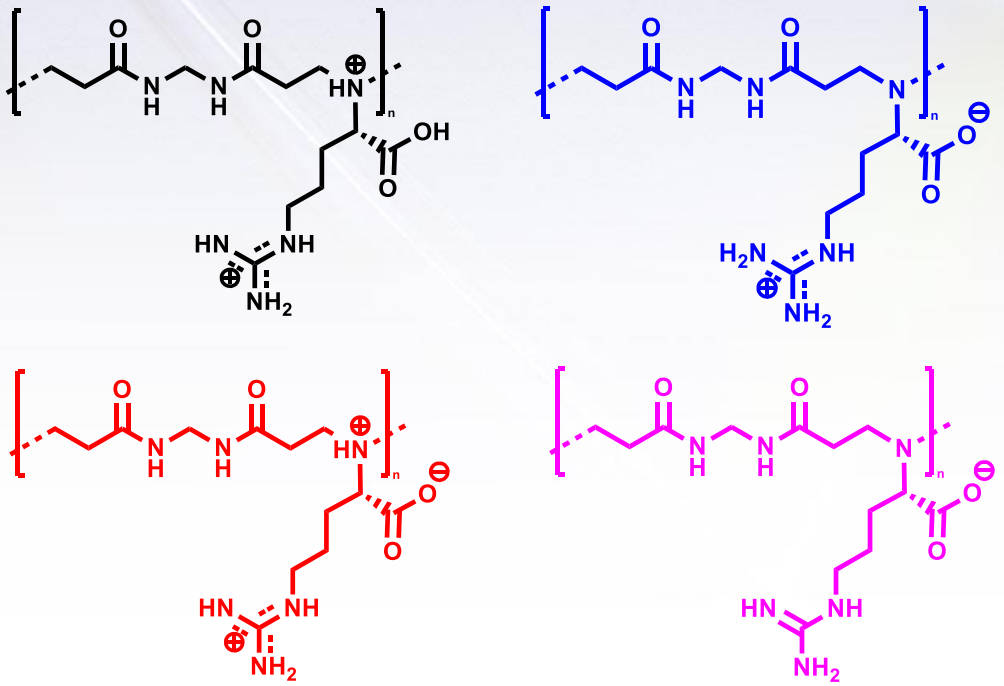
SPECIATION DIAGRAM

The speciation diagram for L-ARGO consists of four ionization states and indicates isoelectric point (I.P.) 9.8 and 0.25 positive net average charge per repeating unit at pH = 7.4.





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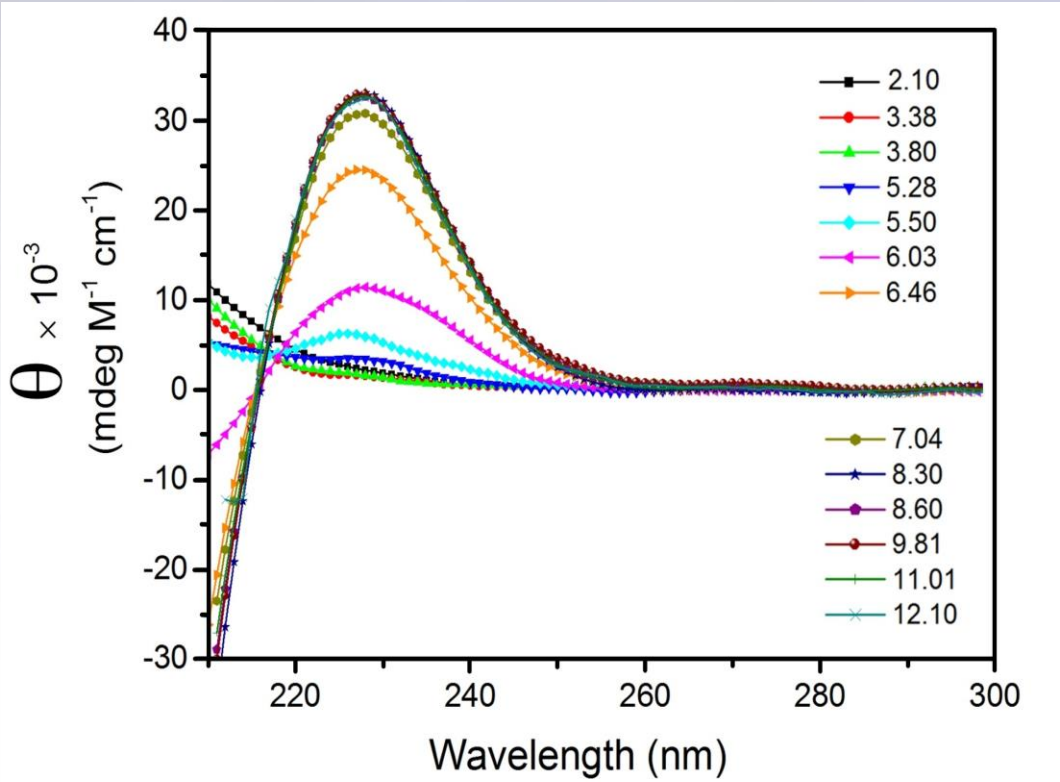
CIRCULAR DICHROISM



Spectra at 25 °C in 0.1 M NaCl and in the 2.1÷12.1 pH range exhibited patterns and intensities consistent with ordered secondary structures.

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 approximately constant up to pH 12.1

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CONCLUSIONS

1. Amphoteric polyamidoaminoacids were obtained with fairly high molecular weights by polyaddition in aqueous solution of (D)-, (L) and (D,L)-arginine with N,N'-methylenebisacrylamide.

2. The polymers' pKas were evaluated: pKa_1 (COOH) value resembled that of arginine, whereas pKa_2 (main chain *tert*-amine) decreased by two units affected by the conversion into a *tert*-amine group. Both constants exhibit deviations from ideal behaviour, more pronounced for the carboxyl group.

3. From circular dichroism analysis, D- and L-ARGO7 gave in the pH range 2-12, spectra consistent with pH-dependent conformation transitions.

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