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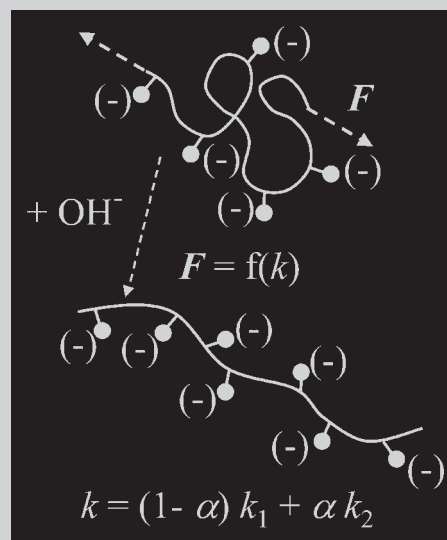
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**Summary:** The paper reports a study on the flexibility of a family of 1:1 hydrolysed maleic anhydride (maleic acid, MA)–olefin copolymers in dilute aqueous solution. The copolymers were MA–ethene, MA–propene and MA–isobutene. The study was carried out in the absence and in the presence of monovalent salts and at different polymer concentrations. Experimental data showing the negative logarithm of the ‘apparent’ dissociation constant,  $pK_a$  versus the degree of dissociation,  $\alpha$ , experimental data were obtained from potentiometric titrations using NaOH, KOH or tetramethylammonium hydroxide as the base. The  $pK_a$  data were fully described with calculated curves obtained using an extension of the counterion condensation theory of linear polyelectrolytes, in which a semiflexible model for the polymers was introduced. Under the present experimental condition, no relevant specificity of the monovalent counterions was apparently observed for the different copolymers. The calculated  $pK_a$  versus  $\alpha$  curves allowed the derivation of both the intrinsic dissociation constants of the first and the second dissociation steps for the different copolymers and the corresponding stiffness parameters built into the model. The agreement between the experimental and calculated data shows an appreciable success of the model. The results pointed to an increase of stiffness parallel to the increase of size of the olefin comonomer, in qualitative agreement with already published findings. Furthermore, for all copolymers the chain rigidity was larger in the  $\alpha$  range of the first dissociation than in that of the second one. The former rigidity was attributed to the formation of intramolecular hydrogen bonds upon the first

ionisation of the MA repeating units, followed by an increase of rotational freedom upon breaking of the H-bond in the second dissociation step. Comparison of the rigidity parameters of the MA copolymers with the data obtained for other polyelectrolytes, both natural—poly(L-glutamic acid) and pectic acid—and synthetic—poly(acrylic acid) and poly(methacrylic acid)—was also performed.



# Potentiometric Titrations of Maleic Acid Copolymers in Dilute Aqueous Solution: Experimental Results and Theoretical Interpretation

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## Introduction

The dissociation in aqueous solution of weak polycarboxylic acids has been extensively studied for many decades as a simple but effective tool for the physico-

chemical characterisation of these polyelectrolytes. The polyelectrolytes of interest to us are the copolymers of hydrolysed maleic anhydride (actually, maleic acid, MA), that were initially studied by Bianchi and coworkers,<sup>[1]</sup> by Strauss and coworkers,<sup>[2]</sup> by our own

group,<sup>[3]</sup> by Kawaguchi and coworkers,<sup>[4]</sup> and, more recently, by other research groups.<sup>[5–8]</sup>

Among the experimental techniques used, there is no doubt that acid–base potentiometric titrations were the most extensively applied. Provided that the concentration and the value of the intrinsic dissociation constant of the polycarboxylic acid are known, potentiometric titrations allow the computation of the values of the apparent dissociation constant and hence the values of the excess electrostatic free energy of dissociation,  $\Delta G_{\text{diss}}$ , as a function of the degree of dissociation,  $\alpha$ . The intrinsic (at  $\alpha = 0$ ) and the ‘apparent’ dissociation constants (at  $\alpha > 0$ ) are usually expressed through their negative logarithm, that is,  $\text{p}K_0$  and  $\text{p}K_a$ , respectively. Once other thermodynamic parameters (for example,  $\Delta H$  or  $\Delta V$ ) are obtained through independent experimental or theoretical ways, the knowledge of  $\Delta G_{\text{diss}}$  enables full thermodynamic characterisation or interpretation of the behaviour of the polyelectrolyte in solution.

In some cases, the inspection of the curve of  $\text{p}K_a$  versus  $\alpha$  was used to ascertain the presence of cooperative conformational transitions occurring by varying the pH of the polymer solution. In fact, the presence of anomalous trends, and in particular the presence of ‘bumps’ in the potentiometric curve profile, are usually considered as the experimental evidence of a conformational transition,<sup>[9]</sup> unless such peculiarities can be attributed to aggregation–disaggregation phenomena occurring as a function of pH.<sup>[10a,10b]</sup> For some ‘hydrophobic’ polyelectrolytes, including certain MA copolymers,<sup>[3i,3j]</sup> and poly(methacrylic acid),<sup>[11]</sup> it is very difficult (and sometimes even impossible) to discriminate between chain separation and dissolution of aggregates, on one side, and cooperative conformational transitions, on the other, both of which can occur on increasing pH. This is a severe limit for a correct interpretation of the experimental evidence of potentiometric titrations of these systems. The introduction of suitable methods for determining  $\text{p}K_0$ , which is one of the aims of this paper, could be very helpful in this respect.

Since many biopolymers of high biological relevance are polycarboxylic acids, the physicochemical characterisation of their conformation in solution takes a practical, as well as a theoretical, importance, being related to their biological activity. The present attempt at simulating the experimental data of  $\text{p}K_a$  (or  $\Delta G_{\text{diss}}$ ) with theoretical curves, obtained using suitable expressions derived from physical modelling, must be seen in the same light.

In the present study, we compared the experimental titration data obtained for three different 1:1 MA copolymers with  $\alpha$ -olefins (namely, ethene, propene and isobutene), hereafter labelled MAE, MAP and MAiB, respectively,<sup>[3c]</sup> with the curves calculated from our model, in which the flexibility characteristic of each copolymer was explicitly considered.

The counterion condensation (CC) theory, based on first principles,<sup>[12]</sup> which has been elaborated on mainly by

Manning,<sup>[13]</sup> has been judged by many researchers to be simple and reliable. As a matter of fact, it was successful in describing different types of experimental data.<sup>[10a,14a]</sup>

Our laboratory has been active in developing extensions of the CC theory to improve its range of application.<sup>[14]</sup> We have also presented a model of semiflexible polyelectrolytes to take into account the thermodynamic conformational averaging that takes place in a polymeric solution.<sup>[15a]</sup> This model was introduced to make the CC model assumption on the chain conformation a more realistic one. In its original form, it considers the polymer as an (infinite) line of equally spaced charges, that is, an infinite rigid rod, ignoring the rich conformational space usually available to a polymer in solution. Model analysis of potentiometric titration data of highly charged weak polyelectrolytes, such as poly(glutamic acid) (PGA)<sup>[15b]</sup> or more recently poly(acrylic acid) (PAA),<sup>[15c,15d]</sup> has shown that properly averaging the thermodynamic functions over the available conformational space is mandatory in order to efficiently compare experimental data and model calculations. These studies also showed that such a sampling of the conformational space can be represented just by an energy term of the Hooke type, that is simply added to the polyelectrolytic excess energy terms provided by the CC theory.

Another significant development of the theory was more recently reached, to account for possible differences in the intrinsic ionisation of the different functional groups present on a polyelectrolytic chain.<sup>[14d]</sup> As a matter of fact, the theoretical analysis of the experimental titration data of copolymeric polycarboxylates is more complicated than that of the corresponding data for homopolymers. In the former case one has to determine the number of different types of ionisable functional groups,  $i$ , their corresponding fractional abundances,  $X_i$ , and the values of  $\text{p}K_0^i$  for each type of functional group. In a first application of this model, Paoletti et al were able to interpret the bump that appears in the  $\text{p}K_a$  versus  $\alpha$  curves of the ionic polysaccharide succinoglycan, without invoking any conformational transition induced by the change of pH, in agreement with experiment.<sup>[14d]</sup>

This extension of the CC theory of linear polyelectrolytes for the dissociation process of a copolymer with different functional groups was the prelude to another refinement of the theory proposed by Porasso et al that allowed for the analysis of potentiometric titration data of natural multifunctional polyelectrolytes, such as humic acid.<sup>[15e]</sup>

The present approach eliminates the necessity of using two different titration equations for the dissociation of the first and the second ionising group, respectively, as performed in the older attempts.<sup>[2]</sup> Also, it overcomes difficulties related with the choice of the most appropriate value(s) for  $\text{p}K_0$ , which is a severe limit in the usual approach.<sup>[3i,8a]</sup>

The inspection of specific polycarboxylate–counterion interactions was outside the aims of the present work.

However, since the potentiometric titrations in the absence of added salts were performed using as the base in some cases NaOH while in others KOH, we have also looked for the possibility of any difference, which could be attributed to specific interactions of these counterions with the copolymers studied.

## Experimental Part

### Materials

The MA-olefin copolymers MAE, MAP and MaiB are known to be of the 1:1 alternating type because of their polymerisation mechanism. They were obtained by hydrolysis at 90 °C in dilute NaOH of the corresponding MA-olefin copolymers received from the Monsanto Chemical Co., at present Dow Chemical Co. After hydrolysis, the basic solutions were dialysed exhaustively against water, passed through a cation-exchange column in the H<sup>+</sup> form, dialysed again, and concentrated under reduced pressure at about 40 °C.

The average number molecular weight was about 10<sup>5</sup> g · mol<sup>-1</sup> in all cases.<sup>[11]</sup> In view of the free radical process of synthesis, the copolymers were believed to be essentially atactic. Stock solutions of the polyacids were prepared by weight. Their concentrations were determined by potentiometric titrations of a small volume of the solution at room temperature, using standard Carlo Erba Normex NaOH, in the presence of 2 M NaCl to enhance accuracy in the detection of the equivalent points. The concentration was expressed in equivalents (*eq*) L<sup>-1</sup>, that is, mole of charge per litre.

Certified 0.1 N Normex from Carlo Erba (NaOH) or Normadose Prolabo (KOH) solutions were used to obtain the titration pH versus (volume of base) curves. HCl solutions used in the cation-exchange column were prepared by dilution of certified 0.1 N Normex from Carlo Erba solutions.

Pure tetramethylammonium perchlorate (TMAClO<sub>4</sub>) was prepared by neutralisation of Fluka cold aqueous tetramethylammonium hydroxide (TMAOH) with Carlo Erba RP concentrated perchloric acid. The white precipitate was collected, washed with cold water, then with methanol, and finally dried under vacuum overnight and stored under vacuum over P<sub>4</sub>O<sub>10</sub>. The purity of the final product was checked by elemental analysis with a Carlo Erba model 1106 Elemental Analyzer. Deionized water which was doubly distilled under special glass was used. Its specific conductivity was approximately 10<sup>-6</sup> ohm<sup>-1</sup> · cm<sup>-1</sup>.

### Methods and Instruments

Potentiometric titrations from this laboratory have already been reported.<sup>[3h]</sup> New ones were performed on freshly prepared solutions in glass vessels, thermostated at 25.00 ± 0.05 °C, using a Radiometer PHM52 digital pH meter with Radiometer GK2301C or GK2321C combined electrodes. Standardisation was checked at pH 4.01 (±0.01) and 7.00 (±0.01) with Radiometer buffer solutions. Pure nitrogen was fluxed only for pH values above 9.

The potentiometric titrations were performed with 1 N KOH or NaOH solutions, so that the polyelectrolyte concentration did not vary significantly during titration. For each copolymer, a number of potentiometric titrations have been carried out. Careful and frequent control of the calibration of the pH meter with standard buffer solutions yielded highly reproducible pH data for all cases. On the basis of this analysis, the pK<sub>a</sub> values may thus be considered to be accurate to within ±0.02 pK<sub>a</sub> units. The titrations were carried out at low polymer concentration in order to avoid any chain aggregation at low α values. The latter was never reported in any previous paper. It was confirmed in this study by the complete absence of turbidity of all the solutions at low pH.

From the titration data points the values of the overall degree of dissociation α were computed with the following expression:

$$\alpha = \alpha_n + \frac{10^{-\text{pH}}}{C_p} = \frac{v(\alpha)}{v_{\alpha=1}} + \frac{V^0 + v(\alpha)}{C_p^0 \cdot V^0} \times 10^{-\text{pH}}, \quad (1)$$

where α<sub>n</sub> is the degree of neutralisation, and C<sub>p</sub> and C<sub>p</sub><sup>0</sup> are the actual and the initial equivalent concentration of the polyelectrolyte, respectively. v(α) and V<sup>0</sup> stay for the volume of titrant (base) and the initial volume of the polyacid, respectively, and v<sub>α=1</sub> is the volume of base necessary for complete neutralisation of the polyacid.

The values of pK<sub>a</sub> were then computed as a function of α using the well-known Henderson-Hasselbalch equation:

$$\text{pK}_a = \text{pH} + \log \left[ \frac{1 - \alpha}{\alpha} \right]. \quad (2)$$

## Theory

### Counterion Condensation Theory

The usual starting point in the CC theory of linear polyelectrolytes is to replace the real chain by an infinitely long charged line.<sup>[10b,16]</sup> The fundamental assumption in this approximation is that the total polyelectrolyte charge, as seen by a probe charge away from the polyelectrolytic domain, can be modelled as uniformly spread over the polymer, which permits a definition of a (mean) structural charge density to be made, ξ, given by:<sup>[14b]</sup>

$$\xi = \frac{l_B}{b} = \frac{e^2}{\varepsilon k_B T b}, \quad (3)$$

where l<sub>B</sub> is the Bjerrum length, b is the average distance between consecutive charges projected onto the polymer axis, e is the value of the elementary charge, ε is the bulk dielectric constant, k<sub>B</sub> is the Boltzmann's constant and T is the absolute temperature.

Following the procedure already described,<sup>[10a]</sup> one can write the analytical expressions for the polyelectrolytic

( $G^{\text{pol}}$ ) and entropic ( $G^{\text{entr}}$ ) contributions to the total (excess) ionic free energy of the polyelectrolyte solution:

$$G^{\text{ion}} = G^{\text{pol}} + G^{\text{entr}}. \quad (4)$$

Proper derivation of this free energy yields the functional form of the thermodynamic function of interest,<sup>[16]</sup> which for the present study is the apparent dissociation constant,  $K_a$ , given in general by:

$$\text{p}K_a(\alpha) = \text{p}K_0 + \Delta\text{p}K_a(\alpha). \quad (5)$$

For a monoprotic weak polyacid,  $\text{p}K_0$  is the intrinsic  $\text{p}K$  characteristic of the isolated ionisable repeat unity making up the polymer, while the change in  $\text{p}K_a$  due to the ionisation of the polyelectrolyte is included in the term  $\Delta\text{p}K_a$ . If the ionic free energy function,  $G^{\text{ion}}$ , is known, we can readily calculate:

$$\begin{aligned} \Delta\text{p}K_a(\alpha) &= \frac{1}{n_p 2.303 RT} \frac{dG^{\text{ion}}(\alpha)}{d\alpha} \\ &= G_{\text{diss}}(\alpha, \xi, C_p, C_1, T, \varepsilon), \end{aligned} \quad (6)$$

where  $n_p$  is the number of polymeric charge units,  $R$  is the gas constant, and  $C_p$  and  $C_1$  stand for the analytical concentrations of polymeric units and monovalent counterions from the supporting 1:1 electrolyte, respectively. Within the framework of CC theory, the analytical expression of the  $\Delta\text{p}K_a$  function has been shown to be the following:<sup>[10a]</sup> a) for  $\xi \leq \xi_{\text{crit}} = 1$

$$\Delta\text{p}K_a(\alpha) = -\frac{\alpha\xi}{2.303} \left\{ 2 \ln \left( 1 - \exp \left[ -\frac{\kappa b}{\alpha} \right] \right) + \frac{\kappa b}{\alpha (\exp[\frac{\kappa b}{\alpha}] - 1)} \left( \frac{\alpha}{2[\alpha + 2R_1]} - 1 \right) \right\}, \quad (7a)$$

where:  $R_1 = \frac{C_1}{C_p}$  and  $\kappa^2 = \lambda (\alpha C_p + 2C_1)$ , with  $\lambda = \frac{8\pi e^2 N_A}{\varepsilon k_B T 10^3}$  ( $N_A$  is Avogadro's number), and b) for  $\xi > \xi_{\text{crit}} = 1$

$$\Delta\text{p}K_a(\alpha) = \frac{1}{2.303} \left\{ -\frac{1}{\alpha\xi} \left[ 2 \ln \left( 1 - \exp \left[ -\frac{\kappa b}{\alpha} \right] \right) + \frac{\kappa b SU}{\alpha (\exp[\frac{\kappa b}{\alpha}] - 1)} \right] + r \log \left( \frac{\alpha r}{r + R_1} \frac{1}{V_p C_p} \right) + (1-r) \log \left[ \frac{\alpha + R_1 - \alpha r}{(\alpha + R_1)(1 - V_p C_p)} \right] \right\}, \quad (7b)$$

where:

$$SU = \frac{1}{2} \left( \frac{\alpha(1-r)}{2(\alpha(1-r) + 2R_1)} \right) - 1$$

and

$$\kappa^2 = \lambda [\alpha C_p (1-r) + 2C_1]$$

$\xi_{\text{crit}}$  is the critical value of  $\xi$  at which a fraction  $r$  of counterions is condensed into the condensation volume,  $V_p$ , surrounding the polymer axis. For monovalent counterions of the titrating base (for example, MeOH, with  $\text{Me}^+$  an alkali metal cation or TMA<sup>+</sup>),  $\xi_{\text{crit}} = 1$ . For details of the derivation of these equations, see reference [7].

We have previously shown that, for heterogeneous weak polyelectrolytes made by any number,  $i$ , of different functional groups ( $i$  is 2 for our copolymers) having fractional abundance  $X_i$  and intrinsic dissociation constant  $\text{p}K_0^i$ , the overall intrinsic  $\text{p}K_0$  is a function of the degree of ionisation  $\alpha$  given by:<sup>[15e]</sup>

$$\text{p}K_0(\alpha) = \text{p}K_0^i + \log \left[ \frac{\beta_i}{(1 - \beta_i)} \frac{(1 - \alpha)}{\alpha} \right], \quad (8)$$

where  $\beta_i$  corresponds to the ionisation degree of the  $i$ th functional group.

The insertion of Equations (7a) or (7b) and (8) into Equation (5) allows for the calculation of theoretical potentiometric titration curves.

### Chain Stiffness and Potentiometric Titration

In order to compare experimental data and model calculations, previous studies from our group<sup>[15a–15d,17]</sup> have shown that it is necessary to consider the thermodynamic averaging of Equation (5) over the conformational space available to the polyelectrolyte in solution.

In principle, according to the predictions of Manning's theory, the  $\text{p}K_a(\alpha)$  curve of a weak polyacid – having

the charge density  $\xi_{\text{max}}$  at  $\alpha = 1$  – monotonously increases from  $\text{p}K_0$ .<sup>[15f]</sup> Actually, that theory is able only to make predictions on the difference between  $\text{p}K_a(\alpha)$  and  $\text{p}K_0$ ,  $\Delta\text{p}K_a(\alpha)$ , which is directly related to the increase of the polyelectrolytic free energy upon charging (that is, increase of  $\alpha$ ).  $\text{p}K_0$  is considered as an intrinsic property of the polyacid chain, stemming from the overall balance of chemical interactions of the dissociating group, and is



supposed to be independent of the ionisation state of the polyelectrolyte. The  $\Delta pK_a(\alpha)$  curve increases very rapidly from  $\alpha = 0$  to  $\alpha_1 = (\xi_{\max})^{-1}$ , corresponding to  $\xi = 1$ . Beyond  $\alpha_1$ , the predicted curve increase is very small indeed. The peculiar aspect is that although the  $pK_a(\alpha)$  curve is continuous, its first derivative,  $\frac{dpK_a(\alpha)}{d\alpha}$ , is discontinuous at  $\alpha_1$ . No  $pK_a(\alpha)$  curve of a weak polyacid has ever been reported showing such a behaviour. However, if one assumes a family of several  $pK_a(\alpha)$  curves having a distribution (for example, without loss of generality, a Gaussian distribution) of  $\xi_{\max}$  values around a central value  $\xi_{\max}^0$ , the ensuing convoluted  $pK_a(\alpha)$  curve, which is derived from thermodynamic averaging, will be smoother with no discontinuity of  $\frac{dpK_a(\alpha)}{d\alpha}$ , perfectly resembling the experimentally determined  $pK_a(\alpha)$  curves. The smoothness of the convoluted curve clearly depends on the width of the distribution: a very broad distribution will produce slowly increasing  $\Delta pK_a(\alpha)$  curves, whereas a narrow distribution will give rise to more 'asymmetric'  $\Delta pK_a(\alpha)$  curves. The curve predicted by Manning's theory in its simple form corresponds to the limiting case of a Dirac's delta distribution curve. In our previous works on the matter,<sup>[15a]</sup> we assumed that such a distribution reflects a distribution of pseudohelical segments of different extension. In turn, such distribution was supposed to derive from the interplay of the polyelectrolyte potential (which depends on intercharge distance) and the conformational energy of the polyacid chain, since, in general, different values of the conformational rotation angles correspond to different values of chain elongation. Passing from potentials to forces, the ionisation process of a weak polyacid can be visualised as follows. The increase of chain charging is expected to bring about a parallel increase of chain segment extension: the polymer counterbalances the increase of the (polyelectrolyte) ionic free energy,  $G^{\text{ion}}(\alpha)$ , by increasing the average distance between the charges. However, such a (polyelectrolytic) expansion force which stretches the chain segments is contrasted by the retreating force originating from the intrinsic stiffness of the polymer chain.

The system can be visualised as an electrically charged spring undergoing (electrostatic) stretching, responding with a conformational restoring force of the Hooke's type, characterised by a rigidity constant,  $k$ , which reflects the distribution of (or, more precisely, the possibility of distributing over) conformational states. So far, no systematic attempts at correlating the values of  $k$  with properties such as the persistence length (or the Kuhn's length), or the so-called Smidsrød's 'B' empirical parameter of stiffness' from viscosity/ionic strength studies have been reported. Still, in the case of carboxymethylamylose the predictions of the viscosity behaviour by the theoretical approach derived from the configurational statistics of the (uncharged) amylose chain were in excellent agreement with the experiments.<sup>[15a]</sup>

Moreover, this finding clearly points to the fact that  $k$  should be correlated with the 'intrinsic' or 'bare'

persistence length, and not with the 'total' or with its 'electrostatic' part. In our works it was shown that a single value of  $k$  was sufficient to provide an excellent fit of the  $\Delta pK_a(\alpha)$  curves of a weak monoprotic polyacid, even including the case of pH-induced conformational transition.<sup>[15b]</sup> The fitting of the  $pK_a(\alpha)$  curve of a polyacid - the  $\xi_{\max}^0$  value of which is supposed to be known - is basically modulated by two parameters, namely  $pK_a^0$ , corresponding to the *intrinsic* dissociation constant, and  $k$ , accounting for the smoothness of the  $\Delta pK_a(\alpha)$  curve and related to the intrinsic chain stiffness. The task at hand in this paper is to extend the approach to the case of weak diprotic polyacids. For the present case of perfectly alternating copolymers, it seems reasonable to assume that the polymeric conformational states are affected by the state of ionisation of the two types of functional groups, with the resulting dependence of polymer flexibility upon the ionisation state. Therefore, in the present analysis, the total flexibility of the polymeric chain is considered to be a combination of two limiting stiffness values, one corresponding to the flexibility of the polymer at low values of the degree of dissociation (initial dissociation of the first functional group), and another representing the polymer flexibility at high values of the degree of dissociation, that is, when only the dissociation of the second functional group is taking place. Hence, we consider an overall stiffness parameter  $k$  given by:

$$k = (1 - \alpha)k_1 + \alpha k_2, \quad (9)$$

$k_1$  and  $k_2$  being the stiffness parameters in the regions of low and high values of the degree of ionisation, respectively.

The fitting of the experimental data with the thermodynamic function  $pK_a(\alpha)$  was obtained using a MatLab computer program written by the authors. Basically, this program uses one of the minimisation subroutines in the MatLab environment, applying least square fitting to the set of nonlinear Equations (5–8) in order to obtain the best values of the free parameters of the model, that is the  $pK_a^0$  of each functional group and the stiffness parameters  $k_i$ . The procedure was iterated until convergence of the four parameters was reached, which always occurred in no more than four iterative steps.

### Determining the Free Parameters of the Model

For the copolymers studied here, the fraction  $X_i$  of both dissociating groups was fixed at 0.5 for structural reasons, which reduced the parameters to be obtained by the model analysis.<sup>[15c]</sup> With this simplification, in the present case the titration curves of all copolymers were successfully reproduced by the model using only four free parameters

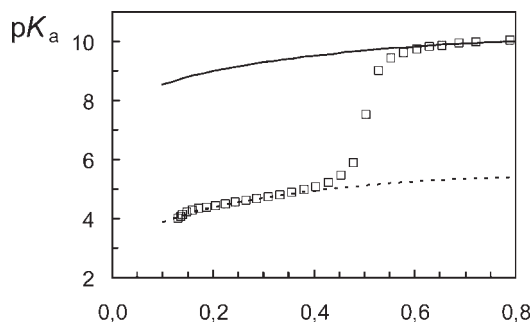


Figure 1. Experimental  $pK_a$  versus  $\alpha$  data (squares) for MAiB in aqueous solution at 25 °C without any added salt, using KOH as the titrant base. The initial polymer concentration was  $9.933 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ . The lines show the theoretical calculations in the low (-----) and high (—) ionisation regions. They show the asymptotic behaviour that yields the initial values of the stiffness parameters  $k_1$  and  $k_2$ .

corresponding to the two stiffness parameters,  $k_1$  and  $k_2$ , and the two intrinsic dissociation constants,  $pK_0^1$  and  $pK_0^2$ .

We first evaluated the two stiffness parameters by separately fitting the limiting behaviour of the potentiometric titration data in the low ( $\alpha \leq 0.35$ ) and high ( $\alpha \geq 0.65$ ) ionisation regions of the potentiometric titration with the proper  $pK_a(\alpha)$  function. This procedure essentially considers the copolymer as monoprotic in these limiting ionisation regions. In practice, it therefore applies the type of data analysis successfully used with PGA<sup>[15b]</sup> and PAA.<sup>[15c,15d]</sup> The result for the potentiometric titration of MAiB is represented in Figure 1 as a sample case. It is readily seen that a good fit of the data was obtained in both limiting ionisation regions. The procedure not only provided an independent initial determination of the two stiffness parameters, but also gave support to the assumption that the chain flexibility changes upon ionisation.

Using this set of initial values of the two stiffness parameters, the intrinsic ionisation constants  $pK_0^1$  and  $pK_0^2$  of the two types of functional groups were determined by least square fitting of the whole titration. Due to the complexity of the conformational averaging of the theoretical  $pK_a(\alpha)$  function [Equation (5)], it was necessary to develop a program in MatLab in order to match the thermodynamic average of the calculated  $pK_a$  function with the experimental data points, as explained above.

## Results and Discussion

Previous potentiometric titrations carried out on different MA-olefin copolymers in the presence of 1:1 added salt showed that the pH jump in the half neutralisation region ( $\alpha = 0.5$ ) could reach as much as a few units, the

phenomenon being more and more relevant on passing from MAE to MAP to MAiB.<sup>[3h]</sup> As a consequence, the  $pK_a$  values also changed up to several units of  $pK_a$  at full ionisation with respect to the value at  $\alpha = 0$ . On the basis of calorimetric, potentiometric and dilatometric data, this effect was essentially attributed to the variations in the enthalpy of ionisation of the second dissociating group, that becomes more unfavourable by about  $3.3 \text{ kcal mol}^{-1}$  on passing from MAE to MAP to MAiB.<sup>[3c]</sup> These data indicate that the intrinsic  $pK_0$  of the second dissociating group of MAiB is clearly higher than the intrinsic  $pK_0$  of the second group of MAP, which in turn is higher than the corresponding value for MAE.

Figure 2 shows new experimental  $pK_a$  data, plotted as a function of  $\alpha$ , for the three MA-olefin copolymers, obtained in water solution (that is, without any added simple salt). Concentrated KOH was used as the titrating base. The initial polymer concentrations are reported in Table 1. In the case of MAiB, the presence of two different ionisable groups is clearly shown in the potentiometric titration data, namely, a large increase in  $pK_a$  occurs around  $\alpha = 0.5$ , and there is a notable total change in  $pK_a$  (about 6  $pK_a$  units), from low to high ionisation. With MAP and MAE, the total change in  $pK_a$  was also noticeable, but progressively smaller than in the previous case (about 4 and 3  $pK_a$  units, respectively). In the same figure the results of the theoretical calculations are also included. These results, presented as curves, were obtained using the  $pK_0^1$  and  $pK_0^2$  values (reported in Table 1) and the  $k_1$  and  $k_2$  values (reported in Table 2), which resulted from the fitting process described in the *Experimental Part*. The total change in  $pK_a$  of the MAE copolymer, although the smallest of the series, is much larger than for PAA, which is a monoprotic weak carboxylic polyelectrolyte with the same charge density as MAE in the *trans* conformation ( $\xi_{\text{PAA}} = \xi_{\text{MAE}} = 2.828$ ).<sup>[31]</sup>

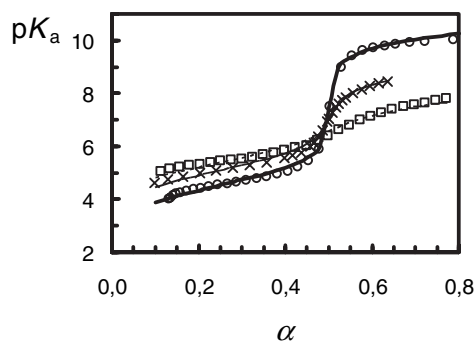


Figure 2. Experimental  $pK_a$  versus  $\alpha$  data for the MA copolymers in aqueous solution at 25 °C without any added salt, using KOH as the titrant base. The polyelectrolytes initial equivalent concentrations were: (□) MAE,  $10.046 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ ; (×) MAP,  $10.080 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ ; (○) MAiB,  $9.933 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ . The corresponding theoretical curves: (-----) MAE; (-.-.-) MAP; (—) MAiB, were calculated with the parameters reported in Table 1 and 2.

Table 1. Intrinsic ionization values for three MA-olefin copolymers in aqueous solutions at 25 °C.  $\xi = 2.828$  in all cases.<sup>a)</sup>

Property	MAE ( $m = 0$ ) <sup>b)</sup>			MAP ( $m = 1$ ) <sup>b)</sup>			MAiB ( $m = 2$ ) <sup>b)</sup>		
	water solvent	aq. 0.05 M TMAClO <sub>4</sub> solvent		water solvent	Aq. 0.05 M TMAClO <sub>4</sub> solvent		water solvent	aq. 0.05 M TMAClO <sub>4</sub> solvent	
$C_p$ ( $eq \cdot L^{-1} \times 10^3$ )	10.04 <sub>6</sub> ( $K^+$ ) <sup>c)</sup>	44.7 <sub>8</sub> ( $Na^+$ ) <sup>c)</sup>	10.01 <sub>9</sub>	10.08 <sub>0</sub> ( $K^+$ ) <sup>c)</sup>	9.95 <sub>3</sub>	9.93 <sub>3</sub> ( $K^+$ ) <sup>c)</sup>	39.8 <sub>4</sub> ( $Na^+$ ) <sup>c)</sup>	9.64 <sub>1</sub>	
$pK_0^1$	4.0	3.8	3.7	3.6	3.4	3.0	2.7	2.9	
$pK_0^2$	6.3	6.1	6.3	7.4	7.4	8.7	8.6	8.7	
$\Delta pK_0 (= pK_0^2 - pK_0^1)$	2.3	2.3	2.6	3.8	4.0	5.7	5.9	5.8	
$\sigma^d)$	0.12	0.14	0.14	0.10	0.20	0.13	0.12	0.51	

<sup>a)</sup> The structural charge density,  $\xi$ , was 2.828 in all cases.<sup>[14b]</sup>

<sup>b)</sup>  $m$  denotes the number of side methyl groups in the repeat units.

<sup>c)</sup> Cation of the titrant base. In aqueous TMAClO<sub>4</sub>, the titrations were performed using TMAOH as the base.

<sup>d)</sup> Quality of fit, given by  $\sigma = \sqrt{\frac{1}{n} \sum_{i=1}^n (pK_{exp}^i - pK_{theo}^i)^2}$ , where  $pK_{exp}^i$  and  $pK_{theo}^i$  stand for the experimental and the calculated  $pK^i$ , respectively.

The MAiB and MAP experimental data and the corresponding calculated curves are in very good agreement, as indicated by the low value of the root mean square deviation between experimental data and calculated values ( $\sigma$ ), shown in the bottom row of Table 1. The agreement between experimental data and the calculated curves is good also for the MAE copolymer, for which, however, some differences in the central ionisation range are detectable. It should be noted that the  $pK_0^i$  values of MAiB obtained with the present model analysis are in close agreement with data recently obtained on similar systems.<sup>[5c,8a]</sup>

In order to analyse the effect of the ionic strength, we studied the same systems in the presence of 0.05 M TMAClO<sub>4</sub>. This salt was chosen because of its well-known low tendency for giving ion pairs. The experimental  $pK_a$  versus  $\alpha$  data for MAiB, MAP and MAE copolymers in the presence of TMAClO<sub>4</sub> are reported in Figure 3. The trend of the data preserved the overall characteristics of the

corresponding copolymer potentiometric titration in water, but a general decrease of pH, and hence of  $pK_a$ , throughout all the  $\alpha$  range was noted. This effect is well known and is usually interpreted as a direct consequence of the screening of the fixed charges on the polyion by the ionic atmosphere.<sup>[18]</sup> The same explanation also reasonably holds for the observed slightly smaller total change in  $pK_a$  from low to high ionisation, that is, the 'steepness' of the  $pK_a$  curve. The latter being a direct measure of  $(dG^{ion}/d\alpha)$ , or  $(dG^{ion}/d\xi)$ , it is immediately evident that any increase of the ionic strength, that is, of  $\kappa$ , would smooth the increase of the electrostatic potential, as already noticed in the potentiometric titration data of monoprotic polyelectrolytes.

From Table 1 it can be seen that the values of  $pK_0$  of the first dissociating groups show a very slight decrease upon increase of the ionic strength and upon an increase of substituent hindrance (namely, 0.3, 0.2 and 0.1 for MAE,

Table 2. Stiffness parameters for different polycarboxylates in aqueous solutions at 25 °C.

Polymer	Condition	$\xi$	Solvent	$k$
				$kcal \cdot mol^{-1} \cdot \text{\AA}^{-2}$
MAE	1 <sup>st</sup> dissociation, MAE-I	2.828	Water or aqueous 0.05 M TMAClO <sub>4</sub>	0.10
MAE	2 <sup>nd</sup> dissociation, MAE-II			0.05
MAP	1 <sup>st</sup> dissociation, MAP-I	2.828		0.19
MAP	2 <sup>nd</sup> dissociation, MAP-II			0.10
MAiB	1 <sup>st</sup> dissociation, MAiB-I	2.828	Water	0.24
MAiB	2 <sup>nd</sup> dissociation, MAiB-II			0.14
PAA		2.828		0.24
PMA	$\alpha > 0.3$	2.828		0.24
PLGA	Ordered, helix	4.76	Water or aqueous 0.01 M NaCl	0.7
PLGA	Disordered	2.09		0.34
PA	Ordered ( $\alpha < 0.4$ )	1.64	Water or aqueous 0.05 M NaClO <sub>4</sub>	0.3
PA	Disordered ( $\alpha > 0.4$ )	1.64		0.1



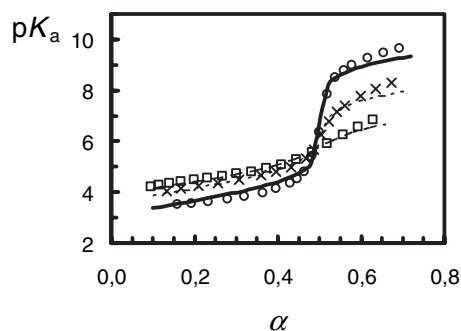


Figure 3. Experimental  $pK_a$  versus  $\alpha$  data for the MA copolymers in aqueous 0.05 M  $\text{TMAClO}_4$  at 25 °C, using TMAOH as the titrant base. The polyelectrolytes initial equivalent concentrations were: ( $\square$ ) MAE,  $10.019 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ ; ( $\times$ ) MAP,  $9.953 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ ; ( $\circ$ ) MAiB,  $9.641 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ . The corresponding theoretical curves: (-----) MAE; (- - -) MAP; (—) MAiB, were calculated with the parameters reported in Tables 1 and 2.

MAP and MAiB, respectively). No difference could be found for  $pK_0^2$ . Likewise, no difference was found for the  $k$  values between pure water and 0.05 M  $\text{TMAClO}_4$  (see Table 2), in agreement with the assumption that  $k$  should be related with the 'intrinsic' persistence length of the polymer. The above findings about  $pK_0$  and  $k$  and the excellent agreement between the calculated curves and the experimental data is a clear indication that the effect of adding a simple salt is properly considered by the CC theory through the expression of Equation 7(a) and (b).

The effect of polymer concentration and the nature of the monovalent counterion were also tested in the absence of added salt. In fact, potentiometric titrations were also carried out at a polymer concentration of about  $4 \times 10^{-2} \text{ eq} \cdot \text{L}^{-1}$ , using concentrated NaOH as the titrant. The experimentally derived  $pK_a$  data for the MAiB and MAE copolymers under these conditions are shown in Figure 4, with the corresponding calculated curves.

Probably the most important result is that also in this case  $k$  did not change from the previous values in pure water or in the presence of supporting salt.

As for  $pK_0$ , the effect of increasing the polymer concentration resulted in decreasing the values of  $pK_a$  over the whole range of  $\alpha$  values. It is well known that an increase of the polyelectrolyte concentration brings about a decrease of  $pK_a$  from the contribution to the ionic strength. The phenomenon was quantified by Cesàro and Benegas.<sup>[19]</sup> The good agreement between the theoretical curve and the experimental data indicates that polymer concentration, introduced into the CC theory from first principles, properly takes this effect into account, as has already been verified for other polyelectrolytes.<sup>[17]</sup> In this case, the decrease of both  $pK_0^1$  and  $pK_0^2$  was probably slightly larger than that expected from the contribution of the ionic strength only. Therefore it is possible that such a difference

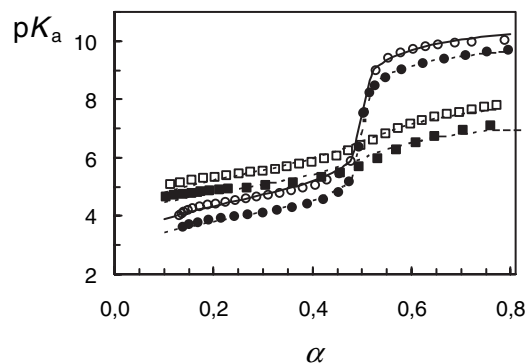


Figure 4. Experimental  $pK_a$  versus  $\alpha$  data values for MAE and MAiB in aqueous solution at 25 °C in the absence of any added salt. The polyelectrolytes initial concentration and titrant base were: ( $\square$ ) MAE,  $10.046 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ , KOH; ( $\blacksquare$ ) MAE,  $44.78 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ , NaOH; ( $\circ$ ) MAiB,  $9.933 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ , KOH; ( $\bullet$ ) MAiB,  $39.84 \times 10^{-3} \text{ eq} \cdot \text{L}^{-1}$ , NaOH. The theoretical curves were calculated with the parameters reported in Tables 1 and 2.

(albeit small) is diagnostic of a small effect of counterion specificity. In the previously quoted work,<sup>[19]</sup> the model provided a very good description of the potentiometric titration obtained using either NaOH or KOH as the titrant (base) without the introduction of any parameter to account for possible specific interactions between the polyions and the counterions in the range of polymer and salt concentrations of the present work. It has to be noted that the occurrence of specific interactions between MA copolymers and divalent cations – and even monovalent cations such as  $\text{Na}^+$  and  $\text{K}^+$  – was shown in a previous paper.<sup>[5a]</sup> In that case, however, the experimental conditions were far from the present ones: those measurements were carried out at very high salt – and hence counterion – concentration, that is, up to 1 N.

The dependence of  $pK_0^1$  and of  $\Delta pK_0$  on the number of side methyl groups is also reported in Table 1. An almost linear dependence on the number of side methyl groups is argued both for the first and for the second  $pK_0$ : the former one decreases whereas the latter one increases. This dependence on the chemical nature of the olefin comonomer, already discussed in the first papers concerning MA copolymers,<sup>[1,2]</sup> is attributed to an increase of the H-bond character of the monoprotonated form ( $\text{HA}^-$ ) upon first dissociation (i.e.,  $\text{H}_2\text{A} \rightleftharpoons \text{HA}^- + \text{H}^+$ ) parallel to increasing substitution.<sup>[14]</sup> This event comparatively reduces the free energy of the  $\text{HA}^-$  form, eventually producing a decrease of  $pK_0^1$ . Interestingly, the corresponding, expected, increase of  $pK_0^2$  is *not* exactly parallel: the ratio of the (absolute) change of  $pK_0$  per unit change of side methyl group is about 2.5:1 for the second dissociation (i.e.,  $\text{HA}^- \rightleftharpoons \text{A}^{2-} + \text{H}^+$ ) versus the first one. This corresponds to an inversion of the relative thermodynamic stability of the  $\text{A}^{2-}$  units. Ionic strength produces a slight tendency towards an increase of the difference between the values of  $pK_0$  of the first and

the second dissociating groups ( $\Delta pK_0 \equiv pK_0^2 - pK_0^1$ ), a characteristic already noted when analysing the potentiometric titration data of humic acids,<sup>[15e]</sup> which are also multifunctional natural polymers.

As for the  $k_1$  and  $k_2$  parameters, it can be concluded that the semiflexible characteristics of the MA copolymers persist in the whole range of polymer and salt concentrations explored in the present study (see Table 2). However, the  $k_2$  values are smaller than the corresponding  $k_1$  values for all three copolymers (see Table 2). The straightforward explanation is that the higher rigidity of  $k_1$  stems from the intramolecular H-bond which characterises the first dissociation with respect to the second one, as postulated on the basis of the above  $pK_a$  results. This hypothesis also accounts for the observation that an increasing number of side methyl groups increases both  $k_1$  and  $k_2$  (see Table 2), but with a significantly larger effect on  $k_1$ . In practice, the absolute value of  $\Delta k$  (i.e.,  $k_2 - k_1$ ) increases from MAE to MAiB, much like  $\Delta pK_0$ , pointing to the nicely converging, albeit independent, information provided by the two sets of different parameters.

It should be recalled that the comparison of calorimetric experiments with theories<sup>[31]</sup> already led to the conclusion that the chemical nature of the comonomers influenced the rigidity of the chain. In particular, the observed behaviour was found to be compatible with a linear (wormlike) chain model and with rigidity varying according to composition in the order  $MAP < MAiB$ , i.e. proportional to the amount of hindering methyl groups. It was proposed that in the case of MAE the chain flexibility may lead to very flexible coiled conformations, due to the lack of large substituents on the olefinic part. This could explain the small discrepancy between the calculated curve and the experimental  $pK_a$  values at low  $\alpha$  for this copolymer (see Figure 2).

Finally, the stiffness parameters here derived were compared with the results of previous studies, performed on both synthetic and natural polyelectrolytes under similar experimental conditions (see Table 2). In spite of the uncertainty in the calculated values of the  $k$  parameters, which averaged  $\pm 0.05$  in all cases, this comparison can help by providing some indications to properly place the copolymers of this family in the correct order of relative flexibility, on the basis of the chemical structure of the corresponding polyelectrolyte. The main findings are as follows.

(i) The MA copolymers share the value of the average structural charge density with the monoprotic polyelectrolytes PAA and poly(methacrylic acid) (PMA) that were recently studied by Benegas et al.<sup>[15c]</sup> and by Porasso et al.,<sup>[15d]</sup> respectively. In particular, PAA and PMA are structural isomers of MAE and MAiB, respectively. Because of this, at a first glance one could expect that the two poly(monoprotic) acids and the corresponding two poly(diprotic) acids have similar flexibility in aqueous

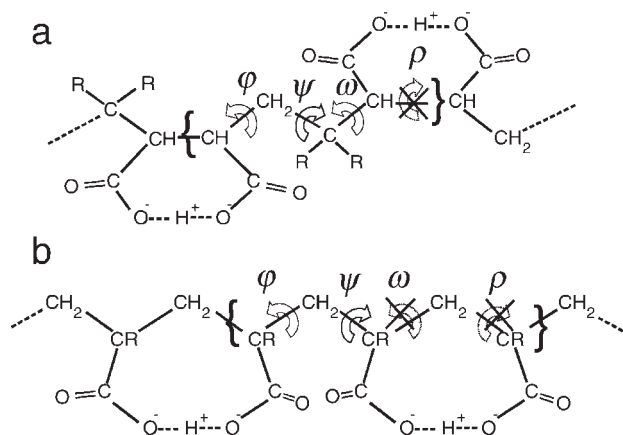


Figure 5. Rotation of the bonds along the polymer chain at half neutralisation: (a) in MA copolymers and (b) in polyacrylic ( $R = -H$ ) and polymethacrylic ( $R = -CH_3$ ) acids. In (a) the two residues  $-R$  denote two  $-H$ ,  $-H$  and  $-CH_3$ , and two  $-CH_3$  in MAE, MAP, and MAiB, respectively.

solution, mainly because of the same number (four) of single bonds in the corresponding dicarboxylic repeating unit (see Figure 5). In fact, it is well known that the number of single bonds in a polymer repeating unit, with the associated rotational freedom, ranks first among the factors influencing chain flexibility. The detailed dependence of the conformational energy on linkage rotation, albeit important, is certainly less so. Possibly, in this respect, just a slight difference could be expected in the present comparison to stem from the nonexact conformational correspondence of the linkage sequence  $\phi_2, \psi_2$  in PAA and PMA with the sequence  $\omega, \rho$  in the MA copolymers.

Inspection of Table 2 shows that only the stiffness parameter of MAiB in the low ionisation regime ( $k_1$ ) is similar to those reported for PAA and for PMA after transition ( $\alpha > 0.3$ ),<sup>[15d]</sup> all the others being from slightly to significantly lower. This observation, taken together with the already assessed presence of an intramolecular H-bond in the structures  $H_2A$  and  $HA^-$  involved in the first dissociation of MAiB (conventionally called here 'MAiB-I', whereas MAE-I, MAP-I, MAE-II, MAP-II, and MAiB-II in the following text stand for the first dissociation of MAE and MAP and for the second dissociation of MAE, MAP and MAiB, respectively), leads to the postulation of a rather different structural (and/or conformational) view of both the MA copolymers and the acrylic polymers, schematically depicted in Figure 5(a) and 5(b), respectively. In MAiB-I the rotational freedom of linkage,  $\rho$ , is very likely close to zero, because of the H-bond which enables the formation of a six-member ring involving the two carboxylic groups (the hydrogen atom is ignored, as usual). This structure, coupled with the presence of two methyl groups on the neighbouring C atom, is similarly expected to dramatically reduce the rotational freedom of linkage,  $\omega$ . In practice, only two rather

unconstrained rotations are left, i.e. the  $\phi$  and  $\psi$  ones at the out-of-ring  $\text{CH}_2$  group. A similar condition could be achieved by PAA/PMA, but postulating that a seven-member ring is formed involving the carboxylic groups of two contiguous repeating units [see Figure 5(b)]. This structure could also nicely explain the surprisingly small difference in  $k$  values between PAA and PMA after the conformational transition ( $\alpha > 0.3$ ; see Table 2). In fact, in this instance the  $\alpha$  carbons – the sites of methyl group substitution – would be involved in the ring, possibly affecting the conformation of the latter, but not the rotational freedom of the out-of-ring  $\text{CH}_2$  group, which likely is the main determinant of chain flexibility.

An interesting indication of the reliability of the above hypothesis may come from the experimental viscosity behaviour. It only apparently differs for the two classes of polyelectrolytes. In the case of PAA and PMA the intrinsic viscosity continuously increases with increasing  $\alpha$ ,<sup>[20]</sup> while the experimental data of the reduced viscosity and the calculated intrinsic viscosity show a maximum at half neutralisation for the MA copolymers.<sup>[4,5c]</sup> In fact, the correct comparison must consider the whole  $\alpha$  range for the acrylates with the first dissociation only of the MA copolymers, i.e. the intervals of proposed existence of the H-bonded ring structures. For the MA copolymers, the presence of the latter was convincingly proposed as the reasonable explanation for this finding.<sup>[1,2,3c]</sup> After 50% dissociation, their conformation becomes increasingly more flexible because of the breakdown of the ring structures and, as a consequence, also less extended. This is manifested as a decrease of viscosity.

(ii) The rigidity of MA copolymers (1<sup>st</sup> dissociation) was found to be smaller than that of the disordered conformation of poly(glutamic acid), PGA ( $k_{\text{coil}} = 0.34 \text{ kcal} \cdot \text{mol}^{-1} \cdot \text{\AA}^{-2}$ ),<sup>[15b,17]</sup> accounting for only one third of it for MAE-I up to two thirds for MAiB-I. For a comparison between the two sets of structures it is convenient to start by setting the substituted CH group in the main chain as the central reference (see Figure 6). The substituent is a hydrogen atom in the MA copolymer series, whereas it is the bulky carboxyethyl group in PGA. Such an enormous difference of steric hindrance is a first important issue to be taken into account. However, the main difference becomes apparent if one considers that the two peptide bonds flanking C- $\alpha$  in polypeptides are truly rigid bodies, so that PGA actually shows only the two well-known degrees of rotational freedom,  $\phi$  and  $\psi$  [Figure 6(b)]. At variance, a residual, albeit very small, torsional flexibility of the  $\rho$  linkage cannot be excluded in the MA copolymers, especially so for MAE-I [Figure 6(a)]. In addition, and more important, the determining difference should be clearly traced back to the rotational freedom of linkage,  $\omega$ , which also strongly increases from a small - but nonzero - value in MAiB to full strength in MAE. Finally, as expected, the difference of flexibility between poly(glu-

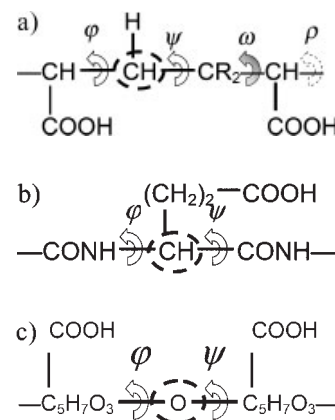


Figure 6. Rotation of the bonds along the polymer chain: (a) in the MA copolymers; (b) in poly(glutamic acid); and (c) in pectic acid. R stems for  $-\text{H}$  (twice) in MAE, for  $-\text{H}$  and  $-\text{CH}_3$  in MAP, and for  $-\text{CH}_3$  (twice) in MAiB.

tamic acid) in the very rigid  $\alpha$ -helical form ( $k_{\text{helix}} = 0.7 \text{ kcal} \cdot \text{mol}^{-1} \cdot \text{\AA}^{-2}$ )<sup>[15b]</sup> and the MA copolymers is even larger.

(iii) The repeating unit of pectic – or poly(galacturonic acid) (PA) – is schematically reported in Figure 6(c). The galactopyranosidic ring is usually considered a rigid body which can be treated as a single virtual bond. This will leave the two well-known degrees of rotational freedom,  $\phi$  and  $\psi$ , around the glycosidic oxygen, much like the case of PGA. Comparing the disordered conformations, that of PA is associated with a much lower value of  $k$  than that of PGA. It is immediately apparent that this is the consequence of the very large difference in size (and hence in rotational mobility) between the bare O atom in PA and the large structure of  $-\text{CH}((\text{CH}_2)_2\text{COOH})-$  in the polypeptide. The comparison with the copolymers is interesting: the  $k$  value of PA coincides with those of MAE-I and MAP-II; it is larger only than that of MAE-II, being smaller than those of all other conformations of the MA copolymers, indicating that the disordered conformation of PA can be considered as a comparatively flexible one, albeit not a flexible coil.

Pectic acid is known to undergo a (cooperative) intramolecular disorder-to-order conformational transition upon decrease of pH.<sup>[21]</sup> The ordered conformation was indicated as a rather rigid one, also on the basis of an accompanying relative increase of viscosity. The recent calculations of Benegas et al,<sup>[22]</sup> based upon the analysis of the pH titration of PA, confirmed the existence of such a transition, providing a relative increase of the stiffness parameter  $k$  from 0.1 (disordered) to  $0.3 \text{ kcal} \cdot \text{mol}^{-1} \cdot \text{\AA}^{-2}$  for the helical conformation. The latter form is then significantly stiffer than any other conformation of the MA copolymers, likely due to the important contribution of an extensive network of intramolecular, interresidue, hydrogen bonds.



## Conclusion

In this work a combined experimental/modelling approach has been used to study the polyelectrolytic and conformational characteristics of a family of MA-olefin copolymers.

Potentiometric titrations of MAE, MAP and MAiB copolymers at different polymer concentrations, with and without addition of simple 1:1 salt, and with three different monovalent counterions ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{TMA}^+$ ), have been analysed with an extension of the CC theory of linear polyelectrolytes.

The very good agreement found between experimental and calculated  $\text{pK}_a$  versus  $\alpha$  curves indicated that the very simple modelling provided by the CC theory was able to reproduce the polyelectrolytic behaviour in solution of this family of MA-olefin copolymers in the wide range of  $\text{pK}_a$  values covered. This agreement showed that the ionic strength and polymer concentration effects were properly built into the theory from first principles. As a consequence, our approach improves the CC theory for linear (and not hydrophobic) polyelectrolytes and promotes its application to other experimental cases of interest.

It is also worth noting that potentiometric titration data, when  $\text{K}^+$ ,  $\text{Na}^+$  or  $\text{TMA}^+$  were used as the counterions, were properly handled by the model. A very small, specific effect, if there was any at all, was detected between the MA copolymers and the said counterions under the present experimental conditions.

Regarding the modelling of the semiflexible polymeric characteristic of the MA copolymers, the present analysis consistently showed that the copolymers are more rigid in the low ionisation than in the high ionisation regime, as indicated by the values of  $k_1$  and  $k_2$  shown in Table 2. This finding was interpreted postulating that an extra interaction, due to the presence of an intrarepeat units hydrogen bond, stabilises a much stiffer polymer conformation at low  $\alpha$  values. As a matter of fact, the presence of the hydrogen bonds has been proved by IR spectroscopy, at least in the case of MAiB.<sup>[4b]</sup> In the second dissociation step the hydrogen bonds are disrupted. Consequently, during the ionisation of the second functional group the polymeric chain are more flexible than at low  $\alpha$  values. This finding, as well as the noted differences in the flexibilities of these MA copolymers, calls for further studies by appropriate experimental techniques (e.g., scattering methods) and molecular modelling.

Finally, it is worth pointing out that this work has shown, by direct comparison, that the simple experimental/modelling approach can be readily extended to the study of other weak polycarboxylates, both natural and synthetic, in which the evaluation of the polyelectrolytic and conformational characteristics of the polymeric chain are of interest.

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