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Tuning the pK_a of the antihistaminic drug chlorpheniramine maleate by supramolecular interactions with water-soluble polymers

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Abstract

The interaction of the pharmacologically important chlorpheniramine maleate (CPM) with polyanions containing sulfonate groups such as poly(sodium 4-styrenesulfonate) (PSS), poly(sodium vinylsulfonate) (PVS), and the more hydrophobic poly(sodium 2-(*N*-acrylamido)-2-methyl-propanesulfonate) (PAMPS) has been studied by ^{1}H NMR. It was found that the pK_a of the low-molecular weight molecule (LMWM) may be modified by its interaction with the polyanions, changing from 3 to 5, due to electric charge compensation. Interestingly, the interaction of CPM with PSS produces changes in CPM resonances, such as a general broadening and upfield shifts of the signals, and NOE effects between the LMWM and the water-soluble polymer (WSP) that indicate the presence of π–π interactions.

Keywords: Chlorpheniramine maleate; Water-soluble polymers; π–π interactions

1. Introduction

The pharmacological activity of drugs depends on their acid–base properties, since drugs may present enough solubility in water, as well as the necessary lipophilicity to trespass hydrophobic barriers. Association of drugs with hydrophilic carriers presents advantages in controlling both the release time and localization [1–8]. By association of drugs with nanocarriers, different barriers that determine their bioavailability can be overcome, as the enzymatic barriers, the absorption barrier, and, in the case of nasal administration, the mucociliary clearance [9–11]. The physical and chemical properties of the hydrophilic matrices, in terms of their interactions with the environment and the drugs, are pivotal for release performance. The release of the drug from a pharmaceutical form is mediated by the ability of the matrix to hydrate, swell and erode, as well as by diffusion of the water-soluble drug through the hydrophilic gel network thus formed. Thus, the specific interactions between the drug and the excipients including the hydrophilic polymers that may constitute the matrix are important in the diffusion of the drug through the hydrated network.

Among the possible interactions between drugs and matrices, electrostatic interactions may be relevant. Measurements of the drug binding capacities of some polyelectrolytes were related to the release profiles of matrix tablets containing the same drug–polyelectrolyte system [6]. However, long-range electrostatic interactions are screened by the presence of excess of electrolytes in solution. Better control of drug release kinetics may be potentially achieved if more specific interactions between the drug and the matrices are provided.

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Anionic polyelectrolytes may be used in drug delivery systems with the advantage of increasing the mucoadhesivity by means of chemical interactions with the mucus in mucous membranes. In a previous paper we have shown that the binding constant of the antihistaminic drug chlorpheniramine maleate (CPM), is more intense for poly(sodium 4-styrenesulfonate) (PSS), polyanion that contains aromatic rings, than for several polyions that do not contain aromatic rings in their structure [12]. Moreover, for the latter polyanions, the corresponding binding constants were very similar and tended to zero in the presence of excess of NaCl, indicating non-specific long-range electrostatic interactions. In the course of subsequent investigations, we have noticed the high affinity of PSS towards binding molecules containing aromatic rings, both negatively charged as triphenyltetrazolium chloride (TTC) [13,14], or zwitterionic as rhodamine B (RB) [15–17]. Aromatic–aromatic interactions were invoked in which π–π interactions play an important role.

Aromatic–aromatic interactions are one of the principle noncovalent forces governing molecular recognition and biomolecular structure. They are important in the stabilization of DNA by means of both π–π stacking and a consequent increase in the hydrogen bonding capacity of the DNA bases [18,19]. Another important property is the change in the pK_a of the substrates produced as a consequence of these interactions. An enzymatic catalysis mechanism has been recently proposed in which the hydrolysis of a nucleotide occurs once its basicity is increased by π–π interaction with two tryptophan residues [20]. Although aromatic–aromatic interactions have been found in many biological and synthetic systems by crystallography, not many studies have been done to date to characterize these interactions in aqueous solution in laboratory model systems containing water-soluble polymers (WSP) and low-molecular weight molecules (LMWM).

In this paper, we analyze the interaction of CPM with several polyanions containing sulfonate groups as PSS, poly(sodium vinylsulfonate) (PVS), and the more hydrophobic poly(sodium 2-(*N*-acrylamido)-2-methyl-propanesulfonate) (PAMPS), by ^{1}H NMR. We show the change in the pK_a of CPM produced by the interaction with the different polyanions, and give evidence of the π stacking of CPM onto PSS.

2. Experimental section

2.1. Reagents

Commercially available PSS (Aldrich, synthesized from the para-substituted monomer, Average Mw 70 000) and CPM (TCI Tokyo Kasei, provided as a racemic mixture) were used as received. Commercial solutions of PVS (Aldrich, 25 wt.% solution in water) and poly(2-(*N*-acrylamido)-2-methyl-propanesulfonic acid) (Aldrich, Average Mw 2 000 000, 10 wt.% solution in water) were weighted on gold plates, and successively evaporated and dissolved in D_2O (Acrōs, 99.8% *d*-content) for 8 cycles at 333 K. The pH was adjusted with minimum amounts of NaOH and DCl (Acrōs, +99% *d*-content). NaCl (Merck) was used to adjust the ionic strength. The structures of CPM and the different WSP are shown in Fig. 1.

2.2. Equipment

The pH was controlled on a Horiba F-15 pH meter. ^{1}H NMR measurements were made in a JNM-Lambda500 spectrometer (JEOL, 500 MHz) and in an AVANCE600 (Bruker, 600 MHz) spectrometer.

2.3. Procedures

Conventional procedures have been followed, and the experimental conditions are given in the figure captions. After preparation of the samples in D_2O (5 mL) the pH was adjusted with minimum amounts of DCl or NaOH. Appropriate conditions for transfer-NOE (TRNOE) experiments were chosen as $[CPM] = 10^{-3}$ M, $[PSS] = 2 \times 10^{-3}$ M, $[NaCl] = 5 \times 10^{-2}$ M, and pH 4. A total of 256 FIDs of 2K were collected with 24 scans per FID. Each scan was taken with a 90° pulse width of 9.7 μs, pulse delay time of 3.0 s, and mixing time of 200 ms. Experimental data (2K × 256) were Fourier transformed to give final data matrices of (2K × 512) data points.

3. Results and discussion

3.1. CPM alone

^{1}H NMR spectroscopy allows following molecular changes in CPM at different conditions. In order to assign the ^{1}H NMR signals to the protons outlined in Fig. 1 COSY experiments were done. Fig. 2 shows the aromatic region for the experiment at $[CPM] = 10^{-3}$ M and pH 7.6, where the cross-peaks clearly allow identifying the chemical shifts with the protons corresponding to the pyridyl and chlorophenyl rings. The complete

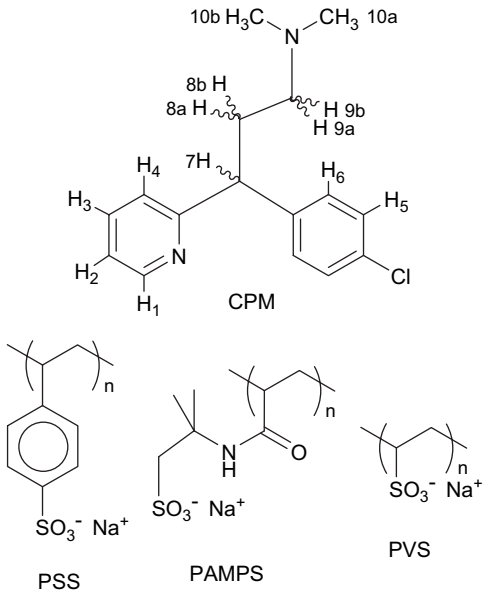

Fig. 1. Molecular structures.

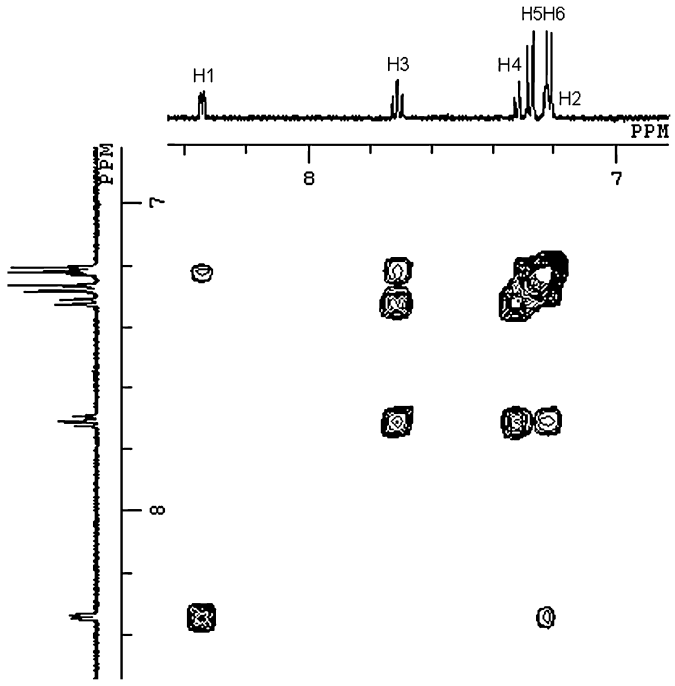

Fig. 2. ^{1}H NMR COSY spectrum (500 MHz) in D_2O of CPM 10^{-3} M at pH 7.6.

CPM spectra at pH 7 and 2 are shown in Fig. 3, and the signals are related with the protons in Fig. 1 (Hm corresponds to the maleate protons). Evaluating the influence of the pH on this molecule in a pH range between 11 and 2, we observe shifting of most signals, as can be also seen in Fig. 4. For CPM two pK_a values (9.2 and 4.0) have been reported [21] corresponding to the protonation of both nitrogen atoms in the molecule. Our results verify approximately this information. At pH between 9 and 11, the most shifted signals correspond to protons H9 and H10, indicating that the tertiary amino group protonates in this pH range. On the other hand, at pH between 2 and 4, the most affected signals are those of the pyridyl ring (protons H1–H4), indicating the protonation of its nitrogen. Note that

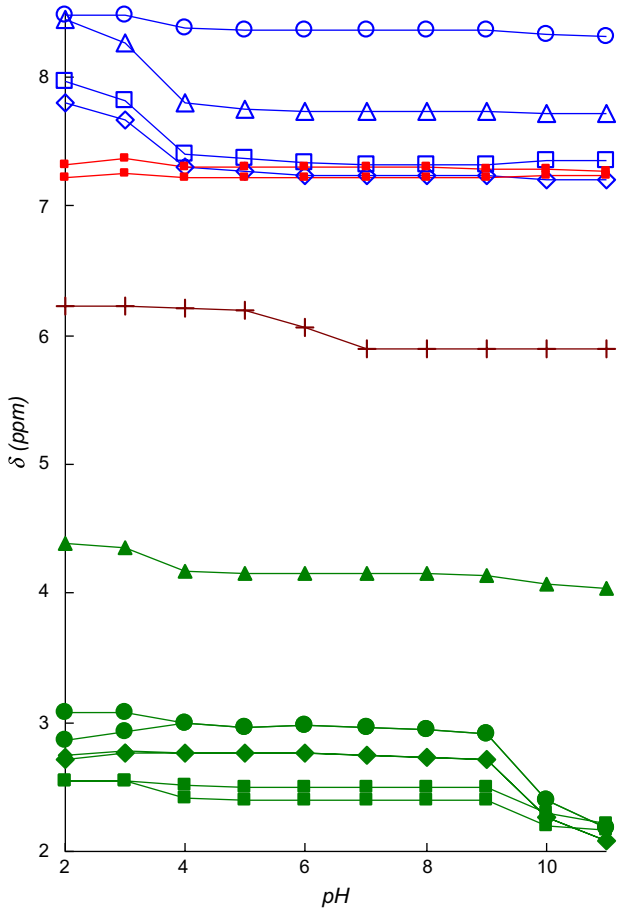

Fig. 4. Chemical shifts of CPM protons as a function of the pH: (○) H1; (△) H3; (□) H4; (◇) H2; (▪) H5 and H6; (+) Hm; (▲) H7; (●) H9aH9b; (◆) H10aH10b; (■) H8aH8b.

the chlorophenyl protons (protons H5 and H6) are less significantly affected by the pH. The low value for the pK_a of the pyridyl nitrogen is justified by the presence of the positive charge in the aliphatic amino group. On the other hand, the maleate counterion also protonates at pH between 5 and 7. The *cis*

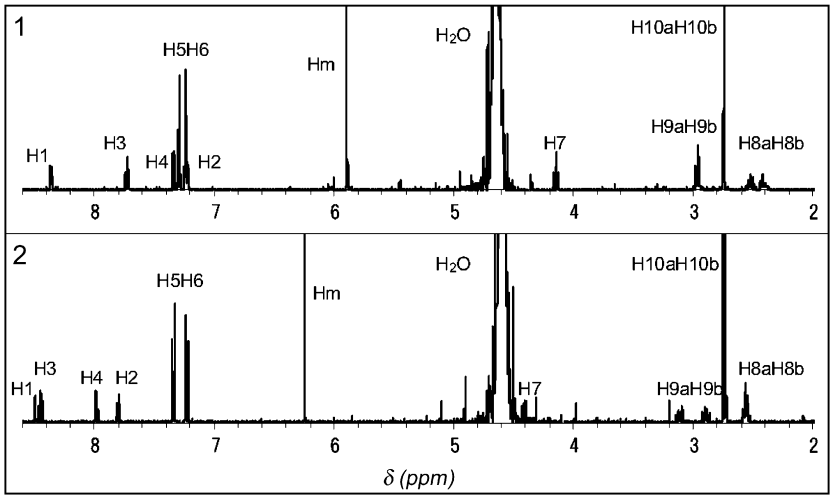

Fig. 3. ^{1}H NMR spectra (500 MHz) in D_2O of CPM 10^{-3} M at pH 7 (1) and 2 (2).

geometry of maleic acid makes it to ionize in two steps, since the monoanion is stabilized by intramolecular hydrogen bonding. As a result, the first ionization takes place very easily ($pK_{a1} \approx 2$) and the second requires low acidity ($pK_{a2} \approx 6$) [22].

Protonation of the pyridyl group produces that the diastereotopic protons H8aH8b exhibit the same chemical shift, whereas the diastereotopic protons H9aH9b, which were indistinguishable before protonation, become very well resolved with differences in the chemical shifts of 0.2 ppm (Figs. 3 and 4). On the other hand, both methyl groups also become distinguishable, with differences in the chemical shifts of 0.02 ppm, which may suggest that the interconversion between both methyl groups by means of inversion of the configuration of the aliphatic nitrogen is slow. These effects may be accompanied by conformational changes, as for example a more extended configuration in order to minimize electrostatic repulsions between the two positively charged groups.

3.2. CPM in the presence of different WSP

In Fig. 5 the aromatic region for the corresponding spectra of CPM can be seen at pH 7 and 2 in the presence of PSS (Fig. 5a1 and a2, respectively), PAMPS (Fig. 5b), and PVS (Fig. 5c). For the polyanions that do not contain aromatic groups (PAMPS and PVS), the spectra are not changed by comparison with CPM alone at pH 7, while at pH 2, downfield shifts are found for some protons, and, contrary to the tendency in the absence of any WSP, the magnetic environment of H5 and H6 become similar. On the other hand, as it has been seen for other systems [14,16,17], a general broadening together with an upfield shift of all the signals is found in the presence of PSS. These changes reveal the interaction of the three WSP with CPM. However, the significant changes in the ^{1}H NMR spectra for CPM in the presence of PSS witness that aromatic–aromatic interactions are taking place, while for the other polymers, it is probable that the interaction is driven mainly by electrostatic and hydrophobic forces which are not producing specific short-distance contacts.

The immediate discussion arising from these results is whether the changes found for the PSS–CPM system are due to non-specific interactions, where the small molecule is confined in a more hydrophobic environment, or if specific interactions such as π–π interactions are also taking place. Several considerations may be envisaged to think in the existence of specific π–π interactions: (1) the general broadening of the signals, which may indicate close contacts between the LMWM and the WSP that produce different magnetic microenvironments; (2) the upfield shift of the aromatic protons due to the influence of the π-electronic currents of the stacked rings; (3) the differences in the values of the relative shifts that may indicate the most probable contacts; (4) NOE signals that evidence specific contacts between the polymer and the LMWM.

As mentioned before, in the case of the PSS–CPM system, both a broadening of the signals and upfield shifts are found. At pH between 7 and 9, where the CPM presents a positive charge at the aliphatic amino group, the highest shifts found in the presence of the WSP correspond to protons H2, H3, and H4 (0.56, 0.56, and 0.42 ppm, respectively), followed by H5 and H6 (0.42, and 0.36 ppm, respectively), while at pH 2, where the pyridyl group is protonated, the most shifted signals correspond to protons H3 and H4 (0.58, and 0.5 ppm, respectively): H2 is shifted 0.39 ppm. So it is likely that both aromatic rings may undergo π stacking onto PSS. Definite evidence of the π stacking of CPM onto PSS and more insights for the geometry of the complexation should be given by NOE experiments. However, NOE effects between a LMWM and

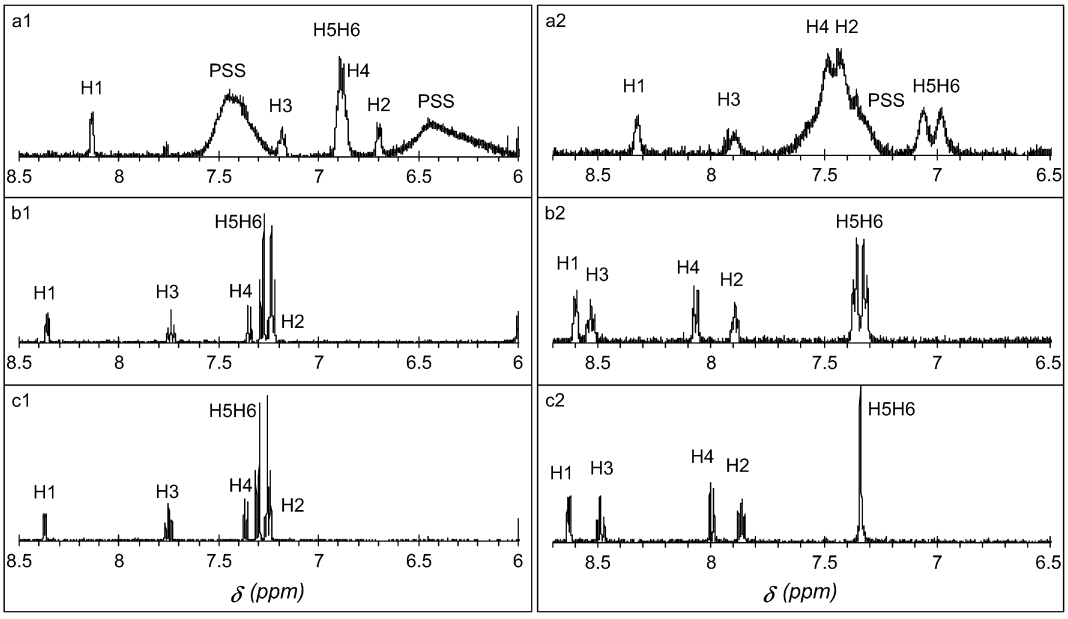

Fig. 5. Aromatic region of 500 MHz ^{1}H NMR spectra in D_2O of CPM 10^{-3} M at pH 7 (1) and 2 (2), in the presence of: (a) PSS 10^{-2} M; (b) PAMPS 10^{-2} M; (c) PVS 10^{-2} M.

a macromolecule are difficult to detect, since the relaxation rates are very high. Conditions must be found that ensure the presence of significant amounts of free LMWM in a fast equilibrium with the bound state. Thus, intense negative NOE effects generated in the bound state can be transferred to the free state by chemical exchange, under the so-called transfer-NOE (TRNOE) [23,24]. The conditions found for TRNOE experiments are related with a low PSS/CPM ratio and relatively high ionic strength [13]. In Fig. 6 negative NOE cross-peaks can be seen between the polymer protons and CPM protons that reveal intermolecular association. Due to possible spin diffusion effects during the mixing time (200 ms) [25] and the broadness of the PSS signal, the cross-peaks found neither allow proposing a geometry for the intermolecular contact, nor calculate effective proton–proton distances.

As stated in Section 3.1, protonation of the pyridyl group occurs at a low pH (around 3), since the positively charged aliphatic amino group inhibits the protonation of the pyridyl nitrogen and subsequent increase in the total charge. However, by the interaction of CPM with the negatively charged polyelectrolytes at pH below 9, the positive charge is neutralized. That produces an increase in the basic character of the pyridyl group. To show this, the chemical shift of H3 versus the pH has been plotted in Fig. 7. For the three polyelectrolytes, the apparent pK_a of CPM becomes around 5. Concerning the effects on the diastereotopic protons, both PVS and PAMPS produce a difference up to 0.02 ppm in the methyl signals at pH 2–4 (equivalent as in the case of CPM alone at pH 2–3). On the contrary, the two methyl groups are distinguishable yet at pH 6 in the presence of PSS, with differences in the chemical shifts that may reach 0.09 ppm, as can be seen

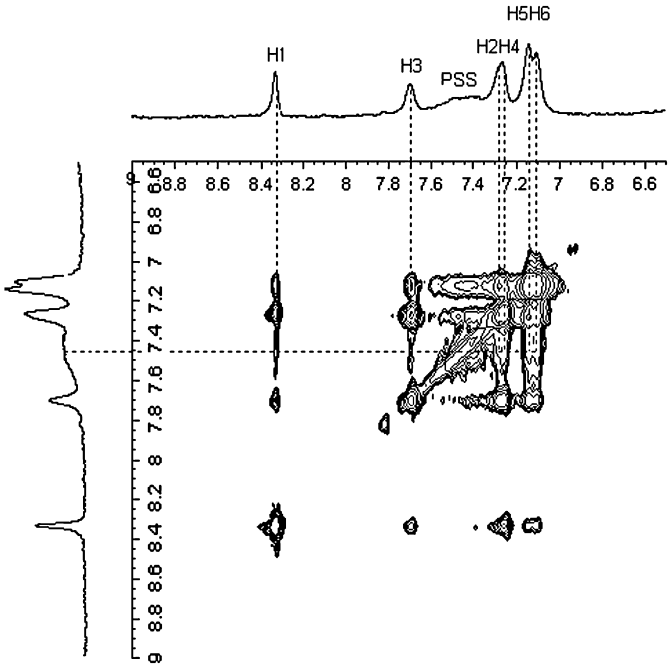

Fig. 6. Aromatic region of 600 MHz ^{1}H NMR TR-NOESY spectrum in D_2O of CPM 10^{-3} M at pH 4, in the presence of 2×10^{-3} M PSS and 5×10^{-2} M NaCl.

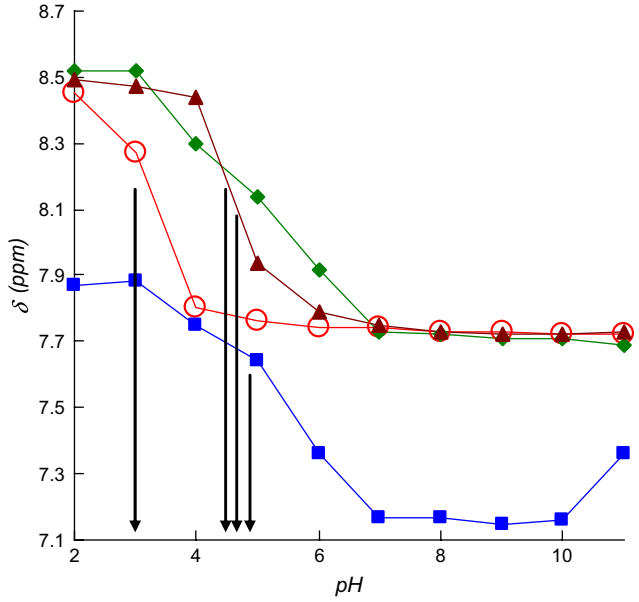

Fig. 7. Chemical shifts of H3 as a function of the pH: (○) CPM alone; (■) CPM 10^{-3} M in the presence of 10^{-2} M PSS; (▲) CPM 10^{-3} M in the presence of 10^{-2} M PVS; (◆) CPM 10^{-3} M in the presence of 10^{-2} M PAMPS.

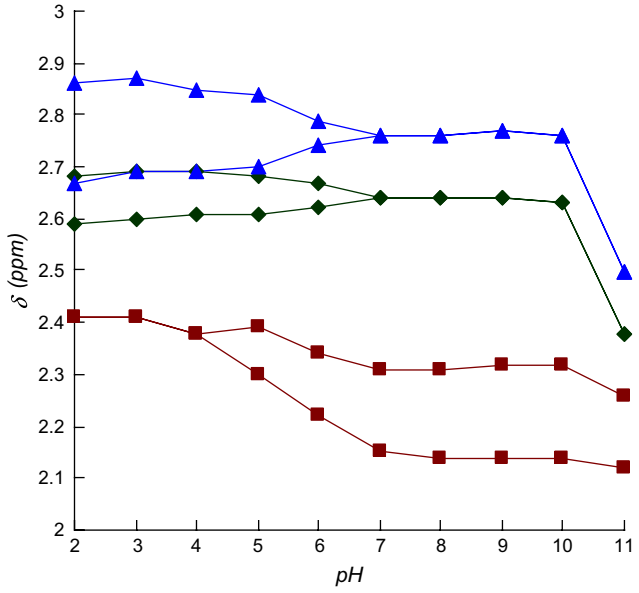

Fig. 8. Chemical shifts for a CPM 10^{-3} M solution in the presence of 10^{-2} M PSS as a function of the pH of: (■) H8aH8b; (▲) H9aH9b; (◆) H10aH10b.

in Fig. 8, where the evolution of all the aliphatic diastereotopic protons is shown as a function of the pH.

4. Conclusions

The pK_a of the pharmacologically important low-molecular weight molecule (LMWM) chlorpheniramine maleate (CPM) may be modified by its interaction with polyanions containing sulfonate groups such as poly(sodium 4-styrenesulfonate) (PSS), poly(sodium vinylsulfonate) (PVS), and the more

hydrophobic poly(sodium 2-(*N*-acrylamido)-2-methyl-propanesulfonate) (PAMPS). Protonation of the pyridyl aromatic ring is achieved at pH 5 in the presence of the water-soluble polymers, whereas it is produced at pH 3 in the absence of the polyanions. $\pi-\pi$ interactions take place between CPM and PSS as seen by a general broadening and upfield shifts of the signals in ^{1}H NMR experiments, together with NOE cross-peaks between the water-soluble polymers and the LMWM.

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