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Polymer 45 (2004) 2127–2132
polymer
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Hydrolytic degradation of polyamidines and its potential application in controlled release of active agents

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Received 11 August 2003; received in revised form 26 January 2004; accepted 29 January 2004

Abstract

Hydrolytic degradation of an aliphatic polyacetamidine at different pH values was studied using ^{1}H NMR spectroscopy. It could be shown that degradation occurred at neutral and basic conditions whereas under acidic conditions the polyamidine was stable. In the course of degradation, aliphatic acetamide, amine and acetic acid are formed. The ability of the polyamidine to release active compounds bonded ionically to the polymer backbone by hydrolytic decomposition was studied by UV spectroscopy. The measurements were performed on a mixture of the polyamidine and salicylic acid as an example. The release behavior of a 1:0.8 mixture at pH 7 was nearly linear within the first 10 h and went to completion after 30 h. This system is discussed with respect to the development of new drug delivery systems based on polyamidines.

Keywords: Polyamidines; Ionic complexes; Release behavior

1. Introduction

Polymers with pronounced basicity are widely used. Among them one can find those containing nitrogen atoms either in the side chain or in the polymer backbone. Well known examples are polyvinylpyridine frequently used as acid scavenger [1] and polyethyleneimine used as additive in cosmetics and in the paper-making process [2]. Less known are polyamidines and polybiguanides [3]. The latter has found its application as water soluble disinfectant [4].

Our interest is focused on polyamidines with the general structure $-(R^1-N{=}C(R^2)-NH)-$. Such polymers are easily available by conversion of diamines with orthoesters [5]. Depending on the residues R^1 and R^2, the basic properties of polyamidines are more or less pronounced. Highest pK_a values (approx. 11) have been found in case of aliphatic polyamidines [6]. It could be shown that such polymers are able to abstract protons from typical proton donors such as carboxylic acids and phenols resulting in the formation of ionic complexes [7–10]. In such complexes, the proton donors are demobilized and are hardly able to release the system. Only at elevated temperatures under vacuum an escape of proton donors was observed [8].

Earlier unpublished results showed that under certain conditions polyamidines are susceptible to hydrolytic decomposition. This raised the question, whether polyamidines could be used as carriers for any active compounds which after hydrolytic decomposition of the polymer leave the system in a controlled manner. Since the decomposition products of polyamidines are assumed to be less toxic, it might even be possible to apply such mixtures in biological systems as a drug delivery system. The properties demanded from a drug delivery system based on polymers are discussed in detail by Andrianov and Payne [11].

This paper concerns investigations about the hydrolytic stability of an aliphatic polyacetamidine and its release behavior in aqueous environment with respect to an imbedded proton donor molecule, namely salicylic acid. This polymer–proton donor complex serves as a model for a potential release system. The preliminary investigations presented here shall help to estimate the potential to develop new effective release systems based on such or similar compounds.

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doi:10.1016/j.polymer.2004.01.055

2. Experimental

2.1. Materials

Salicylic acid and buffer solution of pH 7 (Fluka) were used as received.

2.2. Poly(1,8-octamethyleneacetamidine)

The matrix polymer poly(1,8-octamethyleneacetamidine) (POA) was synthesized as described elsewhere [8]. The number average molecular weight of the polymer determined by NMR terminal group analysis was approx. 2100 g/mol. POA is soluble in ethanol, pyridine and organic acids and does not decompose in solution provided the solvents are free from water. On air, slow decomposition by moisture is observed.

2.3. Hydrolytic degradation of poly(1,8-octamethyleneacetamidine)

Three samples of approximately 10 mg of POA were introduced each with 1 ml of a mixture of CD_3OD/D_2O (3:1, v/v) into NMR tubes. The polymer dissolved completely. One of these samples was mixed with on drop of conc. aqueous HCl (acidic), one with one drop of conc. NaOH (basic), and one was used as received (neutral). After 4 weeks, the extent of hydrolytic degradation was ascertained by ^{1}H NMR spectroscopy.

2.4. Complex formation

3 g of POA (17.9 mmol amidine groups) and 1.96 g (14.2 mmol) of salicylic acid were mixed in a twin screw mini extruder (DACA instrument) at 165 °C for 3 min. The molar ratio of amidine groups to the carboxylic groups in the mixture was approximately 1:0.8.

2.5. Release behavior of polymer/salicylic acid complexes

5 mg of POA/salicylic acid complex were immersed in 1000 ml of a buffer solution with pH $\approx$ 7 at 37 $\pm$ 1 °C. During the storage of the complex, the buffer solution was stirred with 50 rpm. Samples of 5 ml buffer solution were withdrawn at regular time intervals of 2 h initially up to 10 h and then randomly until 50 h. Each withdrawal was replaced by 5 ml of fresh medium. The amount of salicylic acid released was estimated with an UV spectrometer.

2.6. Measurements

NMR measurements were carried out on a Bruker DRX 500 spectrometer operating at 500.13 MHz for ^{1}H. The samples were measured at ambient temperature and referenced on the solvent signal of CD_3OD ($\delta(^1H) = 3.31$ ppm).

For IR spectroscopic investigations, the samples were prepared as thin films on silicon wafers by solvent casting from methanol. All samples were carefully dried under vacuum in a desiccator prior to use. The measurements were carried out on a IFS 66V/S Bruker spectrometer with MCT detector. The spectra were recorded in the spectral range of 4000–600 cm^{-1} with a resolution of 4 cm^{-1}, 5000 scans/spectrum were co-added.

Raman measurements of methanolic solutions of the polyacetamidine, salicylic acid and their mixture were carried out at room temperature with a HoloProbe 785 spectrometer (Kaiser Optical Systems). The Raman laser wavelength was 785 nm. Spectra were recorded with a resolution of 4 cm^{-1}, 50 scans/spectrum were co-added.

UV spectra were recorded with a Varian Cary 100 spectrometer in methanol or in buffer solutions using a 1 cm quartz cuvette.

3. Results and discussion

As an example, the release behavior of aliphatic polyamidines was investigated on a mixture of poly(1,8-octamethyleneacetamidine) (POA) with salicylic acid. The mixture was prepared by melt mixing in a Daca Minimixer at 165 °C. Alternatively, mixtures are also available by solution casting from ethanol. From our earlier investigations, it is known that carboxylic acid groups form ionic complexes with amidine groups almost quantitatively. Such complexes are stable up to at least 150 °C. For the mixture discussed here, complex formation was concluded from IR and Raman spectroscopic investigations.

Our idea to utilize polyamidines as matrix material for a system with release properties presupposes that amidines can be decomposed by hydrolytic cleavage of the amidine groups. It has been assumed that decomposition of the amidine groups would destroy the binding sides of the polymer. This would interrupt the ionic interactions followed by a release of the active compound (salicylic acid).

In the following, investigations concerning the complex formation, the hydrolyzability of pure polyamidine and the release behavior of its mixture with salicylic acid has been described.

3.1. Complex formation of POA with salicylic acid

Evidence of complex formation delivered IR spectroscopic investigations. Although the results are not as unambiguous as described for the interactions of polyamidines with aliphatic carboxylic acids [9], the formation of ionic complexes between POA and salicylic acid could be concluded. In the mixture of both compounds, characteristic bands appear at 1387 and 1570 cm^{-1}, which were assigned to the symmetric $\nu_s(COO^-)$ and antisymmetric $\nu_{as}(COO^-)$

stretching vibration of the carboxylate anion, respectively, formed by proton transfer from salicylic acid to POA.

Ionic interactions in methanolic solution were studied by Raman spectroscopy. The respective region of the spectra of POA, salicylic acid and their mixture are shown in Fig. 1. Pure salicylic acid shows a band at 1673 cm^{-1} corresponding to the C=O vibration of the acid functional group, whereas, the C=N vibration band of POA appears at 1630 cm^{-1}. Both bands disappear in the mixture, which gives a clear indication of the interaction between the amidine groups and the carboxylic acid group resulting in the formation of ionic complexes. Instead, the band of the carboxylate anion also appears at 1385 cm^{-1} (not shown) as already found in the IR spectrum.

3.2. Hydrolytic degradation of POA

Hydrolytic degradation of POA was carried out in a mixture of CD_3OD/D_2O (3:1, v/v) at pH ≈ 1 (acidic), pH = 7 (neutral), and pH ≈ 11 (basic). The ^1H NMR spectrum of POA after 4 weeks exposure to hydrolysis at pH ≈ 1 is shown in Fig. 2(a). The spectrum is completely in accordance with the spectrum of a protonated polyamidine [8]. Under acidic conditions, the amidine groups are able to form amidinium salts the spectrum of which shows some differences compared to the spectrum of the unprotonated species. Thus, the methyl group signal of –NH–$C(CH_3)$=N– at 2.25 ppm appears in CD_3OD as solvent only in protonated polyamidines whereas in unprotonated ones, this signal disappears nearly quantitatively because of proton–deuteron exchange of the CH_3 group protons with the OD deuterons of the solvent. This behavior has only been observed in case of aliphatic polyacylamidines and the mechanism was discussed earlier [8].

Moreover, the ^1H NMR spectrum in Fig. 2(a) shows two separated signal groups at 3.37 and 3.21 ppm corresponding to the H^1, $H^{1'}$ protons of the methylene groups adjacent to the amidine group. In unprotonated polyamidines coalescence of these signals is observed showing that proton exchange between the nitrogens within the amidine groups occurs. Protonation results in a ZE configuration with nonequivalent CH_2 groups [8,12]. Small signals at 3.0 and 1.9 ppm could be assigned to amino and acetamido end groups which are already present in the starting material. The most important result concluded from the NMR spectrum is that aliphatic polyamidines are stable under acidic conditions.

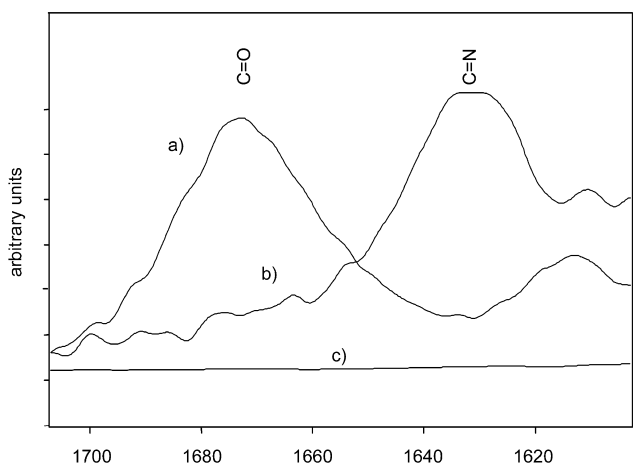


Fig. 1. Raman spectra of (a) salicylic acid, (b) POA, and (c) complex of POA and salicylic acid in methanolic solution.

The ^1H NMR spectrum of POA after four weeks exposure to hydrolysis at pH ≈ 7 (neutral) is shown in Fig. 2(b). In contrast to the spectrum of the sample stored under acidic conditions (Fig. 2(a)), drastic changes can be seen. Practically, no signal of the former polyamidine appears. Instead, new signals belonging to the hydrolysis products can be recognized. The signals at 1.95 (H^2) and 3.13 ppm (H^1) could be assigned to the CH_3 and CH_2 residue, respectively, of an acetamide group, whereas the signal at 2.61 ppm (H^3) belongs to a CH_2 residue attached to a primary amino group. Nearly equal intensities of the signals at 2.61 and 3.13 ppm show that hydrolysis of one amidine group obviously results in the formation of one acetamide and one amino end group. From the spectrum, one can conclude that the hydrolysis was quantitative. The other aliphatic proton signals were observed at their characteristic positions.

At pH ≈ 11 (basic), a similar degradation process is observed. The respective ^1H NMR spectrum is shown in Fig. 2(c). Beside the signals already seen in Fig. 2(b), a new signal appears at 1.91 ppm (H^1) which could be assigned to acetate anion. Simultaneously, the intensities of the signals belonging to the acetamide groups at 1.95 and 3.13 ppm decrease. This can be explained by subsequent hydrolysis of the acetamide groups caused by the excess of OH^- ions present in the system. Basic hydrolysis of the amide group results in the formation of an amine group and an acetate ion. The hydrolytic degradation of the POA is represented in Scheme 1(a).

According to the literature [13–15], hydrolysis of amidines proceeds through an attack by a hydroxide ion at the amidinium cation in the course of which a tetrahedral intermediate is formed. Subsequent deprotonation of –OH and C–N cleavage results in the formation of an amine and an amide. This mechanism explains the commonly observed stability of amidines under acidic conditions [15] were the lack of hydroxide ions prevents decomposition.

3.3. Release behavior of POA

Taking into account the decomposition behavior of POA, the release behavior of POA/salicylic acid mixtures was investigated in aqueous buffer solutions at pH = 7. For this, the samples were stored in the buffer solution and exposed to hydrolysis over a certain time. It has to be mentioned that the samples were inherently insoluble in water. Gradual dissolution is only observed with progressive degradation of

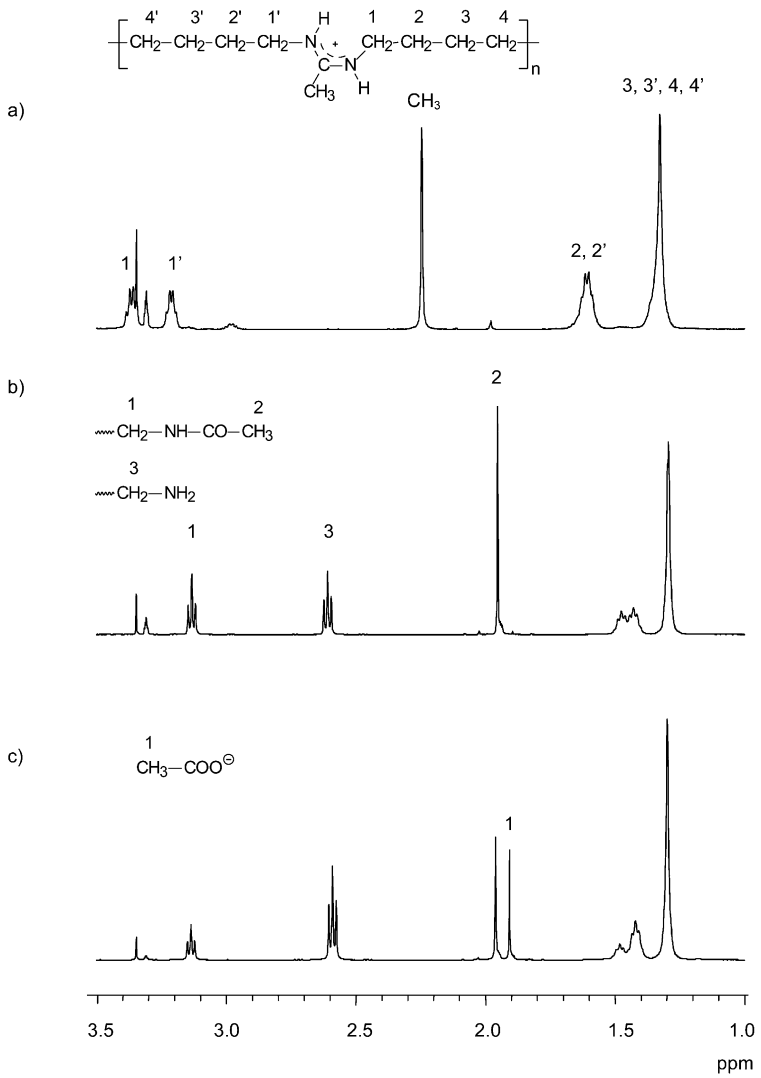
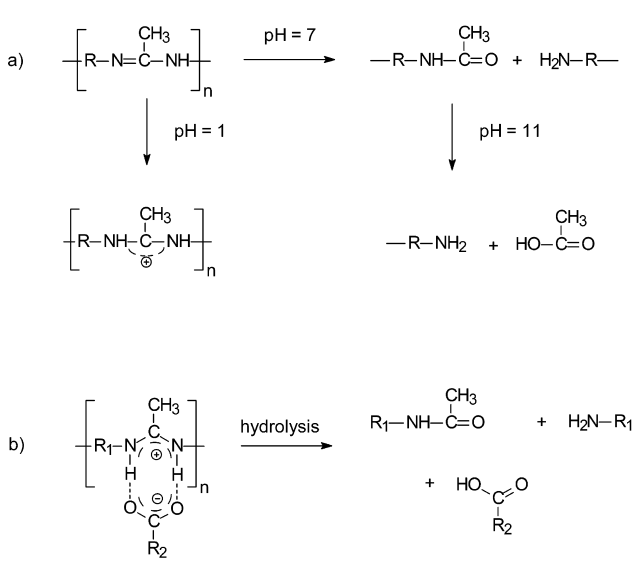


Fig. 2. ^{1}H NMR spectra of POA after four weeks exposure to hydrolysis at (a) pH 1, (b) pH 7, and (c) pH 11.



Scheme 1. Hydrolytic degradation of (a) POA and (b) its mixture with carboxylic acids.

the polymer. The release of salicylic acid into the buffer solution over the time according to Scheme 1(b) was followed by UV spectroscopy.

The UV spectra of salicylic acid, POA and the mixture containing 80 mol% of salicylic acid with respect to the amidine groups in the polymer measured in methanol are shown in Fig. 3. POA shows only one absorption maximum at 220 nm whereas salicylic acid absorbs at 210, 275 and 300 nm. The absorption at 300 nm is ascribed to the $n \rightarrow \pi^{*}$ transition and appears also well resolved in the spectrum of the mixture. Since the buffer solution has no characteristic absorption in this region, this band can be used for a semi-quantitative analysis of the salicylic acid release of POA.

In the buffer solution, the intensity of the absorption maximum varies with the concentration of the released salicylic acid. Fig. 4 shows the UV spectra of samples taken out from the buffer solution at regular time intervals of 2 h initially up to 10 h and then randomly until 60 h. The initial volume of the medium was maintained by replacing the sample volume with the same volume of buffer solution.

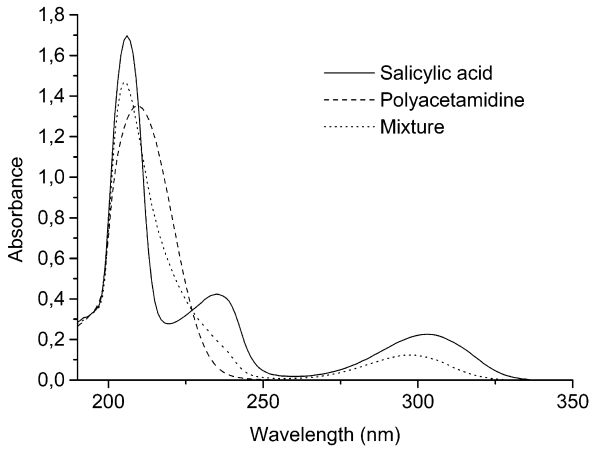


Fig. 3. UV spectra of salicylic acid, POA and their mixture in methanol.

The respective plot of the absorbance maxima versus time is shown in Fig. 5. From both figures, it is clearly seen that the concentration of salicylic acid in the buffer solution increases with time. The liberation proceeds nearly linear within the first 10 h and reaches a level of approximately 75%. Complete release of salicylic acid is observed after approximately 30 h.

It is obvious that the hydrolytic stabilities of the POA salicylic acid complex under the conditions chosen and that of pure POA in acidic solution (see above) differ significantly although in both cases the amidine groups exist in their protonated form. The hydrolytic instability of the amidinium salt under neutral conditions results from the positively charged amidinium moiety, which is highly susceptible to an attack of hydroxide ion [13,14]. Although the concentration of hydroxide ions under neutral conditions is low, it is by orders of magnitude higher compared to the concentration under acidic conditions. This explains the difference in decomposition behavior of the pure polyamidine in solution at lower pH values.

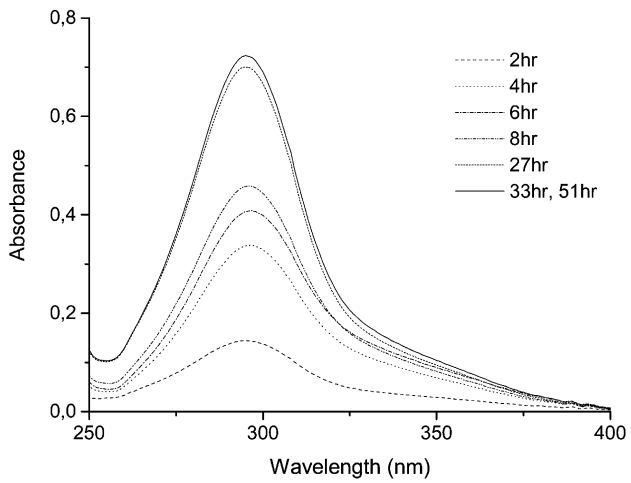


Fig. 4. UV spectra of salicylic acid released from POA/salicylic acid mixture into the buffer solution at pH 7 after different times.

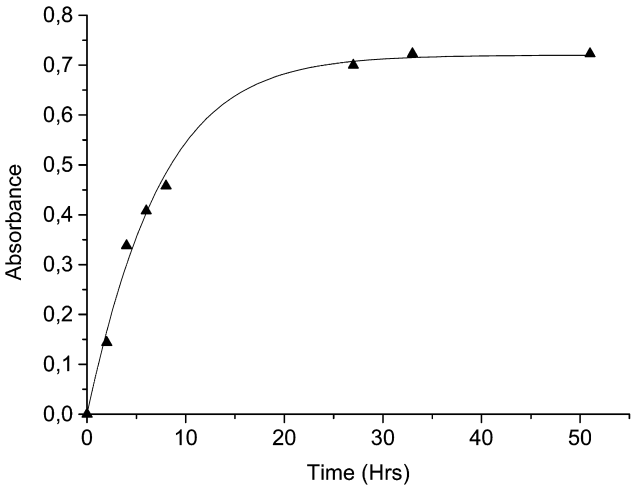


Fig. 5. Release of salicylic acid from a POA/salicylic acid mixture into the buffer solution at pH 7 (quantification by UV spectroscopy).

4. Conclusions

The results show that aliphatic polyamidines are potential carriers for active compounds which can be released by the hydrolytic degradation of the polymer. One requirement for this is that the active compound is a proton donor the interactions of which with the polymer result in the formation of strong ionic complexes. In such complexes, the active compounds are demobilized and are not able to release the sample by diffusion or evaporation. It could be shown that under neutral or basic conditions a hydrolytic decomposition of the polymer took place that resulted in case of neutral hydrolysis in a controlled release of salicylic acid, which served as a model for an active compound. Under acidic condition the polymer has proved hydrolytically stable.

The continuous and controlled release of salicylic acid under neutral conditions could be maintained at least for 10 h. It is assumed that the hydrolytic stability and herewith the release behavior of polyamidines can be tuned by tailoring the polymer structure. Especially, aromatic and cycloaliphatic moieties in the polymer backbone or other substituents at the amidine group are assumed to increase the hydrolytic stability. On the other hand, polyformamidines should have a reduced hydrolytic stability compared to polyacetamidines.

Potential applications for such mixtures described here, are seen in the field of controlled release of drugs, attractants and fertilizers, or for surface finishing with bactericidal or fungicidal agents in a humid environment. The pH dependency of the hydrolytic decomposition may offer the use of the mixtures as an orally administered drug delivery system that passes the stomach region without decomposition because of the acidic conditions (pH = 2). After reaching the small intestine where neutral conditions are predominant (pH = 7), the drug is released to the body since

the polyamidine undergoes hydrolytic decomposition at neutral pH.

Utilization in these fields, however, demand further investigations also with regard to the environmental compatibility.

Acknowledgements

The authors wish to thank Dr Eichhorn and Mrs Adam for FTIR spectroscopic measurements and the Deutsche Forschungsgemeinschaft (DFG) for financial supports.

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