**Summary:** Nucleophilic aromatic substitution of 2,6-*O*-dimethyl-*β*-cyclodextrin (*β*-DMCD)-complexed 4-fluoro-3-nitroaniline derivatives with poly(vinyl amine) (PVAm) in water results in 2-nitro-1,4-benzenediamine-functionalized water-soluble PVAms in one step. The 2-nitro-1,4-benzenediamine moiety linked to the polymer is solvatochromic and undergoes protonation and deprotonation as function of pH as shown by UV-Vis spectroscopy. The occurrence of an isosbestic point in the UV-Vis spectrum is suitable to directly determine the pK_a value using the Henderson-Hasselbalch equation. The influence of the methyl group substitution of the polymer and the 2-nitro-1,4-benzenediamine moiety on the pK_a is discussed.
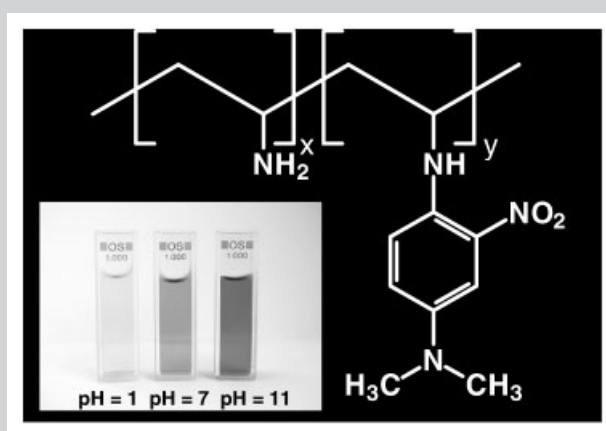
Structure of the 4-*N*,*N*-dimethyl-2-nitro-1,4-benzenediamine-functionalized PVAm and its solution in water at varying pH.

2-Nitro-1,4-diaminobenzene-Functionalized Poly(vinyl amine)s as Water-Soluble UV-Vis-Sensitive pH Sensors

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Introduction

Poly(vinyl amine) (PVAm) is a cationic polyelectrolyte that can potentially bind to anionic polyelectrolytes such as RNA and DNA. The interaction of PVAm with other polymers is determined significantly by the pH of the environment. In addition, variations in pK_a are crucial to the action of enzymes and are important for RNA activity in protein synthesis.[1,2]

The introduction of UV-Vis or electron spin resonance (ESR)-sensitive probes along the polymer backbone of

PVAm is, therefore, of potential interest to sense anionically charged biomacromolecules.

1,4-Diaminophenyl-functionalized polymers are of potential interest in many applications because of their interesting redox and the UV-Vis spectroscopic properties of the chromophoric moiety in the pH range of 4 to 8.[3] Photographic processes, hair dye (henna for hair), and pH sensing are also fields of application.[3–5] Unfortunately, the 1,4-benzenediamine (*p*-phenylenediamine) moiety is sensitive to air (oxygen) and light.[6] This fact means that careful handling of such functionalized polymers under

exclusion of oxygen is required, because polymers functionalized with 1,4-benzenediamine undergo cross-linking reactions to form insoluble products in an oxygen atmosphere.[6]

Nitro groups are suitable to reduce the sensitivity of 1,4-benzenediamine to air as a result of the expected push-pull effect in the *ortho* position which decreases the oxidation potential of the whole chromophore.[4,5] Furthermore, 2-nitro-1,4-diaminobenzene derivatives show solvatochromic as well as different UV-Vis properties as a function of pH.[4,5]

In this communication, an elegant synthetic approach to functionalize PVAms with 2-nitro-1,4-benzenediamine functionalities is reported. The functionalization reaction of choice is the nucleophilic aromatic substitution of PVAm with the fluoro compound in water mediated by cyclodextrins as reported previously (Scheme 1).[7,8]

PVAms (Lupamine®), when functionalized using 4-fluoro-3-nitroaniline and *N,N*-dimethyl-4-fluoro-3-nitroaniline, respectively, are here named polymer **1** and polymer **2**. Polymer **3** is a completely *N*-methylsubstituted PVAm when functionalized with *N,N*-dimethyl-4-fluoro-3-nitroaniline.

To mediate the solubility of the reagents in water, they were used as 1:1 complexes with 2,6-*O*-dimethyl-*β*-cyclodextrin according to established procedures.[7−13]

The choice of the 4-fluoro-3-nitroaniline derivatives was also motivated by the fact that preliminary studies indicated that the reduction of 4-nitroaniline moieties linked to PVAm with $Na_2S_2O_4$ form *p*-phenylenediamine and results in the rapid cross-linking of the polymer in oxygen atmosphere.[14]

Experimental Part

Materials

The aqueous solution samples of PVAm ($\overline{M}_n = 15\,000$ g · mol^{-1}, pH = 11) were kindly provided by BASF AG (Ludwigshafen, Germany).

2,6-*O*-Dimethyl-*β*-cyclodextrin (*β*-DMCD) was donated by Wacker Chemie (Burghausen, Germany). 4-Fluoro-3-nitro-

aniline was commercially available from ABCR and recrystallized from ethanol.

Synthesis of N,N-Dimethyl-4-fluoro-3-nitroaniline (DMFNA)

DMFNA was synthesized by the dimethylation of 4-fluoro-3-nitroaniline with formaldehyde/sodium borohydride in aqueous tetrahydrofuran (THF) according to Giumanini et al. (*N,N*-dimethylation of 4-fluoroaniline).[15] The product was obtained as red crystals in 95% yield with a melting point of 37–41 °C.

^{1}H NMR (CD_2Cl_2): $\delta = 7.21$ (dd, 1H, $J = 5.49$ Hz, $J = 3.30$ Hz), 7.12 (dd, 1H, $J = 10.44$ Hz, $J = 9.34$ Hz), 6.90 (ddd, 1H, $J = 9.34$ Hz, $J = 3.85$ Hz, $J = 3.30$ Hz), 2.97 (s, 6H, –N–$(CH_3)_2$).

Synthesis of Poly(N-methylvinyl amine) (PMVAm)

For acidic hydrolysis, 2 g of poly(*N*-methylvinyl acetamide) with a molecular weight of 196 000 g · mol^{-1} was dissolved in 50 mL of 2 M HCl and refluxed for 12 h. Aqueous sodium hydroxide was then added to the solution of poly(*N*-methylvinyl amine) to increase the pH to 12.

^{1}H NMR (D_2O): $\delta = 1.38 – 1.54$ (2H, –CH_2–CH–), 2.27–2.29 (3H, –NH–CH_3), 2.59–2.70 (1H, –CH_2–CH–).

Synthesis of the Cyclodextrin Complexes

For the formation of the *β*-DMCD, stoichiometric amounts of the fluoronitro-substituted aromatic compound and of *β*-DMCD were dissolved in methanol and stirred overnight at room temperature.[4] The solid complex was isolated by removing the methanol under vacuum.

Synthesis of the Nitroaniline-Functionalized PVAm

For functionalization of PVAm in water, the solid *β*-DMCD complex was dissolved in 50 mL of distilled water and 4.4 mL of an aqueous solution of PVAm containing 0.5 g of the polymer was added. The mixture was refluxed for 8 h. After this time the chromophoric-functionalized PVAm was precipitated into ice-cold acetone (refrigerator) and dried under vacuum.[7,8]
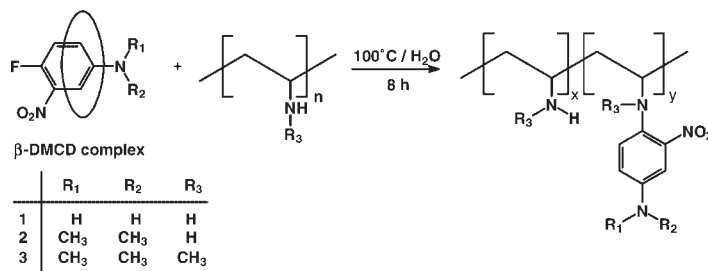
Scheme 1. Reaction of PVAms with *β*-DMCD-complexed 4-fluoro-3-nitroaniline derivatives.

UV-Vis Spectroscopic Measurements

UV-Vis absorption spectra of functionalized-PVAm solutions were measured by means of a UV-Vis MCS 400 diode-array spectrometer (Carl Zeiss) connected to an immersion cell (TSM 5) via glass-fibre optics.

Electron Paramagnetic Resonance (EPR) Spectroscopic Measurements

EPR experiments were performed at room temperature with a BRUKER ESP 300 E spectrometer (X band, $\nu = 9.783$ GHz). The modulation frequency and amplitude were 100 kHz and 5 G, respectively. The spin number of the samples was determined by the sample's absorption peak area compared with a $CuSO_4 \cdot 5H_2O$ standard sample signal.

Results and Discussion

The occurrence of the nucleophilic aromatic substitution reaction at the PVAm backbone can be readily followed by UV-Vis spectroscopy, because the new UV-Vis absorption of the 2-nitro-1,4-benzenediamine moiety shows a typical UV-Vis absorption maxima at about 510 nm.[5,6] Advantageously, the 2-nitro-1,4-benzenediamine-functionalized PVAm is completely soluble in methanol, ethanol, and 1,1,1,3,3,3-hexafluoropropan-2-ol. In water at pH > 7 and dichloromethane the polymers **1** and **2** are sparingly soluble, but polymer **3** is moderately soluble in dichloromethane. In contrast it is poorly soluble in water and methanol. However, the solubility is sufficient to allow the use of the chromophoric polymers in various environments. The UV-Vis absorption maxima of polymers **1**, **2**, and **3**, and of the related model compounds (denoted as **1a**, **2a**, and **3a**),[5] are shown in Table 1.

The respective chromophoric moiety, either the 4-*N*,*N*-dimethylamino-substituted or 4-amino-substituted derivative, shows solvatochromism, which indicates that a push-pull π-electron system has been formed. The push-pull system is attributable to the resonance of the *ortho* substituents. The resonance is lowered by a methyl group at the amino nitrogen atom in the 1-position as a result of twisting of the nitro group as indicated by a significant hypsochromic shift of the UV-Vis absorption maximum of polymer **3** as well as of the related model compound.

Water is a stronger HBD (hydrogen bond donating) solvent compared to methanol. Accordingly, water more strongly stabilizes the *N*,*N*-dimethylamino derivative (polymer **2**) by solvation because of its higher basicity compared to polymer **1**. In contrast, the stronger HBA (hydrogen bond accepting) solvent methanol stabilizes the amino group-functionalized moiety better because of the evident HBD property of an aromatic amino group. Thus, the solvatochromism of both polymers is opposite as a result of the completely different hydrogen bond ability properties of the *para* substituent. For all polymers, a hypsochromic shift is observed in 1,1,1,3,3,3-hexafluoropropan-2-ol. This effect indicates a strong solvation of the amino group by the HBD solvent, which decreases the push-pull character.

Altogether, the UV-Vis absorption maxima of the chromophoric moieties linked to the polymers are significantly bathochromic shifted compared to those of the model compounds. This result is a clear indication that the dipolarity and basicity of the polymer has an effect on the position of the UV-Vis absorption maximum of the linked chromophore.

In addition, a partial radical formation has been evidenced by EPR spectroscopy of the dried solid polymers **1** and **2** and in methanol solution, as shown in Figure 1.

The structures of the two radicals are identical as indicated by the similar signal pattern in the EPR spectra. The signal intensity is quite low, which indicates only a weak concentration of those species. In methanol, radical formation has also been established, whereas no radical formation has been found in water and in acidic media. This effect is discussed in relation to the pK_a measurements later.

To demonstrate the potential of the chromophoric polymer as a pH sensor, UV-Vis spectroscopic measurements were performed as function of pH in water. A typical UV-Vis spectroscopic series for the 4-*N*,*N*-dimethylamino-2-nitroaniline-functionalized polymer (polymer **2**) is shown in Figure 2.

In an acidic environment (pH = 0.85), the UV-Vis absorption of the polymer is completely shifted to $\lambda = 420$ nm. In basic media at pH = 11.2, the UV-Vis band appears at $\lambda = 512$ nm. The long-wavelength UV-Vis absorption at 512 nm is likely attributable to an intramolecular charge transfer (ICT) transition from the amino group in the *para* position to the *o*-nitroaniline moiety.[4–6] The origin of the ICT is indicated by both the broadening of the half width and the typical form of the UV-Vis band. Protonation of the chromophore at the amino group in the *para* position suppresses the CT transition. Thus, the protonated form only shows the typical UV-Vis band of the remaining *o*-nitroaniline moiety at about 420 nm.[8]

The clear separation of the protonated and unprotonated species from each other in the UV-Vis spectrum, and the occurrence of an isosbestic point at $\lambda = 436$ nm, allows the evaluation of the pK_a value of the chromophoric moiety using the well-established Henderson-Hasselbalch equation (Equation (1)).[16]

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \qquad (1)$$

where $[A^-]$ is the concentration of the proton acceptor, and [HA] is the concentration of the proton donor.

The pK_a can be determined either from the x-axis intersection point by plotting the measured $\log [A^-]/[HA]$ as function of pH, or from the turning point of the two intensity curves of the optical absorbance of $[A^-]$ at $\lambda_{max} = 512$ nm and [HA] at $\lambda_{max} = 420$ nm, respectively, for

Table 1. UV-Vis absorption maxima (λ_{max}) of 2-nitro-1,4-benzenediamine derivatives (**1a**, **2a**, and **3a**) and the functionalized PVAms (polymer **1**, polymer **2**, and polymer **3**) measured in five different solvents.

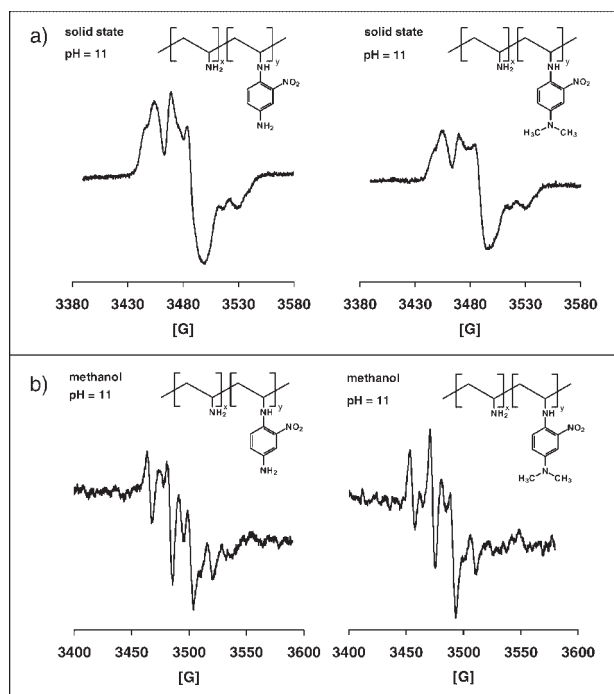| No.	Compound	λ_{max}				
		nm				
		Water	Ethanol	Methanol	Dichloro-methane	1,1,1,3,3,3-Hexa-fluoro-propan-2-ol
1a		–	498[a]	–	500[a]	–
2a		–	505[a]	–	522[a]	–
3a		–	445[a]	–	468[a]	–
1		517(pH = 11)	509	508	512	480
2		510(pH = 11)	520	520	531	468
3		510(pH = 11)	500	500	510	462

[a] Data from ref.[5]

Figure 1. EPR spectra of the 2-nitro-1,4-benzenediamine-functionalized PVAm (polymer **1**, black curve) and the 4-*N*,*N*-dimethyl-2-nitro-1,4-benzenediamine-functionalized PVAm (polymer **2**, blue curve) as the dried solid polymer (a) and in methanol (b).

As expected, the determined pK_a values are in the order of magnitude for aniline derivatives. For example, the pK_a of C_6H_5–NH_3^+ in water amounts to 4.6.[17]

It is a well-established fact that the pK_a value of polysubstituted anilines is a linear function of the sum of the Hammett constant ($\Sigma\sigma$) as shown in Equation (2).[18]

$$pK_a = -288(\pm 0.16) \cdot \sum \sigma + 4.45(\pm 0.16) \qquad (2)$$

where $r^2 = 0.955$, $n = 18$, and $s = 0.251$ (r – correlation coefficient; n – number compounds; s – standard deviation).

Using the pK_a values of substituted nitroanilines from the literature and the above Hammett equation,[17,18] the influence of the nitro group in the *ortho* position on pK_a as function of amino group substitution is finally discussed.

The introduction of an *o*-nitro group and of the two amino groups associated with radical formation can have a manifold influence on the pK_a. The nitro group is an electron-withdrawing group in comparison to the amino groups, which are electron donating. Using the σ_{para} values for the –NH_2 and –$N(CH_3)_2$ group, σ_{ortho} values of the nitro group can be estimated. The calculated σ_{ortho} values for –NO_2 are 0.806 for the chromophoric group in polymer **1** and 0.765 for polymer **2**. These values are close to that of the σ_{para} value of 0.78 for the nitro group. Thus the pK_a values of about 4 for polymers **1** and **2** are scientifically sound.

However, this argument does not allow for the contribution of radical cation forms in acidic media. If a radical cation form is present, then a lower pK_a value would be expected. The slightly lower pK_a value for polymer **1** compared to **2** is logical, because the two methyl groups increase the basicity which leads to a weaker acidity of the protonated form. The significant decrease of pK_a for
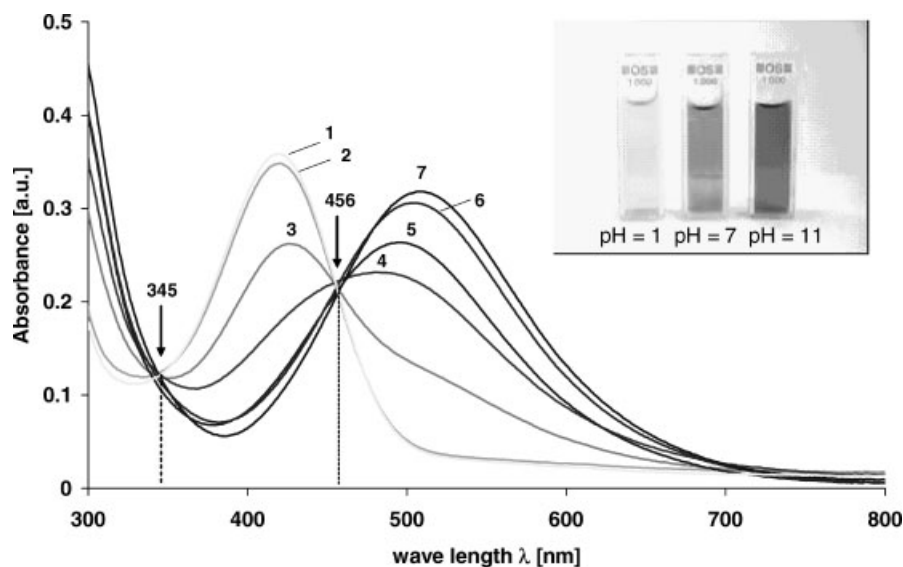
polymer **2**, as a function of pH. Both approaches result in the same pK_a value of 4.06 as shown in Figure 3.

Using the same procedure, pK_a values were determined for three different polymers as compiled in Table 2.

Figure 2. UV-Vis spectra series of 4-*N*,*N*-dimethyl-2-nitro-1,4-benzenediamine-functionalized PVAm (polymer **2**) as function of pH = 0.85 (1), 1.63 (2), 3.45 (3), 4.22 (4), 6.15 (5), 8.95 (6) and 11.19 (7) at room temperature in water (inset: photograph of polymer **2** in water at pH = 1, 7, and 11).
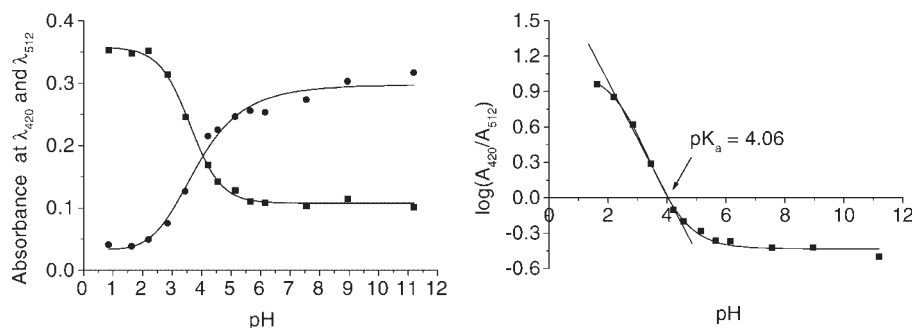
Figure 3. Determination of the pK_a value of the 4-*N,N*-dimethyl-2-nitro-1,4-benzenedi-amine-functionalized PVAm (polymer **2**).

polymer **3** seems surprising, because the methyl group should have a similar influence by considering the inductive effect. The decrease of the pK_a is explained by steric factors, i.e., the *ortho* effect in this way that the nitro group and the *N*-methyl substituent are stronger twisted. Accordingly, the +M effect of the *N*-methyl amino group is decreased. This result is in line with the results of UV-Vis spectroscopy from

Table 1, because polymer **3** and the corresponding model compound, respectively, absorb at a shorter UV-Vis wavelength compared to polymer **1** or **2**.

However, the origin of the formation of radicals is still not clear. It is believed that electron transfer processes from amino groups to the chromophoric moieties may take place since the polymer is strongly associated. This feature has no

Table 2. Determined pK_a values of the protonated form of the nitroaniline moiety of the functionalized PVAms.

	Polymer structure (pH = 11)	Chromophoric moiety (pH = 11)	pK_a
1			4.03
2			4.06
3			2.99−3.10 [a]

[a] The two curves used for the evaluation resulted in slightly different values because the isosbestic point was not so sharp compared to the polymers **1** and **2**.

effect in water, because of the strong solvation of the amino groups.

Conclusion

2-Nitro-1,4-diaminobenzene-functionalized PVAms are suitable pH sensors in aqueous solution as protonation occurs reversibly in the physiological important pH region. In a non-aqueous solution the polymers show solvatochromism. Electron transfer processes take place as indicated by EPR spectroscopy in methanol and in the solid state, and are a subject of further studies.

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