

This article was downloaded by:

On: 24 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of Macromolecular Science, Part A

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597274>

### Effects of Polyethyleneimine Derivatives on 2-Hydroxy-5-Nitrobenzyl Ionization

Elizabeth A. Myatt<sup>a</sup>; Ioannis Scarpa<sup>b</sup>

<sup>a</sup> Loyola University Medical Center Maywood, Illinois <sup>b</sup> Argonne National Laboratory, Biological and Medical Research Division, Argonne, Illinois

**To cite this Article** Myatt, Elizabeth A. and Scarpa, Ioannis(1992) 'Effects of Polyethyleneimine Derivatives on 2-Hydroxy-5-Nitrobenzyl Ionization', Journal of Macromolecular Science, Part A, 29: 2, 153 – 160

**To link to this Article:** DOI: 10.1080/10101329208052158

**URL:** <http://dx.doi.org/10.1080/10101329208052158>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## EFFECTS OF POLYETHYLENEIMINE DERIVATIVES ON 2-HYDROXY-5-NITROBENZYL IONIZATION

ELIZABETH A. MYATT\* and IOANNIS SCARPA

Loyola University Medical Center  
Maywood, Illinois 60153

### ABSTRACT

A chromophoric reporter on polyethyleneimine (PEI) derivatives was shown to ionize at pHs below its intrinsic  $pK$  by varying degrees depending upon its attachment site, other modifications to the polymer, and, in some instances, the buffer. 2-Hydroxy-5-nitrobenzyl bromide (HNB-Br) was reacted with four branched PEI derivatives. In two of these, the parent polymer was substituted with HNB at either primary or tertiary amines. These polymers were not modified further. The other two derivatives contained dodecylbenzyl groups on the same amine type as the reporter group. The tertiary HNB, dodecylbenzyl polymer was further modified by quaternization of all amines with methyl iodide. Tertiary amines of PEI having no alkyl groups perturbed most effectively the  $pK$ s of the reporter groups, with 50% ionization occurring two pH units below the previously reported  $pK$  of 5.9 in solution. Quaternized, tertiary dodecylbenzyl PEI perturbed the  $pK$  of the reporter groups the least, with a 0.4 unit average  $pK$  reduction. The primary HNB PEI having no dodecylbenzyl groups induced 50% ionization at pH 4.5 compared to pH 4.75 upon introduction of 10% total amine of dodecylbenzyl groups to some of the remaining primary amines. These results suggest that the core of the parent polymer effects greater perturbation of HNB ionization than does a matrix of quaternary alkyl ammonium ions.

\*Current address (to which correspondence should be addressed): Biological and Medical Research Division, Argonne National Laboratory, 9700 S. Cass Ave., Argonne, Illinois 60439.

## INTRODUCTION

Polyethyleneimine (PEI) is a weak polyelectrolyte with a complex and poorly understood structure consisting of primary (or terminal) amines, secondary (or bridging) amines, and tertiary (or branching) amines amid its ethylene units. The ratio of primary to secondary to tertiary amines is 1:2:1 [1], but the average length of a 50,000 molecular weight polymer in its fully extended conformation, that is, the complexity of branching, is not known. The amines possess a wide range of  $pK_s$ , but contrary to their small molecule counterparts, the order of basicity decreases from primary to secondary to tertiary amine [2]. The basicity and steric accessibility of primary amines result in their being preferentially substituted by alkyl halides and acylating agents [3]. However, terminal amines would seem the least likely sites for exploitation of the polymer's microenvironment. A recently developed synthetic scheme permits modification of PEI on tertiary and/or secondary amines [4]. This study compares the  $pK$  perturbation of 2-hydroxy-5-nitrobenzyl (HNB) reporter groups on each of four different PEI derivatives. In the first two, the reporter group was reacted with the polymer's primary or tertiary amines. In the last two, linear alkyl groups (dodecylbenzyl) were introduced into the same amine type that contained HNB. The tertiary dodecylbenzyl, HNB PEI derivative was further modified by complete quaternization of the polymer amines by using methyl iodide, thereby converting the polymer into a strong polyelectrolyte which is inert to electrophilic substitution.

## MATERIALS AND METHODS

### Polymer Derivatization

*Tertiary HNB PEI (3-HNB PEI).* An ethanol solution of PEI 600 (Dow, Midland, Michigan) was vacuum dried, redissolved in chloroform, and reacted with methyl trifluoroacetate followed by trifluoroacetic anhydride as described previously [4]. The secondary amines were further blocked with 2-(trimethyl silyl) ethyl carbonate, para-nitrophenyl ester (TEOC-O-Np) (Fluka, A.G. Buchs SG, Switzerland). The blocked polymer, TFA-TEOC PEI, contained less than 1% total amine as unblocked primary and secondary amines [4]. The titratable reporter group, 2-hydroxy-5-nitrobenzyl bromide, HNB-Br (Aldrich Chemicals, Milwaukee, Wisconsin), was added to a nitromethane solution of TFA-TEOC blocked polymer (1 M in amine) to the extent of 2% total amine. It was reacted at 50°C for 20 h with stirring. The polymer was deblocked as described in Ref. 4.

*Primary HNB PEI (1-HNB PEI).* Two hundred micromoles of HNB-Br were added to 10 mmol of PEI amines in 10 mL anhydrous ethanol. The solution was stirred at 23°C for 20 h.

*Primary Dodecylbenzyl, HNB PEI (1-HNB/D PEI).* Ten milliliters of 1 M PEI amines in anhydrous ethanol was reacted with 0.2 mmol HNB-Br at 50°C overnight with stirring. This was followed by addition of 1.0 mmol of dodecylbenzyl

chloride (Stauffer Chemicals, Shelton, Connecticut). The alkyl halide was allowed to react for 48 h at 50°C with continuous stirring.

*Permethylated Tertiary Dodecylbenzyl, HNB PEI (3-HNB/D PEI+)*. TFA-TEOC PEI, prepared as described previously [4], was dissolved in nitromethane or tetrahydrofuran to an amine concentration of 1 M. HNB-Br was then added to a final concentration of 0.025 M and the solution was stirred for 20 h at 55°C. This was followed by addition of diisopropylethylamine (DIEA) (Aldrich Chemicals) and dodecylbenzyl chloride to final concentrations of 0.25 M each. The mixture was stirred at 55°C for an additional 48 h. The polymer was deblocked and quaternized with methyl iodide as described previously [4].

*Removal of Unreacted HNB-Br and Alkyl Halides*. The unreacted HNB-Br was removed from the polymer solutions by passing them through a chloride-containing anion-exchange column (Dowex-1 X8-200) with ethanol as an eluent. Purified PEI eluted without being retained whereas the unbound reporter group adhered strongly to the matrix, which was discarded after each use.

The alkylated polymers were vacuum concentrated to ca. 3 M amine in ethanol. The polymer solution was then applied to an LH-20/ethanol column. The void volume contained purified polymer devoid of unreacted alkane. Unreacted dodecylbenzyl chloride was detected in the eluent by thin layer chromatography on fluorescent plates (Whatman Ltd., Clifton, New York) using hexane as the mobile phase ( $R_f = 0.9$ ). LH-20 chromatography also removed DIEA and methyl iodide.

### Spectrophotometric Data Collection

The polymers' spectra were collected in a Perkin-Elmer 320 spectrophotometer as follows: The purified, lyophilized polymers were brought up in deionized water to a final concentration of 4 mg/mL. One hundred microliter aliquots of each polymer was delivered to a 3-mL quartz cuvette containing 1.9 mL buffer. Buffers were used at the concentrations and pHs listed in Tables 1-4. The scanning spectra were collected between 500 and 250 nm.

TABLE 1. PEI-Tertiary HNB Spectral Results

Buffer, 0.02 M	pH	$\lambda_{\max}, \Phi-O^-$	$A_{320}$	$A_{400}$	% Ionized
Barbital	8.2	405	0.070	0.363	87
Barbital	7.2	405	0.083	0.375	83
MES	7.2	405	0.080	0.396	86
MES	6.2	405	0.087	0.363	81
MES	5.2	402	0.102	0.349	76
Acetate	5.2	402	0.103	0.342	75
Acetate	4.2	395	0.152	0.263	54
Acetate	3.6	395	0.195	0.167	34

TABLE 2. PEI-Primary HNB Spectral Results

Buffer, 0.02 <i>M</i>	pH	$\lambda_{\max}, \Phi-O^-$	$A_{320}$	$A_{400}$	% Ionized
Barbital	8.2	405	0.114	0.364	73
Barbital	7.2	405	0.110	0.339	72
MES	7.2	405	0.125	0.376	72
MES	6.2	405	0.135	0.365	68
MES	5.2	398	0.140	0.290	60
Acetate	5.2	395	0.149	0.289	58
Acetate	4.2	390	0.182	0.189	39
Acetate	3.6	Shoulder	0.215	0.130	26

TABLE 3. PEI-Primary Dodecylbenzyl and HNB Spectral Results

Buffer, 0.02 <i>M</i>	pH	$\lambda_{\max}, \Phi-O^-$	$A_{320}$	$A_{400}$	% Ionized
Barbital	8.2	405	0.035	0.900	94
Barbital	7.2	405	0.168	0.885	88
MES	7.2	405	0.145	0.935	93
MES	6.2	405	0.275	0.780	70
MES	5.2	401	0.280	0.651	63
Acetate	5.2	400	0.308	0.493	52
Acetate	4.2	390	0.443	0.308	29
Acetate	3.6	Shoulder	0.495	0.193	18

TABLE 4. Quaternized PEI-Tertiary Dodecylbenzyl and HNB Spectral Results

Buffer, 0.02 <i>M</i>	pH	$\lambda_{\max}, \Phi-O^-$	$A_{320}$	$A_{400}$	% Ionized
Barbital	8.2	405	0.120	0.440	78
Barbital	7.2	405	0.130	0.365	69
MES	7.2	405	0.132	0.418	73
MES	6.2	405	0.141	0.333	64
MES	5.2	405	0.167	0.288	54
Acetate	5.2	405	0.217	0.230	40
Acetate	4.2	Shoulder	0.326	0.128	18
Acetate	3.6	Shoulder	0.367	0.040	5.7

## RESULTS

Results of the scanning spectra are given in Tables 1–4. They were analyzed as follows.

*Determination of the Nitrophenolate Concentration.* The isosbestic point of the two phenolate forms of the reporter group (on protonated versus unprotonated amine) is 400 nm [5]. At this wavelength the total concentration of ionized reporter group is equal to the absorbance at 400 nm divided by its extinction coefficient at this wavelength,  $18,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ . The protonated reporter group does not absorb at 400 nm.

*Determination of the Nitrophenol Concentration.* The absorbance maximum of the protonated form of the reporter group,  $\Phi\text{-OH}$ , occurs at 320 nm where it possesses an extinction coefficient of  $10,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ . The ionized form,  $\Phi\text{-O}^-$ , has a small but significant absorbance at this wavelength, with an extinction coefficient of  $2000 \text{ M}^{-1} \cdot \text{cm}^{-1}$  [5]. The total absorbance at 320 nm is equal to the sum of the extinction coefficient multiplied by the concentration of protonated species and the extinction coefficient times the concentration of the deprotonated species.

$$A_{320} = \epsilon_{320, \Phi\text{-O}^-} [\Phi\text{-O}^-] + \epsilon_{320, \Phi\text{-OH}} [\Phi\text{-OH}]$$

The equation can be solved in terms of the protonated HNB concentration,  $\Phi\text{-OH}$ , the only remaining unknown in the equation.

$$[\Phi\text{-OH}] = \frac{A_{320} - \epsilon_{320, \Phi\text{-O}^-} [\Phi\text{-O}^-]}{\epsilon_{320, \Phi\text{-OH}}}$$

This analysis of the scanning spectra of each polymer derivative at each pH tested gave the results in Tables 1–4. The percent ionization of the four polymer derivatives over the pH range studied are summarized graphically in Fig. 1.

This mathematical treatment of the scanning spectra of each polymer derivative at each pH tested gave the results in Tables 1–4. The percent ionization of the four polymer derivatives over the pH range studied are summarized graphically in Fig. 1.

## CONCLUSION

The anionic form of the 2-hydroxy-5-nitrobenzyl group is stabilized by PEI whether it is placed on the primary amines or the tertiary amines. This would be expected based on the net positive charge which this soft polyelectrolyte possesses throughout the pH range over which the reporter group has been titrated. On the parent polymer, the reporter group is half ionized in the pH range of 4 (for tertiary amine placement) to pH 4.5 (for primary amine placement). This is 1.4 to 1.9 pH units below the  $pK$  of *N*-lysyl HNB. Reporter groups on primary amines of primary alkylated PEI (1-HNB/D PEI) generally titrated at pH 4.75, whereas those on tertiary amines of tertiary dodecylbenzyl, perquaternary PEI (3-HNB/D PEI+) titrated to 50% ionization at pH 5.5. Therefore, in 3-HNB/D PEI+, the combined effects of laurylation and quaternization were to depress the  $pK$  of the reporter group by only 0.4 pH unit from its  $pK$  on a small amine in aqueous solution, as

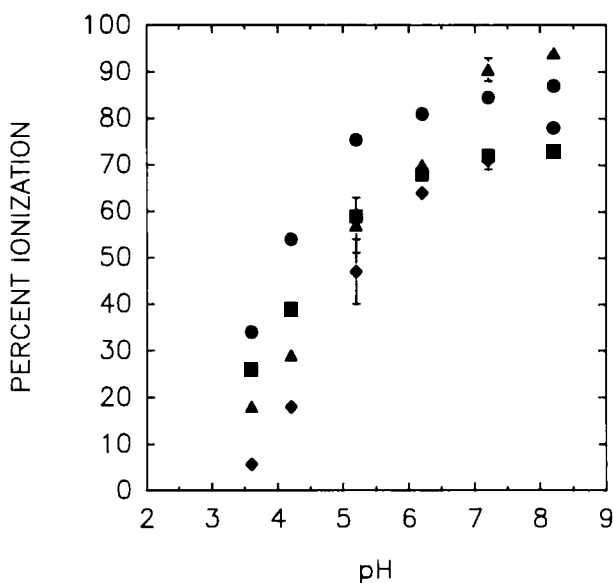


FIG. 1. The percentage ionization of 2-hydroxy-5-nitrobenzyl reporter groups on the polymers as a function of pH. The percentage ionization over the pH range tested is graphed for the four polymer derivatives. Tertiary HNB PEI (●); primary HNB PEI (■); primary dodecylbenzyl, HNB PEI (▲); permethylated tertiary dodecylbenzyl, HNB PEI (◆).

opposed to a 1.9 pH unit depression of the reporter groups'  $pK$  when on the parent polymer's tertiary amines (3-HNB PEI). Surprisingly, then, the quaternized derivative perturbed the reporter group  $pK$  the least of the four polymers studied.

The ranges of  $pK$ s over which the reporter groups ionize are strikingly larger for the nonlaurylated polymers than for those containing hydrophobes (Tables 1-4). Neither complete ionization nor protonation of the reporter group was achieved on 1-HNB PEI and 3-HNB PEI over the range of pH 8.2 to 3.6. On 1-HNB/D PEI, 88% of the reporter groups have titrated by pH 7.2, compared to no more than 83% ionization at pH 7.2 in the other polymers. Despite its fixed positive charge, 3HNB/D PEI<sup>+</sup> contains only 18% of its reporter groups in the anionic form at pH 4.2 compared to 29-54% in the other derivatives studied.

The effect of buffer at pHs 5.2 and 7.2 is minimal in the ionization of the nonlaurylated polymers, 1-HNB PEI and 3-HNB PEI. However, the nature of the buffer at these pHs has dramatic effects on the percent HNB ionization in the two hydrophobe-containing polymers, particularly at pH 4.2. Therefore, alkylation makes the polymer's structure more sensitive to counterion effects.

## DISCUSSION

The broad range of titration of HNB on PEI is reminiscent of the titration of PEI's amines, whose  $pK$ s can be less than 3 or greater than 9 [6]. However, unlike

the titration of the polymer's amines [7], the  $pK$ s of the reporter molecules cannot be grouped on the basis of the amine type to which they have been attached. A broad spectrum of reporter group  $pK$ s exists irrespective of their point of attachment. However, by placing HNB on the tertiary amines, a greater percentage of the total are ionized at all pHs tested compared to placement on primary amines. This might be expected since the local concentration of positive centers (i.e., ammonium groups) would likely be greater at branchpoints in the polymer than at its termini. The clustering of the reporter group  $pK$ s about 5.2 and 7.2 in 1-HNB/D PEI suggests that perhaps in this polymer the alkylation of the polymer's termini results in their being partitioned from the intramolecular hydrogen-bonding network of the parent polymer.

It is noteworthy that for the quaternized polymer, the maximum wavelength of absorption of the ionized form of the reporter group, 405 nm, does not vary with pH. This contrasts with the three nonquaternized derivatives, each of whose maximum changes from 405 to 395 nm as the pH is reduced from 7 to 4–5. The invariance in the phenolate maximum on the quaternized tertiary PEI is consistent with the hypothesis of Kokesh and Westheimer that this shift in the absorption maximum of the ionized reporter group over 10 absorbance units as a function of pH results from hydrogen bonding of the phenolate oxygen to an ammonium group, rather than resulting from coulombic interactions between these two ions [8]. In the perquaternized polymer, ammonium hydrogens have been replaced with methyl groups, and the charge on the amine to which the reporter group is attached is positive irrespective of pH.

Kokesh and Westheimer demonstrated with small molecule amines that the amine site whose protonation alters the absorption maximum of the phenolate can be the same amine to which the reporter group is attached [8]. The fact that nonmethylated 3-HNB PEI also displays the absorbance maximum shift indicates that in PEI the hydrogen bonding interaction necessary to induce the shift can be between the reporter molecule and an ammonium ion other than that to which it is directly attached. Therefore, the shift in the phenolate's maximum absorbance cannot be used to titrate directly the amine to which HNB is attached.

This study illustrates that the  $pK$  perturbation of ionic groups introduced onto PEI can be modified depending on their site of attachment, the nature of other moieties placed on the polymer, and the buffer composition. Over the entire pH range, placement of this ionizable reporter group on tertiary amines favors its deprotonated form compared to placement on primary amines. Alkylation of PEI can promote ionization at alkaline pH (as seen with the primary PEI derivatives, Fig. 1). However, the fixed positive charges of quaternization may be less effective in stabilizing the anionic form of the reporter group than are the polymer's amines with their hydrogen bonding properties, as appears to be the case with the tertiary amine derivatives. The polymers in this study all induced a  $pK$  depression; however, it is possible that introduction of anion containing reagents such as carboxylic acids onto the polymer could stabilize the protonated form of reporter groups and other ionizable compounds. This approach can enhance efforts to control at will the ion-exchange and affinity properties of the polymer in its use as a separation and binding matrix [9].



## REFERENCES

- [1] L. E. Davis, in *Water Soluble Resins* (R. Davidson and M. Sittig, eds.), Reinhold, New York, 1968, pp. 216-226.
- [2] C. Bloys van Treslong and A. Staverman, *Recl. Trav. Chim. Pays-Bas.*, **93**, 171-178 (1974).
- [3] T. Johnson and I. Klotz, *Macromolecules*, **7**, 149-153 (1974).
- [4] E. A. Myatt and I. Scarpa, *Bioorg. Chem.*, **18**, 413-424 (1990).
- [5] P. A. Frey, F. C. Kokesh, and F. H. Westheimer, *J. Am. Chem. Soc.*, **93**, 7266-7269 (1971).
- [6] E. J. Shepherd and J. A. Kitchener *J. Chem. Soc.*, pp. 2448-2452 (1956).
- [7] E. Kokufuta, *Macromolecules*, **12**, 350-351 (1979).
- [8] F. C. Kokesh and F. H. Westheimer, *J. Am. Chem. Soc.*, **93**, 7268-7270 (1971).
- [9] K. Randerath and E. Randerath, *J. Chromatogr.*, **16**, 111-125 (1964).

Received April 5, 1991

Revision received June 25, 1991