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# Poly(*N*-vinylguanidine): Characterization, and catalytic and bactericidal properties

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**Abstract**

Poly(*N*-vinylguanidine) (PVG) is one of the simplest guanidine-bearing polyelectrolytes, but is virtually unknown in the scientific literature. An efficient synthetic route for poly(*N*-vinylguanidine) (PVG) is described that involves free-radical polymerization of *N*-vinylformamide (NVF) followed by basic hydrolysis of the PNVF and guanidinylation of the resulting polyvinylamine (PVAm). The molecular weights can be varied by altering the initial NVF/azo initiator ratio. Characterization of the PVG by $^{1}$H and $^{13}$C NMR, FTIR and FT-Raman spectroscopy methods supports the PVG structure. The PVG possesses an average p$K_a$ of 13.4 and is an active hydrolyzing species for diisopropyl fluorophosphate (DFP), an organophosphate mimic for combat nerve agents. The second-order rate constant of the DFP hydrolysis by PVG at pH 7.8 and 25 °C was measured to be $3.9 \times 10^{-3}$ M$^{-1}$ s$^{-1}$, expressed per concentration of the catalytic amino or imino moieties in each PVG monomer. The hydrolysis occurs via the general $S_N2$ mechanism of base catalysis. The guanidinylation of PVAm affords PVG with 10- to 40-fold lower minimum inhibition concentration (MIC) when tested against four Gram-positive and four Gram-negative bacteria relative to the PVAm itself. Hence, the PVG is effective both as a hydrolyzing agent against toxic organophosphates and as a bactericide, thus exhibiting potential as a material for use in chemical and biological defense as well as a disinfectant in clinical and industrial applications.


*Keywords:* Poly(*N*-vinylguanidine); Synthesis; Organophosphate hydrolysis

## 1. Introduction

The guanidine group defines properties of many biologically active compounds [1,2]. Synthetic guanidines have found applications in the design of molecular recognition devices, sensors, and catalysts as well as disinfectants and antiseptics for clinical use, and in the manufacture and preservation of industrial products [3–5]. Most of the biological and catalytic properties of the guanidine group stem from its unique basicity and its planar, forklike structure. The guanidine group is capable of forming both electrostatic and directed hydrogen bond interactions with polar molecules and anions. Arginine, the only natural amino acid bearing the guanidinium functionality, is the most basic of all natural amino acids and has the highest proton affinity among amino acids by more than 14 kcal/mol, lysine being its nearest neighbor [4]. Since guanidine remains protonated over a wide pH range, including physiological pH (the p$K_a$ of unsubstituted guanidine is 13.5 [4]), and also possesses a geometry enabling it to align well with carboxylates, phosphates, sulfates, nitrates and other anionic groups in water as well as polar compounds in organic solvents, the guanidine-bearing compounds can be utilized in reaction catalysis wherein the ability to bind and thereby activate the guest toward nucleophilic substitution can be exploited. In fact, guanidines have been shown to catalyze the addition of pyrrolidine to α,β-unsaturated lactones [6], additions of nitroalkanes to electrophiles such as unsaturated ketones [7], and esterification and transesterification of fats and oils [8–10]. Because of the remarkable importance of guanidines, their synthesis has been investigated intensively, with the treatment of an amine with an electrophilic amidine species such as cyanamide, *O*-methylisourea hydrogen sulfate, derivatives of

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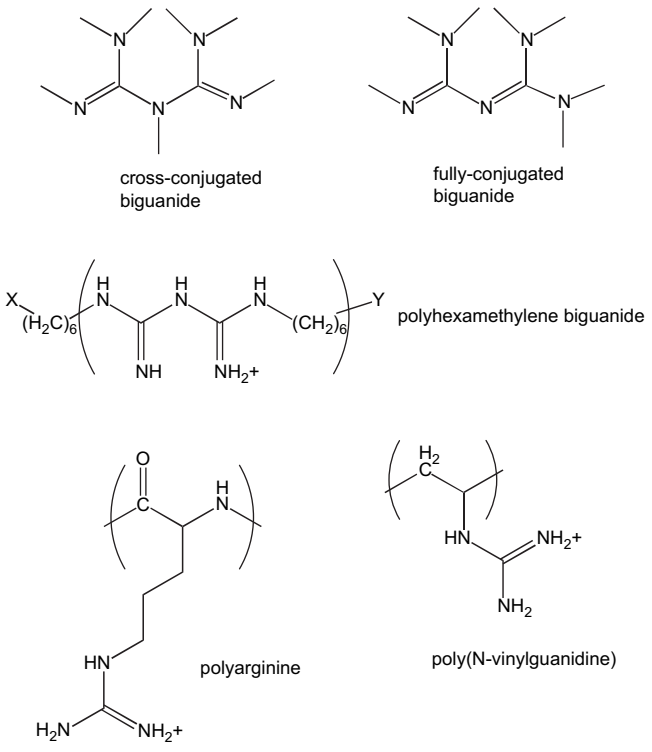
pyrazole-1-carboxamidine, *S*-methylisothiouronium salts, and protected thiourea derivatives emerging as the most common synthetic route [12–15]. Nevertheless, the vast majority of synthetic polymers bearing guanidine groups reported to date have been either polymeric biguanides or polyarginines, with poly(*N*-alkylguanidines) in general and poly(*N*-vinylguanidine) (PVG) in particular (Scheme 1) being practically unknown. The excellent ability of crosslinked PVG gels to bind anions and absorb water has been noted [16–19], but, to the best of our knowledge, any description of the uncrosslinked PVG and its catalytic or antibacterial properties has been lacking. Although the convenient PVG precursor, linear polyvinylamine (PVAm), is one of the simplest synthetic amine-containing polymers, it only received significant attention in the 1990's due to the lack of efficient synthetic methods [20]. With the advent of industrial production of *N*-vinylformamide, and the development of methods of poly(*N*-vinylformamide) (PNVF) synthesis and its subsequent hydrolysis to PVAm, derivatives of PVAm are becoming more widely available [21]. In line with our interest in material development for chemical and biological defense applications [23,24], in this work we set out to characterize, for the first time, the properties of PVG in basic catalysis of organophosphates, as well as to evaluate the potential of PVG as a bactericide. It was shown that PVG can efficiently hydrolyze a nerve agent simulant, diisopropyl fluorophosphate, and effectively inhibit growth of both Gram-positive and Gram-negative bacteria, as described below.




Scheme 1. Structures of biguanide and the most common biguanide polymer, polyhexamethylene biguanide, along with the structures of polyarginine and the subject of the present work, poly(*N*-vinylguanidine).

## 2. Experimental section

### 2.1. Materials

*N*-Vinylformamide (>98%) was received as a gift from BASF Aktiengesellschaft (Ludwigshafen, Germany), distilled at 70 °C under vacuum, and stored at −20 °C prior to use. Cyanamide (50 wt% in water), hydrochloric acid (37%, ACS reagent), 2,2′-azobisisobutyronitrile (AIBN, 98%), 2,2′-azobis(2-methylpropionamidine)dihydrochloride (97%), and chlorhexidine dihydrochloride (CHX, 97%) were all obtained from Sigma–Aldrich Chemical Co. and used as received. All other solvents, gases, and chemicals were obtained from commercial sources and were of the highest purity available.

### 2.2. Syntheses

Two different syntheses of PNVF were implemented, in order to obtain polymeric species of significantly varying molecular weights.

#### 2.2.1. PNVF1 [24]

A mixture of *N*-vinylformamide (2.84 g, 40 mmol) and 2-propanol (12 mL) was deaerated by nitrogen purge. 2,2′-Azobisisobutyronitrile (AIBN, 64 mg, 0.24 mmol) was added to the reaction solution, which was then refluxed for 4 h under nitrogen. The solvent, 2-propanol, was removed under vacuum. The residue was dissolved in a small amount of water and precipitated in acetone; the procedure was repeated. The resulting polymer was dried under vacuum to yield 2.7 g (~90%) of PNVF. The weight-average molecular weight was measured to be 13,600; polydispersity index, 2.4.

#### 2.2.2. PNVF2 [25]

A solution of NVF (10 g, 0.14 mol) in deionized water (100 mL) was deaerated by nitrogen purging for 0.5 h and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (13 mg, 48 μmol) was added. The resulting solution was kept at 60 °C for 24 h under nitrogen purge. The poly(NVF) obtained was dissolved in a small amount of water and precipitated by excess acetone; the procedure was repeated. The resultant polymer was dried under vacuum. Yield: ca. 91%. The weight-average molecular weight was measured to be 60,800; polydispersity index, 2.5. IR (KBr): 1680 $cm^{-1}$ (amide I), 1540 $cm^{-1}$ (amide II). $^1H$ NMR ($D_2O$, ppm): 1.55 (2H, $-CH_2-$), 3.77 (1H, $-CH-$), 7.91 (1H, HCO–).

#### 2.2.3. Hydrolysis of PNVF

PVAm was obtained by basic hydrolysis of the PNVF polymers [20,26]. The polymer samples were dissolved at 10 wt% in deionized water and 1.3 molar excess of 2 M aqueous NaOH was added. The resulting solution was kept at 75 °C, with occasional stirring. Samples were withdrawn intermittently, dialyzed against deionized water and analyzed by $^1H$ NMR for the extent of hydrolysis. The hydrolysis conversion ($F$) was measured as [26] $F(\%) = 100 \times (1 - 2I_{am}/I_{meth})$, where $I_{am}$ and $I_{meth}$ are integrations of the aldehyde side chain

proton signals (centered at 7.85 ppm) and methylene group proton signals of the main chain (centered at 1.6–2.1 ppm), respectively. Complete hydrolysis with a 100% conversion was reached after 48–56 h of the reaction. The resulting PVAm polymer was purified by precipitation from excess acetone at room temperature followed by dialysis (MWCO, 6 kDa) against deionized water and lyophilization. The resulting PVAm samples were analyzed by SEC for molecular weights, which were found to be equal to those of the corresponding parent PVNF polymers. Typical yield: 71–74%. IR (KBr): $\sim$3510 $cm^{-1}$ ((N–H) of $NH_2$), $\sim$2960 $cm^{-1}$ (wide and strong overlapping peak from (N–H) of $-NH_3^+$ and (C–H) of $CH_2$ and CH), 1620 and 1530 $cm^{-1}$ (N–H of $-NH_3^+$). $^1H$ NMR ($D_2O$, ppm): 2.10 (2H, $-CH_2-$), 3.70 (1H, –CH–).

### 2.2.4. Guanidinylation of polyvinylamine

PVAm prepared by 100% hydrolysis of PNVF (6 g, 140 mmol) was dissolved in 100 mL deionized water and 10 mL of 37% HCl, and 200 mL of water were added under stirring. Then 13.8 g (164 mmol) of 50% aqueous solution of cyanamide were added and the resulting solution was kept at 90 °C under stirring for 24 h. The resulting polymer was precipitated in excess acetone, washed by acetone, dried, redissolved in deionized water, dialyzed against water (MWCO, 1 kDa) and lyophilized. No significant changes in the molecular mass of the resulting PVG species compared to the parent PVNF polymers were observed, but polydispersity was lowered somewhat, to 1.9 and 2.2 for the lower and higher MW species, respectively. Yield: 6.4 g (92%). $(C_3H_7N_3)_x$, found (calc): C 42.15 (42.34); H 9.57 (8.29); N 49.12 (49.37). FTIR, FT-Raman, and NMR spectra are given in Section 3.

## 2.3. Polymer characterization procedures

NMR experiments were performed at 25 ± 0.5 °C using Bruker Avance-600 and Bruker DRX 401 spectrometers. $^1H$ and $^{13}C$ resonance frequencies were 600.13 and 100.61 MHz, respectively. Proton decoupling was applied in all NMR measurements and at least 15,000–20,000 scans were collected in $^{13}C$ NMR measurements. Polymer concentrations in $D_2O$ in $^1H$ and $^{13}C$ NMR measurements were set at 10 and 15 wt%, respectively, and pD was adjusted to 7.0 by addition of minute quantities of NaOD and DCl.

Vibrational spectroscopy was performed on polymer samples dried under vacuum at 45 °C from 10 wt% aqueous solution adjusted to pH 7 by addition of 1 M HCl. FTIR spectra were measured in KBr using a Nexus 870 spectrometer (Thermo Nicolet Corp.) in absorbance mode by accumulation of 256 scans with a resolution of 4 $cm^{-1}$. FT-Raman spectra were acquired with 4 $cm^{-1}$ resolution on dry powders using a Varian 3100 spectrometer coupled with a Varian Synergy FT-Raman accessory with a 1064-nm, 2-W Nd:YAG laser. Each interferogram was collected with 3000 scans, apodized, and Fourier transformed before interpretation.

Size-exclusion chromatography (SEC) was run as described previously [27] on a Shimadzu LC-10A Series HPLC set up with a Viscotek SEC$^3$ Triple Detector System, which included a laser scattering detector (scattering angle, 90°; wavelength, 670 nm), differential Wheatstone bridge viscometer (sensitivity, $1 \times 10^{-5}$ $\eta_{sp}$), and a differential laser refractometer (wavelength, 670 nm) and a PL aquagel-OH Mixed, 40, and 60 analytical 3-column system (particle size 8 or 15 μm; dimensions 300 × 7.5 mm, Polymer Laboratories, Inc.) and then eluted at 30 °C using aqueous 0.1 M $NaNO_3$ buffer. The SEC system was calibrated in the $10^3$–$10^7$ Da MW range using polyacrylamide standards (American Polymer Standards Co., Mentor, OH). Potentiometric titration was performed at 25 ± 1 °C using a 736 GP Titrino potentiometric titration system (Metrohm Ltd., Herisau, Switzerland) as described previously [28].

Kinetics of the DFP decomposition were measured at 25 °C with an Orion 96-09 combination fluoride electrode (Thermo Electron Corp., Waltham, MA) and a Model 45 Dual Display Multimeter (Fluke Corp., Everett, WA) connected to a PC with FlukeView Forms software for data processing. The electrode was immersed in a stirred 9-mL aqueous sample and the electrode potential–time output was recorded continuously. No significant changes in pH, set initially at 7.8, were observed in any of the runs. The electrode was calibrated in an independent series of experiments using aqueous solutions of sodium fluoride. While the majority of kinetic measurements were conducted with the polymeric species of a smaller molecular weight ($M_w$, 13.6 kDa), the rate of hydrolysis with the larger PVG species ($M_w$, 61 kDa) at PVG concentrations up to 1–2 mg/mL was not discernibly different from that with the shorter polymer species. We did not measure the rate of hydrolysis with the longer polymer species at higher concentrations in order to avoid effects of the viscosity increases. No significant viscosity increase in solutions of the shorter PVG species was observed at concentrations up to 15 mg/mL.

## 2.4. Microorganisms and determination of minimum inhibitory concentration (MIC)

The microorganisms used were (American Type Culture Collection, Manassas, VA): gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* ATCC 700610, *Staphylococcus epidermidis* ATCC 12228, *Candida albicans* ATCC 10231; gram-negative bacteria *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 15692, *Pseudomonas fluorescens* ATCC 13525, *Klebsiella pneumoniae* ATCC 4352.

The MIC values of polymers and chlorhexidine dihydrochloride (CHX) were determined in vitro using a broth microdilution assay [29]. The polymer and CHX solids were dissolved into small stock samples of the broth, wherein the pH was adjusted to 7.3 as needed. Muller Hinton Broth (MHB, Becton Dickinson, Franklin Lakes, NJ) (pH 7.3 ± 0.1) was used for dilutions. Serial dilutions of compounds between 350–4000 mg/L for polyvinylamine, 8–512 mg/L for poly(*N*-vinylguanidine) and 0.3–8.1 mg/L for CHX final concentration in the liquid medium were dispensed into 96-well polystyrene culture Corning® Costar® cell plates

(Sigma–Aldrich Chemical Co.). The diluted samples were inoculated with a suspension of the test bacterium on the liquid medium to a final concentration of approximately $10^4$ colony-forming units (CFU)/mL. The MIC was defined as the lowest concentration of an antimicrobial compound that inhibited bacterial growth after 24 h at 37 °C. Measurements were conducted in duplicate. Negative control experiments were conducted with the MHB dilutions without bactericidal additives and no reduction in bacterial counts after 24 h was observed. Time-dependent killing measurements of the PVG species were carried out on *S. aureus*, initial viable bacteria count in cell plates $\sim 2 \times 10^6$ CFU/mL. Concentrations of PVG equal to four-fold MIC were used and the measurements were conducted at time points 0 and 5 min, 0.5 h, 1 h, and 8 h. Three independent measurements were performed at each time point.

## 3. Results and discussion

### 3.1. Polymer characterization

The synthetic route toward poly(*N*-vinylguanidine) (PVG) involved free-radical polymerization of *N*-vinylformamide (NVF) to yield poly(*N*-vinylformamide) (PNVF) that was base-hydrolyzed into poly(vinylamine) (PVAm) followed by PVAm treatment with an electrophilic guanidinylation species, cyanamide, to result in PVG (Scheme 2).

The molecular weight of the PNVF species that resulted from the free-radical polymerization of *N*-vinylformamide with 2,2′-azobis(2-methylpropionamidine)dihydrochloride or AIBN as initiators in water or 2-propanol, respectively, was adjusted by a careful choice of the monomer-to-initiator ratio as described previously [30]. Monomer-to-initiator molar ratios of 167 and 2917 resulted in PVNF with $M_w$ of 13.6 and 60.8 kDa, respectively, in accordance with the well-known notion that the sizes of macromolecules produced in a free-radical polymerization without chain-transfer agents are inversely proportional to the square root of initiator concentration [31]. The PVNF was hydrolyzed by 2 M sodium hydroxide as previously described [26] and the resulting PVAm was guanidinylated by cyanamide. The conversion of amines to guanidines, i.e., guanidinylation by cyanamide, is widely used when reactions at elevated temperatures and aqueous acidic conditions are feasible [11]. Herein, the guanidinylation resulted in modification of 100% of the amine groups of PVAm.

Representative $^1$H NMR spectra of a parent PNVF ($M_w$, 61 kDa) and resulting PVAm and PVG polymers are shown in Fig. 1. As is seen, the signal from the aldehyde side group protons centered at 7.86 ppm in PNVF diminished greatly as the hydrolysis progressed (compare with the spectrum of PVAm) and eventually disappeared. As the formaldehyde groups were eliminated and substituted with the primary amino and charged ammonium or guanidinium functionalities at pD 7.0, significant deshielding was observed in the positions

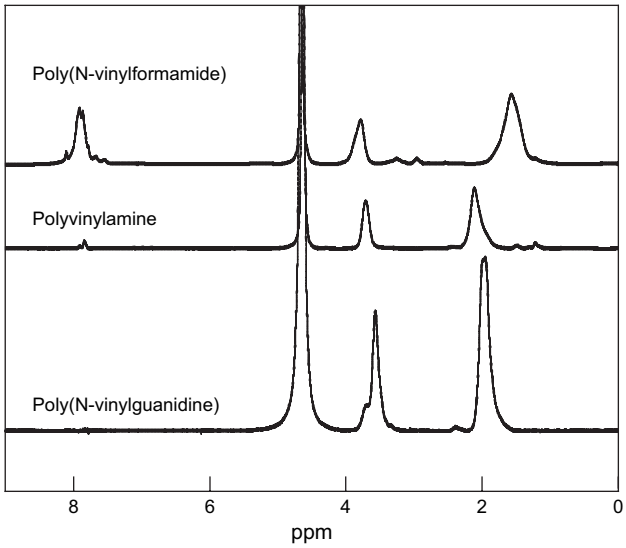
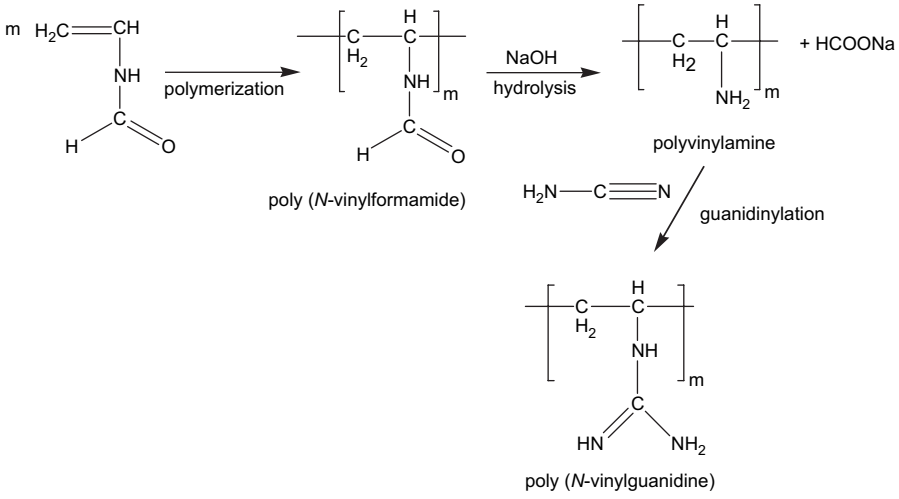



Fig. 1. 600 MHz $^1$H NMR spectra of poly(*N*-vinylformamide), poly(vinylamine) (degree of PVNF hydrolysis, 93%), and poly(*N*-vinylguanidine) in $D_2O$ at pD 7.0.




Scheme 2. Synthesis of poly(*N*-vinylguanidine).

of methylene (>$CH_2$) backbone proton signals (compare chemical shift, $\delta \sim 1.5$ ppm in PNVF with $\delta \sim 2.0$–2.1 ppm in PVAm and PVG). The substitution of the amino groups in PVAm onto guanidine functionalities with their multiple mono- and diprotonated ammonium and iminium structures [32] led to the changes in the methine (>CH–) proton signals ($\delta \sim 3.7$ ppm in PVAm and PNVF). The methine proton signals appeared at 3.58 ppm in the PVG spectrum, with a shoulder that was centered at ~3.66 ppm after deconvolution.

Similarly, significant differences in the $^{13}C$ NMR spectra were observed between the parent, fully hydrolyzed PVAm, and PVG (Fig. 2). The PVAm spectrum consisted of a broad signal ($\delta$ in the range 44–46 ppm) corresponding to both methine and methylene carbons of the backbone [33], with any aldehyde signal in the 160-ppm area lacking, as expected. The PVG spectrum featured signals in the 44–46 and 36–35 ppm areas corresponding to the methylene and methine carbons, respectively, along with a signal at 151.6 ppm belonging to the carbon of the protonated guanidinium group [32].

Overall, the NMR spectra confirmed the course of the reactions in Scheme 2 and corresponded well with the expected structure of PVG. Although detailed NMR studies of polyguanidines with their biguanide structure as well as arginines have been reported [34–38], to the best of our knowledge, ours is the first account of the poly(*N*-vinylguanidine) NMR.

Furthermore, despite a significant body of literature related to natural and synthetic guanidines [39–43], vibrational spectroscopic characterization of poly(*N*-alkylguanidines) in general and PVG in particular is absent. Therefore, herein we provide both FTIR and FT-Raman spectra of the newly synthesized polymer (Fig. 3), with the experimental vibrational wavenumbers and their tentative assignments collected in Table 1. The presence of strong amidine bands in both IR and Raman spectra is illustrative of numerous charged groups present in the polymer. Indeed, potentiometric titration of

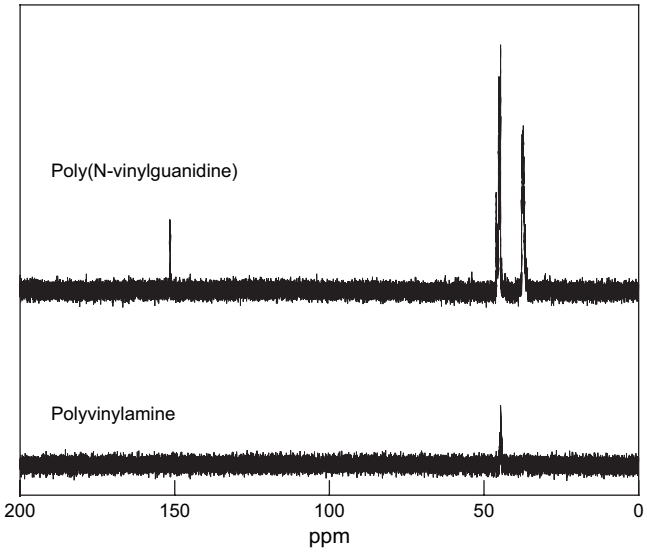



Fig. 2. 100.61 MHz $^{13}C$ NMR spectra of poly(*N*-vinylamine) and poly(*N*-vinylguanidine). Solvent, $D_2O$; pD, 7; 17,000 scans; polymer concentration, 15 wt%.

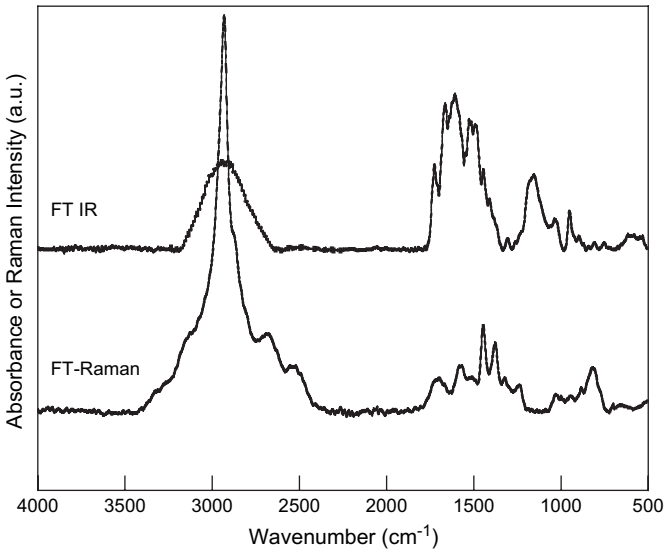



Fig. 3. FTIR (KBr) and FT-Raman (powder) spectra of poly(*N*-vinylguanidine).

PVG yielded an average p$K_a$ of 13.4, irrespective of whether polymer species of $M_w$ 13.6 or 60.8 kDa was used. This p$K_a$ is almost equal to that of unsubstituted guanidine [4]. Thus the basicity of PVG by far exceeds that of the parent polyvinylamine (p$K_a$ 9.4 [44]) as well as polybiguanide (p$K_a = 10.96$ [45,46]) or polyarginines (p$K_a$ 12.5 [4]) and places PVG among the most potent polymeric bases known.

### 3.2. Basic catalysis

Since PVG is a strong polybase, we expected it to exhibit reactivity toward DFP, an inhibitor of acetylcholine esterase that is widely used as a simulant to mimic the properties of more toxic, combat organophosphates such as the nerve agents sarin and soman [22]. In fact, guanidine has been shown to be

Table 1
Assignment of bands found in FTIR and FT-Raman spectra of poly(*N*-vinylguanidine)

| Wavenumber ($cm^{-1}$) | | Intensity | Assignment |
|---|---|---|---|
| FTIR | FT-Raman | | |
| | 830 | m | $NH_2$ deformation (wagging) |
| | 894 | w | |
| 957 | | m | C–N stretching |
| 1040 | 1040 | m/w | |
| 1170 | | s | |
| 1420 | 1380 | s | Aliphatic $CH_2$ deformation (wagging) |
| 1460 | 1450 | s | |
| 1510 | | s | C–N stretching |
| 1530 | | s | |
| 1610 | 1590 | s/m | NH, $NH_2$ deformation |
| 1670 | | s | C=N stretching |
| 1730 | 1730 | s | Amidine hydrochloride $-C(NH_2)_2^+Cl^-$ stretch |
| 2940 | 2875 | vs | Aliphatic $CH_2$ stretching |
| | 2940 | | |
| | 3145 | s | N–H stretching |

phosphorylated by DFP at elevated temperatures, i.e., the hydrolysis products of DFP form salts with guanidine [47]. Epstein et al. [48] correctly pointed out that the reactivity of a nitrogen nucleophile in catalysis is due to its proton basicity, which may not be reflected in a high p$K_a$ and may not correlate directly with the nucleophile's charge. Previously, we have demonstrated catalytic hydrolysis of DFP by oximate compounds acting as oxygen α-nucleophiles, wherein the −C═N−OH groups attacked phosphorus in DFP, cleaving the P−F group [22,23]. However, nucleophilic attack on phosphorus by nitrogen nucleophiles such as amines does not contribute to the overall reaction rate due to the formation of stable zwitterionic intermediates [48]. Instead, the amine-catalyzed hydrolysis of organophosphates such as DFP proceeds via general base-catalyzed bimolecular substitution mechanism ($S_N2$), involving the P−O bond cleavage with the solvated amino group, $RNH_2\cdots H{-}OH$ (Scheme 3) [49]:

According to Scheme 3 and taking $OH^-$-catalyzed, spontaneous hydrolysis into consideration, the governing kinetic equation of the DFP hydrolysis can be written as follows [48]:

$$\frac{d[DFP]}{dt} = -k_2[DFP][amine] - k_{sp}[DFP]\left[OH^-\right] \quad (1)$$

wherein $k_2$ is the second-order rate constant and [amine] is the concentration of the active amine species.

Note that the reaction shown in Scheme 3 is first-order with respect to the concentration of the substrate, [DFP], which is measured in the course of the reaction. The hydrolysis of DFP was studied by monitoring the appearance of the DFP decomposition product, fluoride ion, by an ion-selective electrode, as described in our previous publications [22,23]. The reaction was monitored in solutions of PVG and its parent PVAm under conditions of a molar excess of the active amine species over the initial substrate concentration ($[amine]_o > [DFP]_o = 1$ mM).

Under these conditions and at pH maintained constant at 7.8 by the Tris buffer, the DFP hydrolysis via the reaction shown in Scheme 2 is first-order with respect to the concentration of the substrate, [DFP]. The fluoride electrode potential was converted to the time-dependent fluoride concentration ($C_t$) readings using electrode calibration curves in sodium fluoride solutions. Fig. 4 shows typical DFP conversion vs time kinetics with PVG, which depended on the polymer concentration.

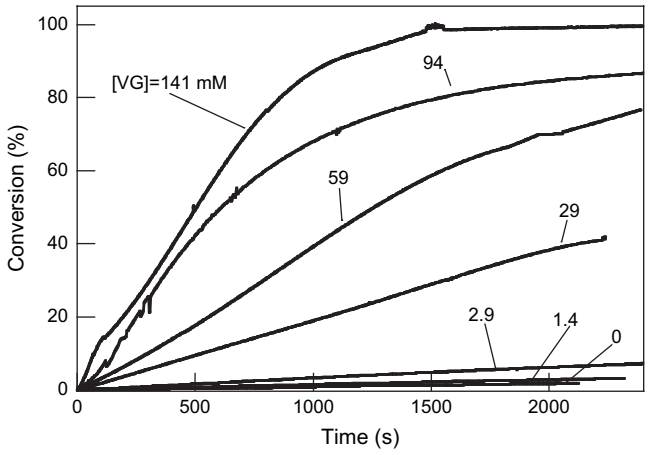


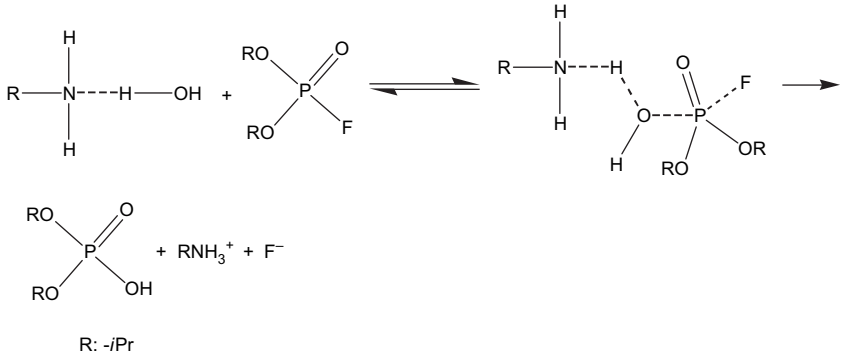

Fig. 4. Kinetics of conversion of diisopropyl fluorophosphate (DFP) in aqueous solutions of PVG. Conversion(%) = 100 × $C_t$/$[DFP]_o$. Tris buffer, 50 mM; pH 7.8; 25 °C; numbers denote PVG concentration in mM; calculated as mol/L of VG units.

The initial slope of the $C_t$ vs $t$ kinetic curves increases with the polymer concentration and gives the initial rate of the DFP hydrolysis, and the rate constant for the DFP hydrolysis ($k_{obs}$) is obtained from the experimental data using the equation [22,23]:

$$-\ln\left(1 - C_t/[DFP]_o\right) = k_{obs}t \quad (2)$$

where $[DFP]_o$ is the initial concentration of the substrate.

The observed rate constant measured without the polymers ($k_{sp}$) was measured to be $(9.3 \pm 0.3) \times 10^{-6}$ ($n = 3$), which corresponded well with the previously reported value for spontaneous DFP hydrolysis at similar pH [50]. Fig. 5 shows the dependencies of the observed rate constant on the effective concentration of the amine species, [amine], in the polymer solutions under study. The [amine] value was calculated as the number of equivalents of nucleophilic groups in one monomer unit of the polymer. Notably, PVG possesses three such groups, while its parent polymer, PVAm, has one amino group in each of their respective monomeric units. As is seen in Fig. 5, the dependencies of $k_{obs}$ on [amine] measured for both PVG and PVAm exhibited excellent linear fit ($R^2 > 0.99$), yielding the second-order rate constant $k_2 = 3.9 \times 10^{-3}$ $M^{-1}$ $s^{-1}$.




Scheme 3. Amine-catalyzed hydrolysis of diisopropyl fluorophosphate.

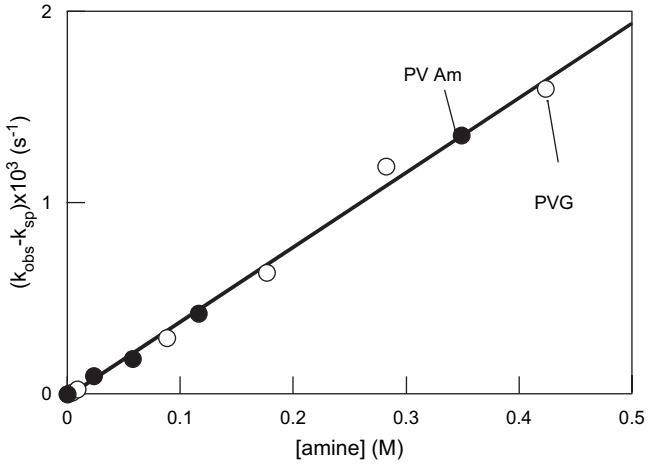



Fig. 5. Observed kinetic constants of DFP hydrolysis ($k_{obs}$) vs monomeric unit concentration in PVG and PVAm solutions. Open and filled points show data for PVG and PVAm, respectively. Polymer $M_w$, 13,600; $[DFP]_o = 1$ mM; pH 7.8; 50 mM Tris buffer; $T = 25$ °C. $k_{sp} = 9.3 \times 10^{-6}$ $s^{-1}$ is the rate of spontaneous DFP hydrolysis at pH 7.8.

This rate constant is approximately equal to the DFP and sarin hydrolysis rate constants with monomeric amines such as ethanediamine [48] and butylamine [49] as well as weak polymeric α-nucleophiles such as poly(4(5)-vinylimidazole) [50] and poly(4-vinylpyridine-*N*-phenacyloxime-*co*-acrylic acid) [22]. It is also remarkable that the values of the rate constants per [amine] measured for either PVG or PVAm coincided, irrespective of the fact that the two polymeric species had different charge densities at pH 7.8 (compare p$K_a$ of 13.6 and 9.4 for PVG and PVAm, respectively). This is in excellent agreement with the notion of the absence of a "charge" effect consistent with base catalysis (Scheme 3) being the hydrolysis mechanism [48]. In practical terms, the ability of PVG to hydrolyze DFP is 3-fold higher than that of PVAm at the same molar concentrations per monomer unit and about 1.5-fold that of PVAm at the same weight concentrations.

### 3.3. Bactericidal properties

There has been a significant amount of work on the bactericidal properties of cationic polymers rich in biguanide, quaternary ammonium, *N*-alkylpyridinium, and *N*-alkylated ethylene imine moieties, which exhibit higher antimicrobial activities than the corresponding low molecular weight compounds [51–59]. The effect of polycations with their large charge densities has been attributed to their excellent capacity to bind onto (negatively charged) cell surfaces and cytoplasmic membranes containing anionic lipopolysaccharides or lipids, respectively [60–63]. It has been observed that due to the great and multifunctional capability of guanidinium groups to bind anions, the guanidinium-rich polyarginines have a superior capacity to bind to and translocate across cellular and bilayer lipid membranes, compared to other cationic polypeptides of lysine or histidine [64,65].

These considerations have led us to hypothesize that PVG can be an efficient bactericide. Data collected on minimum inhibitory concentrations (MIC) of PVG (Table 2) indeed proved this hypothesis. To compare compounds with different molecular weights, MIC values in Table 2 were expressed in the typically used μg/L; data on chlorhexidine hydrochloride (CHX) were measured and are given for comparison. CHX is a bisbiguanide compound that has become a standard for cationic bactericides [5]. As is seen, although the parent PVAm is quite capable of inhibiting bacterial growth, its guanidinylation led to a dramatic 10- to 40-fold lowering of the MIC values. Expressed in μg/L, the MIC of the PVG species for *P. aeruginosa* and *E. coli* was as low as the MIC of CHX or a specifically formulated Akacid plus® bactericide, which is a 3:1 mixture of poly(hexamethylene guanidinium chloride) and poly[2-(2-ethoxy)ethoxyethyl guanidinium chloride] (MIC, 32 μg/mL) [66]. No clearly discernible trend relating MIC to the polymer length was observed. The time-dependent killing measurements conducted on *S. aureus* showed that PVG concentrations exceeding four-fold the MIC (Table 2) lowered the logarithm of the mean viable bacteria counts, or log (bacterial reduction), by 0.4, 1.3, 2.2 and over 5 at time points of 5 min, 0.5 h, 1 h, and 8 h, respectively. No significant differences were observed with either 13.6 or 60.8 kDa PVG species. These results are comparable to those with the biocide Akacid plus® that is being commercialized as an antiseptic [66]. It should be noted that previous studies with aqueous solutions of polycationic bactericides, including polyvinylamine [67], have been conducted with oligomers or polymers of size smaller than ours, with degrees of polymerization typically not exceeding 10–40, except for polymethacrylates with pendant biguanide groups and poly(4-vinylbenzyl ammonium chloride) derivatives that have been shown to be bactericidal with degrees of polymerization as high as 200 [68]. Generally, a significant enhancement of bactericidal and bacteriostatic activity has been reported with an increase in the polymer chain length in polymeric quaternary ammonium salts and polyhexamethylene biguanides (PHMBG) [69]. Bactericidal activity of the polycations that exhibit membranolytic activity, such as polynorbornene derivatives [70], clearly depends on the presence and size of a hydrophobic group in the repeat unit. The increase in antibacterial activity with increasing

Table 2
Minimum inhibition concentrations (MIC) in μg/mL for polyvinylamine (PVAm), poly(*N*-vinylguanidine) (PVG), and chlorhexidine dihydrochloride (CHX)

| Microorganism | PVAm1[a] | PVAm2[b] | PVG1[a] | PVG2[b] | CHX |
|---|---|---|---|---|---|
| Gram-positive | | | | | |
| *Staphylococcus aureus* | 1400 | 2100 | 68 | 68 | 0.6 |
| *Streptococcus mutans* | 700 | 500 | 128 | 128 | 1.8 |
| *Staphylococcus epidermidis* | 1000 | 700 | 68 | 68 | 1.8 |
| *Candida albicans* | 700 | 1000 | 128 | 255 | 4.0 |
| Gram-negative | | | | | |
| *Escherichia coli* | 1000 | 700 | 34 | 34 | 32 |
| *Pseudomonas aeruginosa* | 350 | 500 | 34 | 68 | 32 |
| *Pseudomonas fluorescens* | 1400 | 1000 | 128 | 68 | 4.0 |
| *Klebsiella pneumoniae* | 700 | 700 | 68 | 34 | 4.0 |

[a] PVAm1 and PVG1: number-average degree of polymerization, 166.
[b] PVAm2 and PVG2: number-average degree of polymerization, 642.

hydrophobicity is in accordance with the concept of cell membrane disruption wherein the hydrophobic groups can be anchored. Of note, the PVG tested herein lacks any hydrophobic groups beside the methine and methylene groups in the backbone. It must be the significant charge density that determines the bactericidal activity of the PVG.

## 4. Concluding remarks

Synthesis of poly(*N*-vinylguanidine) (PVG) via polymerization of *N*-vinylformamide followed by basic hydrolysis of the PNVF and guanidinylation of the resulting polyvinylamine (PVAm) is quite efficient (yields in polymerization and guanidinylation are $\geq$90%) and affords a PVG with molecular weight corresponding to that of the parent PVAm. The molecular weights can be varied by altering the initial NVF/azo initiator ratio. Analogous guanidinylation can be performed in one step on a commercially available PVAm sample. The elemental analysis, NMR, and vibrational spectroscopy data, most of which are obtained herein for the first time, all support the PVG structure. This PVG appears to be an ardent hydrolyzing species for diisopropyl fluorophosphate (DFP), an organophosphate mimic of combat nerve agents. The second-order rate constant for DFP hydrolysis by PVG at pH 7.8 was measured to be $3.9 \times 10^{-3}\ M^{-1}\ s^{-1}$, expressed per concentration of the catalytic amino or imino moieties in each PVG monomer. There are three basic catalytic groups in each monomeric unit of PVG, which is consistent with the general $S_N2$ mechanism of the base catalysis governing the hydrolytic reaction. We have demonstrated that guanidinylation of PVAm resulting in PVG affords a 10- to 40-fold lower minimum inhibition concentration (MIC) against tested four Gram-positive and four Gram-negative bacteria as compared to the PVAm, itself a bactericidal and bacteriostatic polymer. Hence, the PVG polymers described herein are bactericides that may be suitable as biocidally active compounds in the production of disinfectants and as active compounds for disinfectants and for the preservation of industrial products such as dispersions, emulsions, colorants, coatings, drilling and cutting fluids as well as in cosmetic products. Combined with the basic hydrolyzing capability of PVG against toxic organophosphates, the bactericidal properties of PVG indicate its value as a promising material in chemical and biological defense applications.

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