Nitroalcohol Induced Hydrogel Formation in Amine-Functionalized Polymers

Marissa R. Solomon,[1] Naphtali A. O'Connor,[2] David C. Paik,[3] Nicholas J. Turro[1]

[1]*The Department of Chemistry, Columbia University, New York, New York 10027*
[2]*The Department of Chemistry, Lehman College-CUNY, New York, New York 10468*
[3]*The Department of Opthamology, Columbia University, New York, New York 10027*

Received 10 April 2009; accepted 8 December 2009
DOI 10.1002/app.31944
Published online 26 March 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Certain β-nitroalcohols degrade under basic conditions or upon heating to form formaldehyde. This reaction provides an elegant approach to generate formaldehyde within a system at a desired time using the stimulus of pH or temperature. Using β-nitroalcohols as a delivery agent for formaldehyde, polymer crosslinking can be induced via stimulus. Such an approach is akin to those used to prepare "self-healing" polymers, which have received much attention recently. Herein, we describe the use of certain β-nitroalcohols as a masked formaldehyde delivery system and demonstrate its use as a crosslinking agent of amine functionalized polymers to form hydrogels. We examine the temperature and pH dependence of 2-nitro-1,3-propanediol and 2-(hydroxymethyl)-2-nitro-1,2-propanediol on the rate and extent of gelation and characterize the resulting gel by swelling and FTIR experiments.
 J Appl Polym Sci 117: 1193–1196, 2010

Key words: hydrogels; crosslinking; nitroalcohol; polyamines; formaldehyde

INTRODUCTION

A great deal of resources and ingenuity have been invested in the study of hydrogels and the possibilities they offer are varied, including materials for tissue engineering, immunoisolation, immobilized biocatalysts, and drug delivery.[1–6] Hydrogels, typically, are gelatinous structures composed of hydrophilic polymers crosslinked through either chemical or physical means.[7,8]

Aldehydes such as formaldehyde and glutaraldehyde are versatile chemical crosslinkers for amine, hydrazide, and alcohol functionalized polymers.[6–9] Meanwhile, β-nitroalcohols (Fig. 1) have been known to decompose back to the relevant carbonyl fragment and the nitroalkane or its salt when heated or in alkaline solution (Scheme 1).[9,10] In particular, the β-nitroalcohols **1** and **2** release formaldehyde under these circumstances. Because the active reagent formaldehyde is masked in these β-nitroalcohols **1** and **2**, crosslinking can occur at any desired time and in a controlled manner by simply increasing pH or temperature. β-Nitroalcohols have recently been shown of use in therapeutic corneoscleral crosslinking.[9,10] The mechanism of this crosslinking is attributed to the action of formaldehyde. β-Nitroalcohols **1** and **2** can be envisioned as agents of repair/healing in formaldehyde crosslinked polymers by encapsulating them within these polymeric networks or as crosslinking agents for variety of biopolymers. Also typical formaldehyde crosslinking conditions for biopolymers are in acidic media,[11–13] and this approach adds an additional dimension to the utility of formaldehyde as a crosslinker.

In this article, we describe the facile crosslinking of amine functionalized polymers using β-nitroalcohols **1** and **2** as formaldehyde surrogates. Formaldehyde is produced *in situ* and reacts with amines to form imines, which inturn react with other amine and amide groups to create the crosslinks (Scheme 2).[14] This communication describes the preparation of polyallyl amine, polylysine, and chitosan hydrogels using β-nitroalcohols **1** and **2** as the crosslinking agents.

EXPERIMENTAL

Materials

Poly(allylamine) hydrochloride (average $M_w \sim 15{,}000$) poly-L-lysine hydrochloride (M_w 15,000–30,000) and poly-L-asparagine (M_w 5000–15,000) sodium phosphate monobasic ($NaH_2PO_4 \geq 99\%$) and sodium phosphate dibasic ($Na_2HPO_4 \geq 99\%$) were obtained from Aldrich. (2-Hydroxymethyl)-2-nitro-1,3-propanenitrodiol[1] and 2-nitro-1,3-propanenitrodiol[2] were obtained from TCI

Correspondence to: N. J. Turro (njt3@columbia.edu).
Contract grant sponsor: NSF; contract grant numbers: CHE 04-15516, CHE 07-17518.

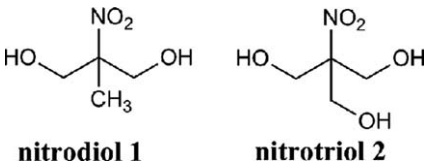
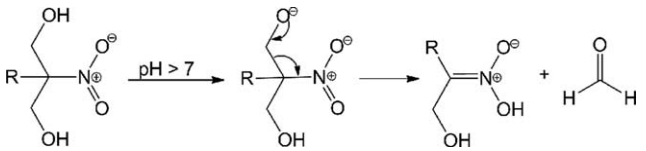


Figure 1 Formaldehyde-releasing β-nitroalcohols.



Scheme 1 Formaldehyde release from the β-nitroalcohol at neutral to alkaline pH.

America, and glycol chitosan was obtained from MP Biomedicals. All these were used as received; for the buffer and salt solutions, 0.23*M* NaH_2PO_4 and 0.2*M* Na_2HPO_4 were combined in the appropriate ratios.

Characterization of gel formation

The swelling properties as well as FTIR spectra were obtained to characterize gel formation. FTIR spectra were collected on a Nicolet Nexus 870 FTIR with a Thermo Multibounce HATR accessory with a ZnSe crystal. The pH measurements were obtained using a WTW Measurements Systems, Chekmite pH-20 Sensor. The swelling is defined as w_0/w, where w_0 is the weight of the dry piece of gel and w is the weight of the hydrogel after sitting in water for 2 h.

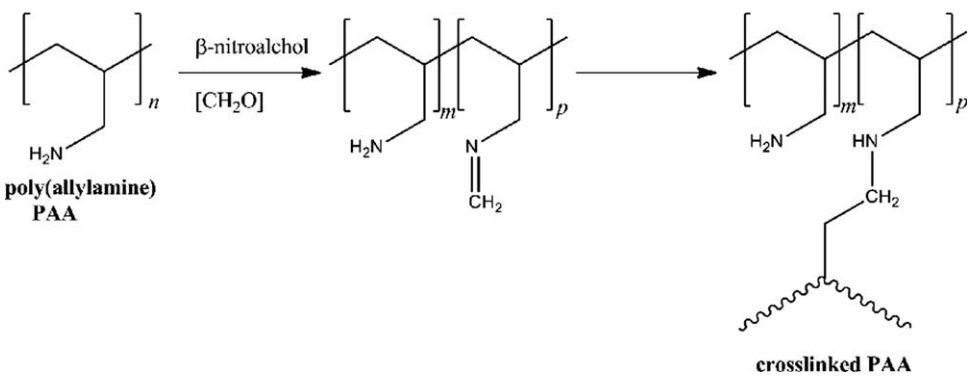
Hydrogel preparation

This is a general description of hydrogel preparation. Polyamines (110 mg) were dissolved along with nitrodiol **1** (110 mg) in 0.3 mL of buffered solution of the desired pH and heated to the desired temperature in a centrifuge tube until a firm gel is formed. The gel was either washed or dewatered with ethanol before drying in a vacuum oven overnight, or dried without dewatering, prior to swelling experiments.

RESULTS AND DISCUSSION

Hydrogels were obtained by dissolving the polyamine polymer along with the nitroalcohol in a buffer solution and heating at specific temperatures between 37°C and 100°C until a firm gel is formed. Deprotonation of the alcoholic proton of β-nitroalcohols and subsequent decomposition to formaldehdye is promoted by alkaline conditions and to that end, we investigated hydrogel formation at various pH with poly(allylamine) (PAA) and nitrodiol **1** at 37°C (Table I). The mass ratio of crosslinker to polymer is expressed by *r* (mass crosslinker/mass polymer) and is 1 for Table I. β-Nitroalcohols are more acidic than normal alcohols, for example, nitrodiol **1** has a pK_a of 5.4 compared with $\sim$ 15 for alkanols. Gelation with nitrodiol **1** was observed to occur above pH 6, with the most favorable conditions being alkaline. The more alkaline the solution, the quicker the gel formation occurred. Gels formed within two days at pH 11.6, but twice as long at pH 7.4. No significant difference was seen in the swelling ratios of these two materials, indicating that pH does not have a strong effect on the swelling ratio. Thus, β-nitroalcohols are a means to crosslinking with formaldehyde in basic media.

The effect of temperature on nitroalcohol-induced gelation was also studied. Gelation was investigated at a variety of temperatures in 0.2*M* Na_2HPO_4 solution (pH 11.6). The results are shown in Figure 2. Optimal conditions for gel synthesis were found to be between 37°C and 50°C at pH 11.6. Additionally, it was determined that gelation is dependent on the concentration of crosslinker. On varying the concentration of the nitrodiol **1**, we observed that at a certain concentration an increase in crosslinker retards gelation (Table II). When the mass ratio *r* of crosslinker to polymer is between 0.6 and 1, a firm gel



Scheme 2 Crosslinking in poly(allylamine) with β-nitroalcohol.

TABLE I
Effect of pH on Gel Formation of Poly(allylamine) and Nitrodiol 1[a]

Sample	Aqueous solution	Gel time (h)	Swelling (w_0/w)
1	pH 2.0	no gel	n/a
2	pH 5.9	no gel	n/a
3	pH 7.4	96	22 ± 4
4	pH 11.6	48	25 ± 5

[a] Phosphate buffer ($I = 0.2$ *M*, 37°C), ratio of crosslinker to polymer r (mg/mg) = 1.

TABLE II
Effect of β-nitroalcohol 1 Concentration on Gelation of Poly(allylamine) within the First 24 h (50°C, pH 11.6)

Sample	r (mg/mg)	24 h	Swelling (w_0/w)
1	0.6	Firm gel	26 ± 5
2	1.0	Firm gel	25 ± 5
3[a]	2.7	Partial gel	12 ± 5
4[a]	3.6	No gel	10 ± 2
5[b]	1.1	Firm gel	22

[a] Gel obtained after 24 h.
[b] Nitrotriol **2** used; gel obtained within 5 h.

was obtained within 24 h. However, when the mass ratio was increased to 2.7, only partial gelation was observed after 24 h, with a gel being observed in the lower portion of the reaction vessel. After 24 h, no gel was observed when the mass ratio was increased to 3.6. These observations are likely due to a greater number of amine functionalities being converted to Schiff-bases with the increased mass ratio. The Schiff bases react with the remaining amine functionalities to create the crosslinks. If too many Schiff bases are generated then there are not enough amines present to react with them, and thus there is a diminishing in crosslinking. After 2–3 days, gels were eventually obtained for mass ratio 2.7 and 3.6. The comparatively lower swelling values in Table II for these two mass ratios substantiate the proposed decrease in crosslinking in these gels.

FTIR spectra obtained for the poly(allylamine) hydrogel before and after washing with a combination of ethanol and water are shown in Figure 3. Peaks at 1540 cm^{-1} and 1351 cm^{-1} are assigned to NO_2 symmetric and asymmetric stretching, respectively, and the peak at 1077 cm^{-1} is assigned to the C—NO_2 bending. These peaks disappear with washing thereby suggesting that the nitroalkyl side product does not react with the polymer chain and is not incorporated covalently into the gel.

The effect of β-nitroalcohols on the polyamine crosslinking was examined further by studying nitrotriol **2** and the rate and degree of gelation. The nitroalcohol **2**, possesses 3 hydroxyl groups as opposed to the two hydroxyl groups of the nitroalcohol **1** and degrades to three equivalents of formaldehyde. The rate of gelation was increased with nitrotriol **2** when compared with nitrodiol **1**. Gelation with nitrotriol **2** occurred in less than half the time it took for the polymer to gel with nitrodiol **1**, yet the degree of swelling was approximately comparable. As the nitrotriol is known to produce 3/2 times more formaldehyde than the nitrodiol **1**[11], it is not surprising that the gelation occurs in less time. However, the high acidic nature of nitrotriol **2** may also be a contributing factor, because deprotonation will occur faster in nitrotriol **2** than in nitrodiol **1** under the same alkaline conditions. However, the gel formed by nitrodiol **1** was colorless, whereas the gel formed by nitrotriol **2** had a yellowish color that could not be removed. This is possibly due to the incorporation of the nitromethane salt (formed as a side product by the nitrotriol) into the polymer. This was confirmed by FTIR, where the NO_2 stretching peaks (1340 and 1530 cm^{-1}) as well as the C—NO_2 bending peak (1050 cm^{-1}) persist after washing.

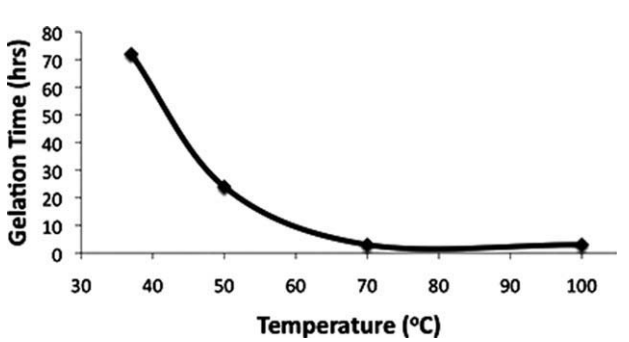


Figure 2 Effect of temperature on gel time of PAA crosslinked with nitroalcohol **1** (phosphate buffer, pH = 11.6, $I = 0.2M$, 37°C; r (mg/mg) = 1).

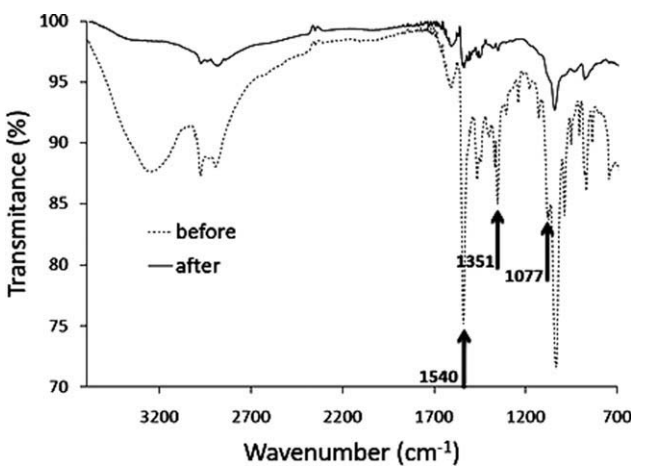


Figure 3 ATR-FTIR of poly(allylamine) crosslinked with nitrodiol **1** at pH 7.4 and 37°C (a) --- before and (b) — after being washed with ethanol and water.

TABLE III
Biodegradable Hydrogels Prepared with β-nitroalcohol 1 (37°C, pH 11.6)

Sample	Polyamine	Gel time (h)	Swelling
1	poly-l-lysine	24	10 ± 4
2	poly-l-asparagine	no gel	n/a
3	glycol chitosan	24	6 ± 1

With favorable conditions for crosslinking determined, we demonstrated hydrogel synthesis of amine functionalized biopolymers using β-nitroalchols. Glycol chitosan, poly-L-lysine, and poly-L-asparginine were selected as model biopolymers. Gels of these biopolymers have been demonstrated in a variety of applications, including drug delivery and tissue engineering.[7,15] Formaldehdye crosslinked gels of these biopolymers have been previously demonstrated under acidic synthetic conditions.[11,13,16] Crosslinking was attempted under basic conditions using β-nitroalcohols at pH 11.6. Gels were not obtained for poly-L-asparginine, even though the formaldehyde crosslinking of amides has been demonstrated.[12] However, we were able to easily prepare hydrogels for glycol chitosan and poly-L-lysine. The conditions and swelling results are listed in Table III. The gelation times were found to be comparable to those of poly(allylamine). This demonstrates the utility of β-nitroalcohols as crosslinking agents for amine functionalized biopolymers.

CONCLUSIONS

We have demonstrated the use of β-nitroalcohols as effective crosslinking agents for polyamine polymers and for selected proteins. We showed that β-nitroalcohols are able to crosslink polyamines and form gels in as little as 5 h. These results demonstrate that β-nitroalcohols can serve as surrogates for formaldehyde and provide a route to the facile delivery of known quantities of formaldehyde. The masked delivery of formaldehyde also provides a mechanism, whereby polyamines can be crosslinked at the desired point by raising the pH or temperature and may allow its use *in vivo*, where the formaldehyde is unmasked at low concentrations only at the desired crosslinking site or as self-healing agent by encapsulating it within the polymer.

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