Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Moszner, N. , Hartmann, M. , Bergmann, H. , Schütz, H. , Schütz, E. , Schwachula, G. , Feistel, L. , Zalupsky, P. and Vegh, D.(1991) 'Polymeric Nitrofuran Derivatives. II. Chemical Modification of Functionalized Resins with Nitrofuran Derivatives', Journal of Macromolecular Science, Part A, 28: 8, 761 — 773
To link to this Article: DOI: 10.1080/00222339108054055
URL: http://dx.doi.org/10.1080/00222339108054055

J. MACROMOL. SCI.—CHEM., A28(8), pp. 761–773 (1991)

POLYMERIC NITROFURAN DERIVATIVES. II. CHEMICAL MODIFICATION OF FUNCTIONALIZED RESINS WITH NITROFURAN DERIVATIVES

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ABSTRACT

Polymeric nitrofuran derivatives have been synthesized by chemical modification of macroporous styrene-divinylbenzene copolymers

with low molecular weight nitrofurans. The 5-nitrofuryl groups are covalently attached to the polymeric carrier by azomethine, ester, *N*-alkyl, and sulfamide links, respectively. Comparative hydrolysis studies and biological tests of the modified resins suggested that the polymeric carrier-bound nitrofurans are antimicrobially active. The polymeric nitrofurans have been characterized by IR and ^{13}C-solid-NMR spectroscopy.

INTRODUCTION

Nitrofuran derivatives have long been known to possess antimicrobial properties, and several such derivatives are currently being used as chemotherapeutic agents [1]. Broader use of nitrofuran-based biologically active agents has been hampered by their low chemical stability. In an attempt to facilitate the problem of instability, cellulose- and polyacryloylhydrazine-bound nitrofuran derivatives were synthesized [2, 3].

We recently reported [4] the homo- and copolymerization of 5-nitrofurfuryl methacrylate that led to the formation of polymers with different hydrophilicities.

We now describe the synthesis of styrene–divinylbenzene copolymers modified by the 5-nitrofuryl moiety and attached to the polymer carrier by a wide variety of chemical linkages.

EXPERIMENTAL

Materials

The crosslinked copolymers poly(4-vinylbenzylamine-*co*-divinylbenzene) (I), poly(4-vinylbenzenesulfonamide-*co*-divinylbenzene) (II), and poly(4-vinylbenzoic acid-*co*-divinylbenzene) (III) were obtained from Chemie AG Bitterfeld-Wolfen, GDR. The divinylbenzene content in the initial mixtures amounted to approximately 5 wt%. These swellable macroporous copolymers showed a water content of 55 (I), 51 (II), and 56 wt% (III), respectively, and were washed with methanol and dried under vacuum before use. Elemental analyses of dry samples were

I: C 80.25, H 8.28, N 6.79, Cl 0.62

II: C 51.82, H 5.44, N 6.17, Cl 1.60, S 14.30

III: C 66.25, H 5.36, N 0.93, Cl 3.73

5-Nitrofurfuryl alcohol was prepared as described previously [4]. 5-Nitrofuraldehyde was obtained by oxidation of 5-nitrofurfuryl alcohol by manganese dioxide [5]. 5-Nitrofurfuryl iodide and 3(5-nitrofur-2-yl)acrolein were prepared according to Refs. 6 and 7, respectively. 5-Nitrofurfurylidenebenzylamine was synthesized from 5-nitrofuraldehyde and benzylamine in ethanol by a conventional method [8].

Modification of the Resins

Reaction of I with Aldehydes

Copolymer I (5.0 g) was swollen in 11 mL ethanol or dimethylformamide. After addition of the appropriate aldehyde, the mixture was stirred at 80°C, usually for 10 h. Then the modified resins were filtered off and purified by Soxhlet extraction with methanol for 40 h. The products were dried at 50°C under vacuum to constant weight. Elemental analyses of dry samples were

Ib: C 69.33, H 5.85, N 6.60, Cl 0.61

Ic: C 69.16, H 6.19, N 5.63, Cl 0.47

N-Alkylation of I

To 3.3 g Copolymer I a solution of the organic halide and an excess of triethylamine (TEA) (with respect of the halide concentration) in 20 mL ethanol was added with stirring at room temperature. Stirring was continued for 16 h at 80°C. The products formed were separated and purified as described above:

Id: C 74.31, H 7.40, N 5.61, Cl 0.71

Sodium Salt of II

Copolymer II (16 g) was swollen in a solution of 2.82 g NaOH in 10 mL water for 2 days at room temperature. The sodium salt (IIa) of resin II was filtered off, washed with water, purified by extraction with methanol for 48 h, and subsequently dried under vacuum.

N-Alkylation of IIa

To 5 g of Copolymer IIa a solution of 27.2 mmol 5-nitrofurfuryl iodide and 3.8 mL TEA in 16.2 mL ethanol was added. Then the mixture was stirred at 80°C for 16 h. The reaction mixture was filtered off and

the polymers obtained (IIc) were washed with water and purified as described above:

IIc: C 53.19, H 4.98, N 11.43, Cl 1.48

Esterification of IIIa

The conversion of III into Resin IIIa containing acid chloride groups was done as reported previously [9]. The acid chloride content of IIIa (62.3 mol%) was calculated from the chlorine content of IIIa (17.78), taking into account a chlorine content of 3.73% in the starting Resin III.

To a suspension of Copolymer IIIa (6 g) in methylene chloride (50 mL), a mixture of 9.2 g 5-nitrofurfuryl alcohols and 5 mL TEA was added with stirring at room temperature. After refluxing for 16 h, the modified resin was filtered off and purified by extraction with chloroform for 16 h and subsequently with hexane for 6 h. The product was dried under vacuum to constant weight.

Measurements

^{13}C-NMR-spectroscopic measurements of resins were performed with a homebuilt spectrometer of the Department of Physics, Friedrich-Schiller-University of Jena. The working frequency was about 15 MHz. The cross-polarization technique was combined with magic-angle spinning (MAS) to minimize the bandwidth. The rotation frequency was 2 kHz. Adamantane was used as the reference material. The spectra were measured with a pulse delay of about 25 ms, the cross-polarization time was 2 ms, and the decoupling time was 40 ms. An infrared spectrometer M 80 (Carl Zeiss GmbH, Jena) was used to record the IR spectra. UV absorptions were measured with a spectrometer, Specord (Carl Zeiss GmbH, Jena).

Hydrolysis of the modified resins was conducted in bidistilled water at 30°C as described previously [9]. The release of nitrofurans was observed by UV-VIS spectroscopy of the hydrolysis solutions.

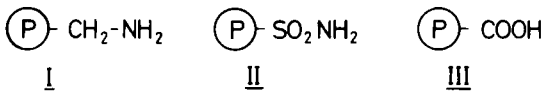
Biological Tests

Antimicrobial effects of nitrofurans were determined by the common agar plate method [10], *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae,* and *Enterobacter cloacae* were the test organisms. In the

case of the suspension test, the polymeric nitrofurans (0.2 g) were suspended in 10 mL water and injected with the test organisms (*E. coli*). After a certain time the survival rates of the separated solutions (0.5 mL) were determined by the agar plate method. The incubation time was 48 h at room temperature.

RESULTS AND DISCUSSION

The polymer carrier itself was a modified styrene–divinylbenzene copolymer, closely related to well-known ion-exchange resins and to resins used for the immobilization of enzymes. On its macroporous structure it carried aminomethyl (I), sulfonamide (II) and carboxyl groups (III):



I II III

P : Poly (styrene-co-divinylbenzene)

Such a polymeric carrier could be further modified via the formation of azomethine, ester, and *N*-alkylated compounds. All the synthesized derivatives possess, by virtue of the newly formed chemical bonds, different stabilities toward natural or enzyme hydrolysis.

The search for optimal reaction conditions of the polymer-modifying reactions was greatly facilitated by the use of selected model compounds.

Fixation of the Active Agent by Means of an Azomethine Linkage

Although the nitro group at the furan ring has been recognized as being responsible for the biological activity in tests, nitrofurans conjugated with an exocyclic polarized multiple bond have shown much lower minimum inhibitory concentration (MIC) values than the parent compound. During optimization of the reaction conditions for the polymer-analogous modification reaction, we employed 4-bromobenzaldehyde as a model. As can be seen from the data in Table 1, variation of the solvent influenced conversion, the highest conversion being achieved in

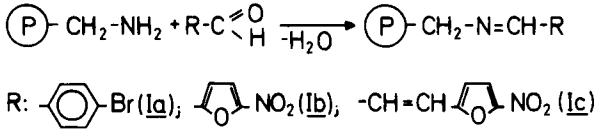
TABLE 1. Modification of Poly(4-Vinylbenzylamine-*co*-Divinylbenzene) I with 4-Bromobenzaldehyde[a]

Solvent	Time, h	Bromine content, wt-%	Conversion, mol%[b]
Ethanol	8	15.47	57
Ethanol	16	18.22	74
Dimethylformamide	8	14.54	53
Acetonitrile	8	19.78	84

[a]Ratio of $[-NH_2]/[\text{aldehyde}] = 1/1.8$ in the starting reaction mixture.
[b]With respect to the initial content of NH_2 groups.

acetonitrile. The bromine content of modified resins (Ia) was not the only criterion of successful conversion, for all modified polymers displayed the characteristic band at 1610 cm^{-1} (valence vibration of the CH=N bond) in their IR spectra.

In an analogous manner, Copolymer I was modified by 5-nitrofuraldehyde (Ib) and 3(5-nitrofur-2-yl)acrolein (Ic):



In these cases the progress of the modification reaction, however, had to be monitored by calculations based on the difference in oxygen content, since the difference in nitrogen content was too small to be reliable. Such calculations indicated a 41-mol% content of the active group in Ib and a 50-mol% content in Ic, corresponding to a 68% conversion of the aminomethyl group in Ib and a 84% conversion in Ic, respectively, both values related to the aminomethyl group content in the parent copolymer (I). Once again, apart from the calculations, successful chemical modification was also confirmed by inspection of the IR spectra, which fortunately showed distinct bands at 1015 cm^{-1} (skeletal vibrations of the furan ring) as well as a band at 1350 cm^{-1} (symmetric valence vibrations of the NO_2 group).

A more detailed picture of the structure of the prepared, modified copolymers has been attained from ^{13}C-NMR spectral data. Figure 1

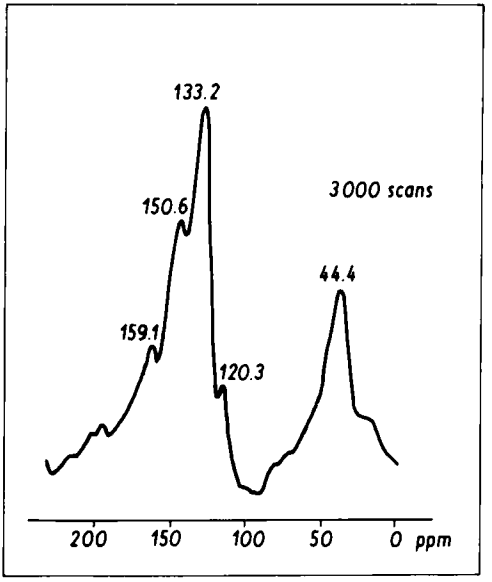


FIG. 1. ^{13}C-NMR solid spectrum of modified Resin Ib.

shows the ^{13}C-solid-NMR spectrum of Ib. Based on comparison with the spectrum taken of the $CDCl_3$ solution of 5-nitrofurfurylidenebenzylamine (Table 2), we have assigned the signal at 120.3 ppm to $C_{3,4}$ of the furan ring, that at 159.1 ppm of the C_{Tot5}azomethine carbon was found at 150.6, whereas carbon atoms of the phenyl ring were at 133.2 and aliphatic carbon atoms at 44.4 ppm.

The solid-state ^{13}C-NMR spectrum of Ic (Fig. 2) displayed signals of $C_{3,4}$ at 115.2, C=N at 147.0, and C_5 at 156.1 ppm. Signals of the exocyclic double bond are overlapped by aromatics.

Fixation of the Active Agent by Means of *N*-Alkylation

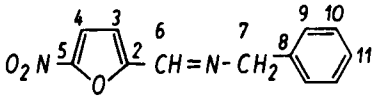
N-Alkylation of the starting Copolymer I was expected to lead to nonhydrolyzable 5-nitrofuryl modified polymers with their biological activity further enhanced on account of the presence of possible quaternary ammonium groups.

As in the reaction described previously, the optimal reaction conditions were ascertained from a model reaction of 5-nitrofurfuryl iodide

TABLE 2. Chemical Shift Data of the ^{13}C-NMR Solution Spectrum of 5-Nitrofurfurylidenebenzylamine[a]

Carbon atom	Chemical shift, ppm
2	137.6
3	112.6
4	113.6
5	153.8
6	149.5
7	65.2
8	136.7
9–11	127.5–128.7

[a]



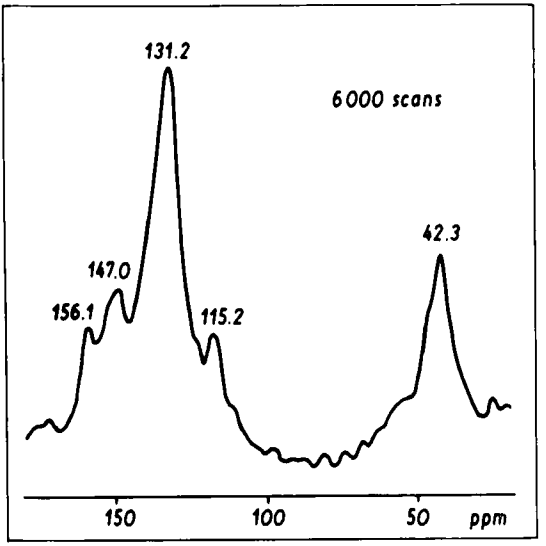
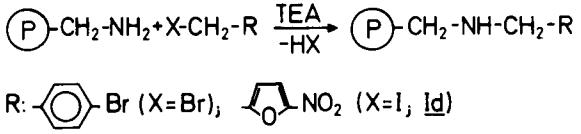


FIG. 2. ^{13}C-NMR solid spectrum of modified Resins Ic.

with benzylamine, as well as from a reaction of 5-brombenzyl bromide with I.

Because the calculated bromine content associated with complete monoalkylation of the polymeric substrate is only 21.78 wt%, the bromine content found in the case when an excess of alkylating agent was used seems to indicate the formation of dialkylated products (Table 3, the last item).

The reaction of I with the 5-nitrofurfuryl iodide (1:1.9 molar ratio) was conducted in alcohol and in the presence of an equimolar amount of triethylamine.



The product obtained after 16 h (Id) was analyzed by the oxygen difference method and found to contain 25 mol% of an active substance, corresponding to 42% alkylation of the starting aminomethyl groups. As in the previous cases, the presence of vibrations at 1350 cm^{-1} in the IR spectrum confirmed the chemical modification of the copolymer, assumed from elemental analysis data.

Fixation by Means of the Sulfonamide Functional Group

The alkylation of both the low-molecular and polymeric substrate (*p*-tolylsulfonamide and II) with 4-bromobenzyl bromide in ethanol and in the presence of an equimolar amount of NaOH was successful, render-

TABLE 3. Alkylation of Poly(4-Vinylbenzylamine-*co*-Divinylbenzene) (I) with 4-Bromobenzyl Bromide in the Presence of TEA

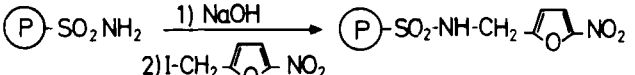
$[-NH_2]$/[bromide]	Time, h	Bromine content, wt-%	Conversion, mol-%[a]
1/1.8	8	10.92	36[b]
1/1.8	8	16.63	64
1/1.8	16	17.25	68
1/5.3	16	22.02	>100

[a]With respect to the initial content of NH_2 groups.

[b]In the absence of TEA.

ing a polymer with a 16.73-wt% bromine content (72 mol% conversion of sulfonamide groups). Unfortunately, the analogous alkylation of II with 5-nitrofurfuryl iodide in ethanol, catalyzed with NaOH, failed.

Because there were well-founded fears that the iodide was destroyed by the action of NaOH before it could react with Polymer II, we first prepared the sodium salt of II (IIa) and subsequently subjected it to reaction with 5-nitrofurfuryl iodide in ethanol. Under such conditions the reaction was successful, substantiating our assumption of the instability of the reagent in basic media.

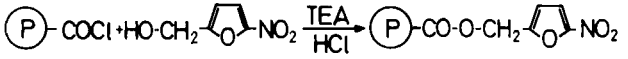


IIc

Calculations based on the sulfur content of IIc, compared with that of II, showed a 31-mol% conversion and a 45-mol% degree of alkylation. In addition, titration of the released iodide ions with silver nitrate corroborated the previous data, giving 40 mol% alkylation. Once again, IR spectral data confirmed successful chemical modification, for they showed a broad band at 1160 cm^{-1}, belonging to the SO_2 group, with a shoulder at 1350 cm^{-1}, resulting from the introduced nitro group.

Fixation by Means of an Ester Linkage

In analogy to previous reports from our group [9], the starting Copolymer III was first treated with $SOCl_2$ to convert it to polymeric acid chloride IIIa. This was then allowed to react with 5-nitrofurfuryl alcohol in methylene chloride in the presence of an equimolar amount of TEA.



The reaction afforded the corresponding ester IIIb. Its nitrogen content of 3.69 wt% indicated 60 mol% of active substance. Neither pyridine as a solvent nor the presence of *N,N*-dimethylaminopyridine as a catalyst produced higher conversions.

The structure of IIIb was studied by IR spectroscopy. The spectra contained an ester carbonyl at 1730 cm^{-1}. Furthermore, there were peaks at 1350 and 1500 cm^{-1}, stemming from the NO_2 group, as well as signals at 1020 cm^{-1} (skeletal vibrations of furan).

Biological Tests

Nitrofuran derivatives show antibacterial activity against a wide spectrum of microorganisms, high kill rates, and low mammalian toxicity [1]. However, little is known about the details of their mode of action, particularly at the molecular level, in which a disruption of cytoplasmic membranes of bacteria or the inhibition of protein biosynthesis caused by nitrofurans occurs [11].

In studying the relationship between the nature of the linkage of nitrofuran compounds on polymeric carriers and the biological efficiency, the synthesized polymeric nitrofuran derivatives were used to carry out the biological tests. From the data of the agar plate method (Table 4), it is evident that all prepared polymeric nitrofuran derivatives were active against the tested organisms, whereas the initial polymers did not show any antimicrobial activity. These results are also confirmed by a suspension test using *Escherichia coli* as test organisms (Table 5), in which the polymeric nitrofurans destroyed nearly all model organisms within 1 day. Furthermore, examination of the hydrolysis of modified Resins Ic, IIc, and IIIb shows that within 300 h, no low molecular weight nitrofurans are released. To elucidate the presence of the nonspecific absorption of nitrofurans, mixtures of starting Polymers I or II with 4-bromobenzaldehyde and mixtures of III with 5-nitrofurfuryl alcohol were extracted with methanol. The extracted products did not contain absorbed 4-bromobenzaldehyde and 5-nitrofurfuryl alcohol, respectively, which was confirmed by elemental analyses and IR spectroscopy,

TABLE 4. The Effect of Polymeric Nitrofuran Derivatives on the Test Organism (agar plate test)[a]

Polymer	*E. coli*	*P. vulgaris*	*K. pneumoniae*	*E. cloacae*
I	–	–	–	–
Ib	+	+	+	+
Ic	+	+	+	+
IIa	–	–	–	–
IIc	+	+	+	+
III	–	–	–	–
IIIb	+	+	+	+

[a] –: No inhibition of growth of bacteria. +: No growth of bacteria.

TABLE 5. Effects of Polymeric Nitrofurans on *Escherichia coli* (suspension test)

Polymer	Survival rate P (%)[a] after			
	4 h	6 h	24 h	48 h
I	100	100	100	100
Ib	55	–	0.5	0
Ic	56	35	0.5	0
IIc	–	25	0.5	0
IIIb	63	–	0.5	0

[a]P = Quotient of the number of colony-forming units (CFU) in the presence of polymers to the number of CFU in the absence of the test substrate.

thus showing the efficiency of extractive separation of free nitrofuran compounds from polymer-bound nitrofurans.

The conclusion of our investigations is that nitrofuran compounds are antimicrobially active in their polymer-carrier-bound state. On the basis of the fact that immobilized agents are not able to pass membranes of organisms, these results suggest that a crucial step in the antimicrobial action of polymer-bound nitrofurans may be disruption of the cytoplasmic membranes of bacterias, followed by release of cytoplasma constituents.

CONCLUSIONS

New polymeric nitrofuran derivatives were synthesized by chemical modification of functional macroporous styrene–divinylbenzene copolymers with low molecular weight nitrofurans. The resins containing covalently bound nitrofurans showed antimicrobial activity. The results suggest that immobilized nitrofuran compounds are antimicrobially active and can be used as polymeric disinfectants.

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Received October 22, 1990
Revision received March 6, 1991