Structure of carbofuran in crosslinked starch matrix by ^{13}C n.m.r.: correlation of release and swelling kinetics with the dynamic behaviour of polymer chains*

Parshuram G. Shukla, Swaminathan Sivaram† and Baijayantimala Mohanty

Division of Polymer Chemistry, National Chemical Laboratory, Pune 411008, India

(Received 10 July 1991; revised 16 October 1991; accepted 16 December 1991)

A solid state ^{13}C cross-polarization/magic angle spinning n.m.r. study was carried out to elucidate the structure of carbofuran inside starch crosslinked by urea formaldehyde. It was confirmed that carbofuran is physically entrapped in the matrix. Linewidth measurements showed the maximum degree of crosslinking occurs for a urea to starch ratio of 0.6 (w/w). The observed trends in relaxation times showed that release and swelling phenomena are partly governed by the relaxation mechanism. This conclusion is similar to that obtained from *in vitro* release kinetics of carbofuran from crosslinked starch matrix.

(Keywords: controlled release carbofuran; solid state n.m.r.; spin-spin relaxation time; release and n.m.r. data correlation; starch–urea formaldehyde encapsulation)

INTRODUCTION

Starch is a naturally occurring polymer which is inexpensive and abundant. A number of modified starch derivatives such as starch xanthate, a calcium adduct and borate have been reported in the literature as encapsulating matrices for agrochemicals[1–5]. Crosslinked starches (waxy corn) with phosphate and adipate groups have been studied and compared with crosslinked pregelatinized starch[6]. It was concluded that crosslinked and modified waxy corn starches, whether pregelatinized or not, were unsuitable as hydrophilic matrices in sustained release theophylline formulations. It has also been observed that crosslinked waxy corn starches showed the same disintegrating properties as potato starch whereas crosslinked pregelatinized waxy corn starches showed better disintegrating properties than crosslinked starch[7]. Pregelatinized and crosslinked pregelatinized starches have been used as binding agents in the formulation of high quality lactose granules[8]. Starch has also been used after crosslinking in aldehyde–starch saturant laminating adhesives[9].

Starch crosslinked with urea formaldehyde (St-UF) has been studied in our laboratory as an encapsulating matrix for a variety of pesticides[10–12]. This matrix is suitable for both water soluble and insoluble pesticides as well as pesticides which are unstable in alkaline pH[13]. Release of the pesticide from the encapsulating matrix has been found to depend on the chemical structure of the polymer, the physical state of the encapsulant and the environment.

We have recently reported a detailed study of the chemical structure and dynamics of crosslinked starch using solid state ^{13}C cross-polarization/magic angle spinning (CP/MAS) n.m.r. spectroscopy[14]. In this paper we report the structure of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), a systemic carbamate pesticide, encapsulated in a crosslinked St-UF matrix and the relationship between molecular motion in crosslinked starch as measured by n.m.r. relaxation time [T_2(C)] with *in vitro* swelling and release kinetics.

EXPERIMENTAL

Materials

Carbofuran (Rallis India Ltd) was purified by a reported procedure[10]. Maize starch powder (Anil Starch Product Ltd) was obtained commercially. Urea (extra pure), formalin solution (37–40%) (S. D. Fine Chemicals Pvt Ltd, India) and formic acid (Loba Chemie Industries Co., India) were used as received. Spectral grade methanol was used for all u.v. measurements. Samples of crosslinked St-UF polymers were synthesized with different degrees of crosslinking[10]. The degree of crosslinking was expressed as the ratio of urea to starch (w/w). The urea to formaldehyde mole ratio was kept at 1:1.5 in all cases.

Encapsulation procedure

Encapsulation of carbofuran in St-UF matrices was carried out as follows[10]. The starch was gelatinized by heating the dispersion of starch powder in distilled water to ~80°C with mixing. Carbofuran was then added to this starch paste and stirred until uniform dispersion was

*NCL communication no. 5224

†To whom correspondence should be addressed

0032-3861/92/173611-05

Table 1 N.m.r. samples

Sample no.	Sample designation	Urea/starch ratio (w/w)
1	B0[a]	–
2	B1	0.2
3	B2	0.4
4	B3	0.6
5	B4	0.8
6	C3[b]	0.6

[a]Pure starch
[b]Contains 3% carbofuran

obtained. This was followed by slow addition of UF prepolymer which was obtained by relfuxing neutralized formalin with urea for 15–20 min. The pH of the mixture was then adjusted to ~3.5 by dropwise addition of dilute formic acid and the mixture was stirred for a further 5 min. After 30 min a rubbery mass was obtained, which was wet sieved through a 2000 μm (ASTM) stainless steel sieve. The wet granules obtained were dried in an air-draft oven at 50°C for 4 h. Dried granules of all samples of mesh size −2000 μm + 700 μm were ground in an analytical mill and the fraction −150 μm + 88 μm was generally used for n.m.r. analysis. The samples used for n.m.r. are listed in *Table 1*.

Analysis

The quantity of carbofuran incorporated in the matrix was estimated by a u.v. method using a u.v.-vis. spectrometer (Hitachi model 220). Swelling and release rate studies were carried out using procedures reported earlier[10].

I.r. spectra were recorded on a Perkin-Elmer 599B i.r. spectrometer. I.r. ν_{max}^{liquid} (cm^{-1}): 1720 (amide carbonyl), 3360 (N–H stretching), 2860–3000 (aromatic C–H stretching).

^1H n.m.r. spectra of 7-hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran (the hydrolysis product of carbofuran), carbofuran and extracted carbofuran, were recorded using a Varian FT-80 A n.m.r. spectrometer. Carbofuran (δ, ppm): 1.5 ($(CH_3)_2\overset{|}{C}-$); 2.9 ($NH-CH_3$); 3.0 (CH_2); 5.0 (NH) and 6.7 (aromatic proton). 7-Hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran (δ, ppm): 1.5 ($(CH_3)_2\overset{|}{C}-$); 3.0 (CH_2); 5.7 (OH) and 6.8 (aromatic proton).

The chemical composition of extracted carbofuran was confirmed by ^1H n.m.r. and elemental analysis. For carbofuran ($C_{12}H_{15}O_3N$), calculated (%): C, 65.19; H, 6.79; N, 6.33. Found (%): C, 65.28; H, 7.02; N, 5.96. For extracted carbofuran (%): C, 65.38; H, 6.88; N, 6.10.

^{13}C CP/MAS n.m.r. spectra were recorded using a Brüker MSL-300 n.m.r. spectrometer (75.5 MHz) with a CP/MAS accessory at 25°C. The sample (~200 mg) was contained in a cylindrical rotor made of zirconia and spun at 3 kHz. The contact time and repetition time were 2 ms and 5 s, respectively. The spectral width and data points were 27 kHz and 8 K, respectively. The ^1H field strength was 2.0 mT for both the CP and decoupling process. The number of accumulations was 160–200. ^{13}C chemical shifts were calibrated indirectly with reference to the higher field adamantane peak (29.5 ppm) relative to tetramethylsilane. The Hartmann–Hahn condition was matched by using adamantane. The experimental error for the chemical shifts was within ±0.1 ppm for broad peaks.

The spin–spin relaxation time (T_2) were obtained using a 90°-τ-180° pulse sequence, with the amplitude of the spin echo (occurring at 2τ) being measured as a function of pulse separation. Care was taken to ensure that there was no contribution to the T_2 decay from self-diffusion in field gradients caused by inhomogeneities in the static magnetic field. Plotting $A(t)/A(0)$ [where $A(t)$ and $A(0)$ are the signal amplitudes at delay times t and 0, respectively] *versus* delay time gave a straight line. The slope ($-1/T_2$) was calculated using the least-squares method.

RESULTS AND DISCUSSION

The i.r. spectrum of pure carbofuran shows distinct peaks at 1720 cm^{-1} (amide carbonyl), 3360 cm^{-1} (N–H stretching) and at 2860–3000 cm^{-1} (aromatic C–H stretching). The i.r. spectrum of extracted carbofuran from the St-UF matrix with urea/starch = 0.6 is identical to that of pure carbofuran, indicating there is no change in chemical structure upon encapsulation. Also, no

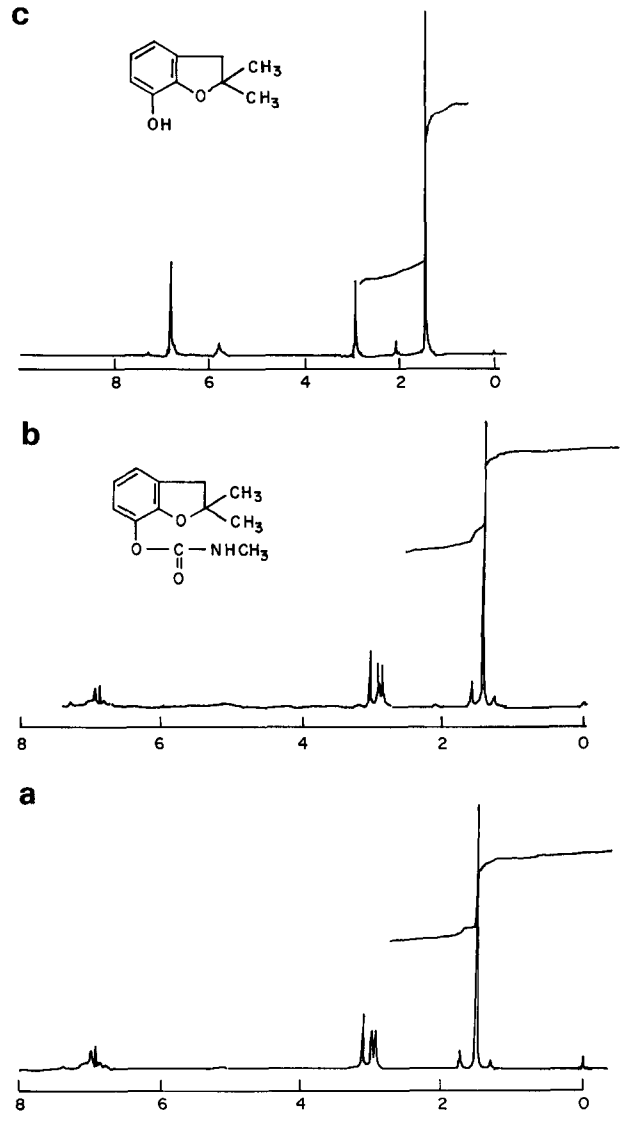

Figure 1 ^1H n.m.r. spectra of carbofuran (a), extracted carbofuran (b) and the hydrolysis product of carbofuran (c)

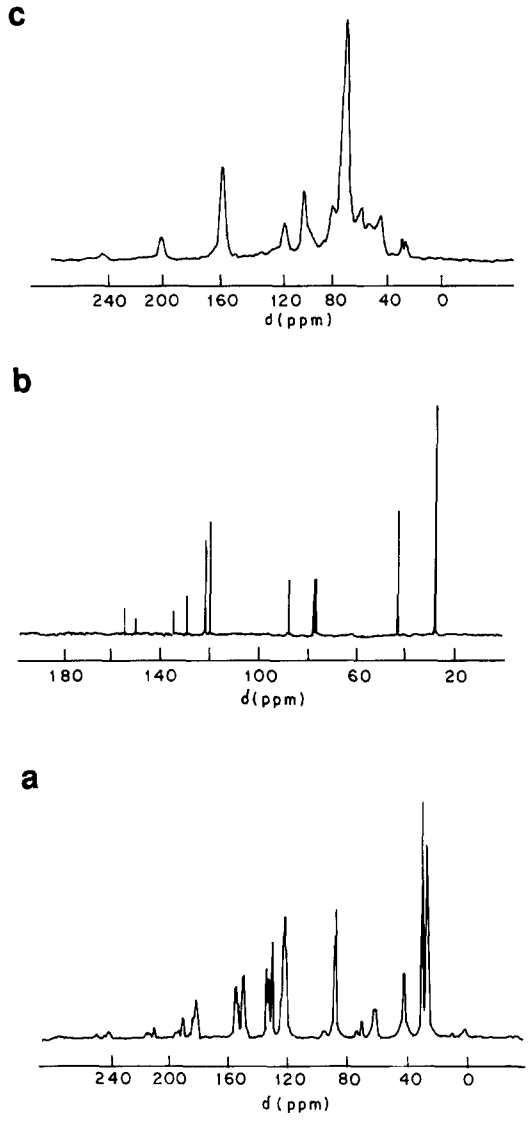

Figure 2 ^{13}C n.m.r. spectra of carbofuran in the solid state (a), in solution (b) and St-UF containing 3% carbofuran (c)

hydrolysis product could be detected. The ^{1}H n.m.r. spectra of extracted carbofuran, pure carbofuran and the hydrolysis product further support this observation (*Figure 1*).

Figure 2 shows the ^{13}C n.m.r. spectra of carbofuran (solid and solution) and 3% carbofuran in the St-UF matrix. The methine, methylene and methyl groups of carbofuran are observed separately by using the DEPT technique and are similar in the solid and solution spectra. The peaks due to carbofuran in *Figure 2c* are identical to those for pure carbofuran (both solid and solution) except at 150.6 ppm. This may be due to low carbofuran loading (3%) in the St-UF matrix.

The solid state ^{13}C n.m.r. chemical shifts of pure starch (B0), blank St-UF with urea/starch = 0.6 (B3) and 3% carbofuran loaded in the St-UF matrix with urea/starch = 0.6 (C3) are shown in *Figure 3* and the peak assignments are given in *Table 2*. *Figure 3a* shows the multiplicity at 98.7, 99.7, 100.9 and 102.3 ppm which is assigned to C-1 of starch as discussed earlier[14]. This is due to contributions from both crystalline and amorphous material in native maize starch[15–17]. The peaks at 61.4 and 71.8 ppm are due to C6 and all the other ring carbons (C-2, C-3 and C-5), respectively.

Crosslinked St-UF polymer (urea/starch = 0.6) shows a predictable spectrum except for the C-1 and C-6 carbons. The C-6 carbon is displaced upfield due to a chemical reaction occurring between the C-6 methylene group of starch with $HO-CH_2-NH-$ of UF resulting

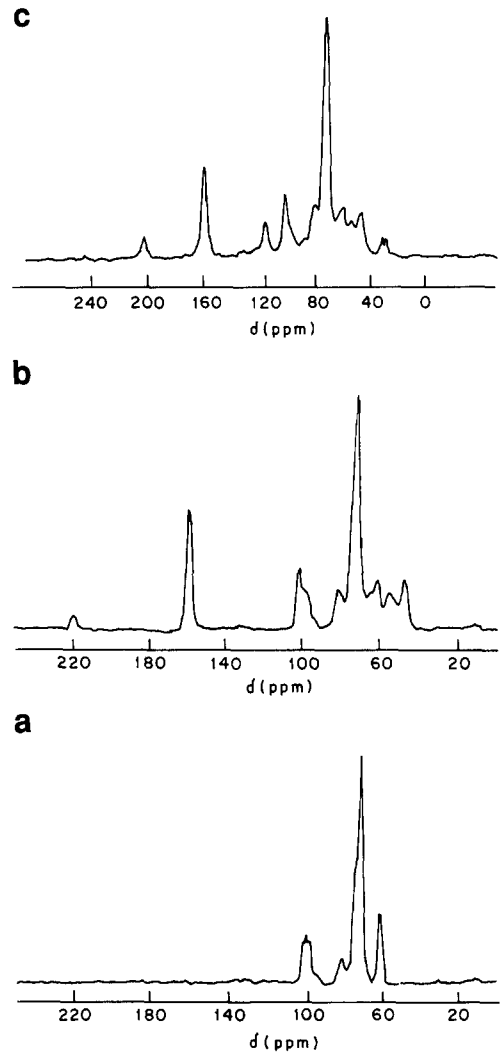

Figure 3 ^{13}C n.m.r. spectra of B0 (a), B3 (b) and C3 (c)

Table 2 ^{13}C n.m.r. chemical shifts of pure starch (B0), blank St-UF with urea/starch = 0.6 (B3) and St-UF containing 3% carbofuran with urea/starch = 0.6 (C3)

Carbon atoms	^{13}C n.m.r. chemical shifts (ppm)		
	B0	B3	C3
C-1[a]	102.3 100.9 99.7 98.7	102.1	102.0
C-2, C-3, C-5[a]	71.8	71.9	71.0
C-4[a]	81.2	81.5	81.0
C-6[a]	61.4	60.4	60.8
$=N-\underset{\underset{O}{\|}}{C}-N=$[b]	–	159.0	159.0
$-N(\|)-CH_2-NH-$[b]	–	54.3	54.4
$-NH-CH_2-NH-$[b]	–	46.7	47.0

[a] Chemical shifts of all carbons of starch are assigned from reference 17
[b] Chemical shifts of carbonyl and methylene groups of UF are assigned from reference 18

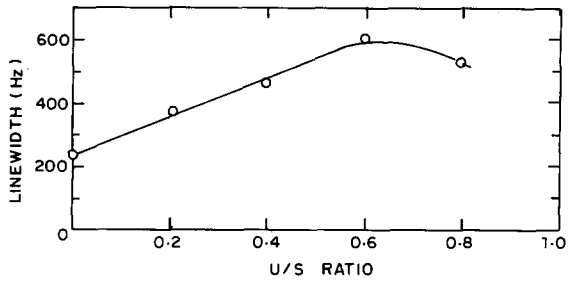

Figure 4 Effect of degree of crosslinking on linewidth measurements of C-6 of starch

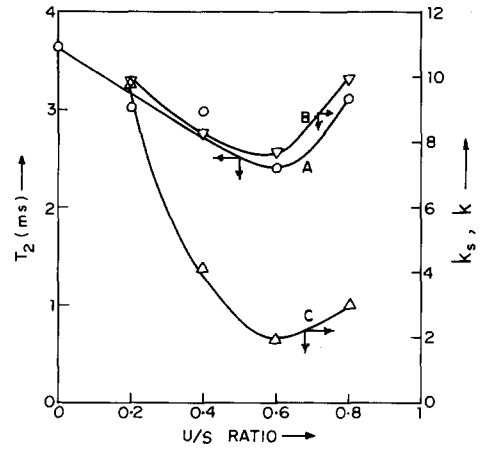

Figure 5 Correlation between T_2 of C-6 of starch (A), release rate constant (k) (B) and swelling rate constant (k_s) (C)

in (CH_2–O–CH_2) and (NH–CH_2) type linkages[14]. The chemical shift displacements at 46.7 and 54.3 ppm in *Figure 3b* are due to –NH–CH_2–NH– and

–N–CH_2–NH- linkages of UF condensates[18]. Sample C3 shows chemical shifts (*Figure 3c*) similar to that of UF crosslinked starch (*Figure 3b*, *Table 2*). This indicates that the blank St-UF and St-UF matrix containing 3% carbofuran are structurally similar.

The crosslinked polymer of starch with UF with various urea/starch ratios was quantitatively analysed by linewidth measurements. The linewidth plot of the primary hydroxyl group at C6 (in Hz) *versus* the urea/starch ratio is shown in *Figure 4*. The plot is linear except for sample B4. The line broadening observed in B1, B2 and B3 is consistent with the hypothesis that the C-6 carbon of starch exclusively takes part in a chemical reaction resulting in CH_2–O–CH_2 and CH–CH_2–NH linkages[14]. Similar line broadening has been observed during n.m.r. studies of a divinylbenzene crosslinked poly(*p*-chloromethylstyrene) system[19,20]. It is known that the reactivity of methylol derivatives of urea with glucose is higher compared to other carbohydrates[21]. However, the self condensation reaction of UF resin is suppressed in B1, B2 and B3 and the reaction between starch and UF predominates. In sample B4 with urea/starch = 0.8 the self condensation reaction of UF seems to predominate over the reaction of starch with UF. Thus, the maximum degree of crosslinking between starch and UF is achieved at urea/starch = 0.6.

It was therefore of interest to examine whether a relationship exists between T_2 of the C-6 carbon with release and swelling kinetics of starch matrix encapsulated carbofuran.

The dynamic behaviour of the St-UF matrices at various urea/starch ratios was visualized by measuring the T_2 of all carbons of starch (C-1–C-6)[14]. The T_2 value of C-6 is more significantly affected compared to other carbons (C-1–C-5). The T_2 values of C-6 of starch decrease monotonously as the urea to starch ratio increases up to 0.6 and then increases when the ratio is 0.8 (*Figure 5A*). This implies that the molecular motion of C-6 progressively decreases because of the increased crosslinking reaction up to urea/starch = 0.6. The further increase of T_2 at urea/starch = 0.8 indicates the presence of free methylol groups of starch which do not take part in the crosslinking reaction.

The St-UF polymers at different urea/starch ratios were used as encapsulating matrices for carbofuran and the release kinetics studied[10,11]. It has been found that water uptake by a hydrophilic St-UF polymer and release of an active agent from this polymer can be analysed by the generalized equations $(M_t/M_\infty)_s = k_s t^n$ and $M_t/M_\infty = kt^n$, respectively[22]. In the former $(M_t/M_\infty)_s$ is the fractional uptake of the penetrant (water) by polymer, t is the diffusion time, k_s is a constant characteristic of the polymer penetrant system and n is an exponent characteristic of the mode of transport of the penetrant. Similarly, in the latter equation, M_t/M_∞ is the fractional release of an active agent at time t, k is a characteristic of the solute–polymer system and n is the diffusional exponent characteristic of the release mechanism. In the case of the St-UF polymer in an aqueous environment, the release of carbofuran from the matrix is governed by a non-Fickian or anomalous mechanism. The non-Fickian mechanism occurs when the release of an active agent (or swelling of polymer) is partly governed by the diffusion of active agent through the swollen polymer (or diffusion of water in the polymer in the case of swelling) and the relaxation of polymer chains at the gel–glassy interface.

Thus, swelling and release characteristics of the St-UF polymer could be related to the molecular motions occurring within a crosslinked polymeric matrix. The overall molecular motion is restricted as the number of crosslinks increases. In the St-UF polymer, when the degree of crosslinking is increased from 0.2 to 0.6, both the swelling rate constant (k_s) and release rate constant (k) were reduced. However, at urea/starch = 0.8 an increase in these rate constants was observed (*Figure 5B* and *C*). The n value remained more or less constant with change in degree of crosslinking indicating that the release mechanism was unaffected by crosslinking. These observations are attributed to the fact that as the degree of crosslinking is increased, the polymer chains become more rigid. Consequently the diffusion of active agent through the polymer decreases as increasing crosslinking hinders the molecular motion of the polymer chains. N.m.r. measurements of St-UF also confirm that the maximum degree of crosslinking is achieved at urea/starch = 0.6.

CONCLUSIONS

Carbofuran does not undergo any chemical reaction in the process of encapsulation as observed by ^{13}C n.m.r. studies. The linewidth measurements showed a maximum degree of crosslinking at urea/starch = 0.6. T_2(C) measurements showed that the relaxation of polymer chains progressively reduces up to urea/starch = 0.6 but

again increases at urea/starch = 0.8. T_2(C) measurements indicate that release and swelling phenomena are partly governed by the relaxation mechanism (non-Fickian). This fact is also confirmed by *in vitro* release kinetics.

ACKNOWLEDGEMENT

We are grateful to Drs S. Ganapathy and P. R. Rajamohanan for many useful discussions and for the n.m.r. measurements.

REFERENCES

1 Shasha, B. S., Doane, W. M. and Russell, C. R. *J. Polym. Sci., Polym. Lett. Edn* 1976, **14**, 417
2 Doane, W. M., Shasha, B. S. and Russell, C. R. *Am. Chem. Soc. Symp. Ser.* 1977, **53**, 74
3 Stout, E. I., Shasha, B. S. and Doane, W. M. *J. Appl. Polym. Sci.* 1979, **24**, 153
4 Shasha, B. S., Trimnell, D. and Otey, F. H. *J. Polym. Sci., Polym. Chem. Edn* 1981, **19**, 1891
5 Shasha, B. S., Trimnell, D. and Otey, F. H. *J. Appl. Polym. Sci.* 1984, **29**, 67
6 Visavarungraj, N., Herman, J. and Remon, J. P. *Drug Dev. Ind. Pharm.* 1990, **16**(7), 1091
7 Visavarungraj, N. and Remon, J. P. *Int. J. Pharm.* 1990, **62**, 125
8 Visavarungraj, N., Herman, J. and Remon, J. P. *Int. J. Pharm.* 1990, **59**, 73
9 Romz, T. A., Mohler, P. J. and Baind, L. *Eur. Pat. Appl. EP 323691*, 1989
10 Shukla, P. G., Rajagopalan, N., Bhaskar, C. and Sivaram, S. *J. Control. Rel.* 1991, **15**, 153
11 Rajagopalan, N., Shukla, P. G., Bhaskar, C., Bankar, V. S., Dharia, J. R. and Khilar, K. C. *J. Appl. Polym. Sci.* in press
12 Shukla, P. G., Rajagopalan, N. and Sivaram, S. *J. Appl. Polym. Sci.* submitted
13 Bhaskar, C., Shukla, P. G., Rajagopalan, N. and Mitra, R. B. *Ind. Pat. Appl. 881/DEL/87*, 1987
14 Shukla, P. G., Sivaram, S. and Mohanty, B. *Macromolecules* in press
15 Dias, P. and Perlin, A. S. *Carbohydr. Res.* 1982, **100**, 103
16 O'Donnell, D. J., Ackerman, J. J. H. and Maciel, G. E. *J. Agric. Food Chem.* 1981, **29**, 514
17 Gidley, M. J. and Brociek, S. M. *J. Am. Chem. Soc.* 1985, **107**, 7040
18 Maciel, G. E., Szeverenyi, N. M., Early, T. A. and Myers, G. E. *Macromolecules* 1983, **16**, 598
19 Mohanraj, S. and Ford, W. T. *Macromolecules* 1985, **18**, 351
20 Ford, W. T. and Balakrishnan, T. *Macromolecules* 1981, **14**, 284
21 Azarov, V. L. and Kononov, G. N. *Nanchn Tr.-Mosk Lesolekh Inst.* 1982, **143**, 85; *Chem. Abstr.* 1983, **99**, 106951r
22 Ritger, P. L. and Peppas, N. A. *J. Control. Rel.* 1987, **5**, 37