Synthesis, Characterization, and Antibacterial Activity of Metal-Containing Polyurethanes

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ABSTRACT: Metal salts of mono(hydroxyethoxyethyl)-phthalate [M(HEEP)$_2$] (M = Ca^{2+}, Cd^{2+}, and Pb^{2+}) were synthesized by the reaction of diethylene glycol, phthalic anhydride, and metal acetates. A series of metal-containing polyurethanes (PUs) were synthesized by the reaction of hexamethylene diisocyanate or tolylene 2,4-diisocyanate with Ca^{2+}, Cd^{2+}, and Pb^{2+} salts of mono(hydroxyethoxyethyl)phthalate using di-*n*-butyltin dilaurate as a catalyst. The PUs were well characterized by FTIR, ^{1}H, and ^{13}C NMR, solid-state ^{13}C-CP-MAS NMR, viscosity, solubility, elemental, and X-ray diffraction studies. Thermal properties of the polymers were also studied by using thermogravimetric analysis and differential scanning calorimetry. The antibacterial activities of these polyurethanes have also been investigated by using the agar diffusion method. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 288–295, 2004

Key words: polyurethanes; thermal properties; viscosity

INTRODUCTION

Studies on polymers containing metals in the main chains are important from the scientific and industrial view points. Difunctional compounds containing ionic bonds are starting materials for preparing the ionic polymers.[1] Incorporation of metal and functional groups into the polymers has led to wide applications such as aqueous thickeners, impregnates, textile sizers, adhesives, additives, resins, and catalysts.[2–6] Metallo polymers containing metals in the backbones of the polymer chains have been prepared.[7,8] Metal-containing polymers with ionic links formed between COO$^-$ and M^{++}, in the backbone have been studied.[9–25] Metal salts of mono(hydroxyethoxyethyl)-phthalate M[HEEP]$_2$ contain the ionic metal linkages and two hydroxyl groups. These are of interest and are useful as difunctional ionic monomers in which metal is firmly incorporated in the backbone. The resulting metal-containing polyurethanes will possess the ionic links, and hence, are expected to have applications in adhesives, coatings, and biomedical fields.[26,27]

It is important to understand the interactions between microbes and heavy metals, their transformation pathways, and resistance mechanism owing to the extensive use of some metals and metal compounds as fungicides, disinfectants, and catalyst in various industries. The Gram-positive and Gram-negative bacteria's have frequently exhibited the resistance to some toxic metals such as mercury, arsenic, and cadmium.[28–30] Metal-containing monomeric and polymeric particles with antibacterial activity has been patented.[31–33]

The present article deals with the synthesis, characterization, and antibacterial activity of metal-containing PUs derived from calcium, cadmium, and lead salts of mono(hydroxyethoxyethyl)phthalate with hexamethylene diisocyanate (HMDI) or tolylene 2,4-diisocyanate (TDI).

EXPERIMENTAL

Materials

Phthalic anhydride (BDH), diethylene glycol (Merck), di-*n*-butyltin dilaurate (DBTDL) (Fluka), HMDI, and TDI (Fluka) and metal acetates (BDH) were used as received without any purification. The solvents such as acetone, methanol, dimethyl formamide (DMF), dimethyl sulphoxide (DMSO), dimethyl acetamide (DMAc), benzene, toluene, *m*-cresol, and chloroform were purified by standard procedures. The M(HEEP)$_2$s were synthesized as reported in our pervious article.[34] The chemical structure of M(HEEP)$_2$ is shown in Figure 1. The organisms such as *Escherichia coli*, *Pseudomonas fluorescence*, *Streptococcus* sp., and *Salmonella* sp. were isolated from different environmental domains.

HO(CH₂)₂O(CH₂)₂OOC   COOMOOC   COO(CH₂)₂O(CH₂)₂OH

M(HEEP)₂
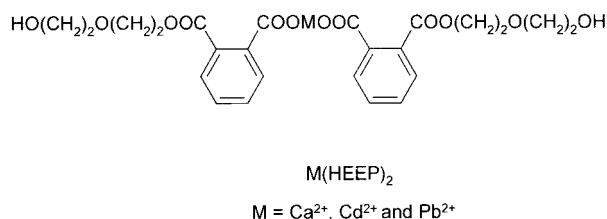
M = Ca²⁺, Cd²⁺ and Pb²⁺

Figure 1 Chemical structure of M(HEEP)₂.

Synthesis of PUs

The metal-containing monomer, M[HEEP]₂ (M = Ca²⁺, Cd²⁺, and Pb²⁺) (0.02 mol) dissolved in DMF (100 mL) was taken in a three-necked round-bottom flask fitted with a water cooled condenser, a nitrogen inlet tube, and a dropping funnel. The solution was heated in an oil bath and the temperature was maintained at 90°C and stirred well using a magnetic stirrer. To this solution, was added 2–3 drops of DBTDL as the catalyst, followed by 0.02 mol of diisocyanates (HMDI or TDI) dissolved in 50 mL of DMF over periods ranging from 25 to 45 min, depending on the diisocyanates used. After the addition of diisocyanate the reaction mixture was stirred continuously at the same temperature for 4 h. The reaction mixture was diluted with 50 mL of DMF and kept overnight. Then

the solution was filtered and the filtrate was treated with methanol to precipitate the polymer. The product obtained was washed thoroughly with water and then methanol. Finally, the product was dried in vacuum at 50°C for 10 h. The reactions involved in the polymer synthesis are given in Scheme I. The synthesis data of PUs are given in Table I.

Antibacterial activity

The metal-containing PUs were screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas fluorescence*, *Streptococcus* sp., and *Salmonella* sp. The antibacterial activity was evaluated using the agar diffusion method.[35] One loopful of bacteria was inoculated in 10 mL of nutrient broth (peptone 5 g/L, pH 6.8) and incubated at 37°C for 28 h in a test tube shaker at 100 rpm. 20 mL of nutrient agar was poured into sterile petriplates and allowed to solidify at room temperature. After solidification 0.1 mL of the bacterial culture was spread on the nutrient agar. A circular well (9 mm) was made at the center of each petriplate with a sterilized steel borer. The polyurethane test samples were prepared using DMSO as the solvent at a concentration of 20 μg/mL. Then 0.1 mL of each test solution was added to the well and incubated at about

M(HEEP)₂ + 2OCN-R-NCO $\xrightarrow[\text{DMF , DBTDL}]{90^0C}$

⎡O(CH₂)₂O(CH₂)₂OOC   COOMOOC   COO(CH₂)₂O(CH₂)₂OCNH—R—NH—C⎤

R = -(CH₂)₆- ,   —(benzene ring with CH₃ groups)—CH₃   M = Ca²⁺, Cd²⁺ and Pb²⁺

I   II
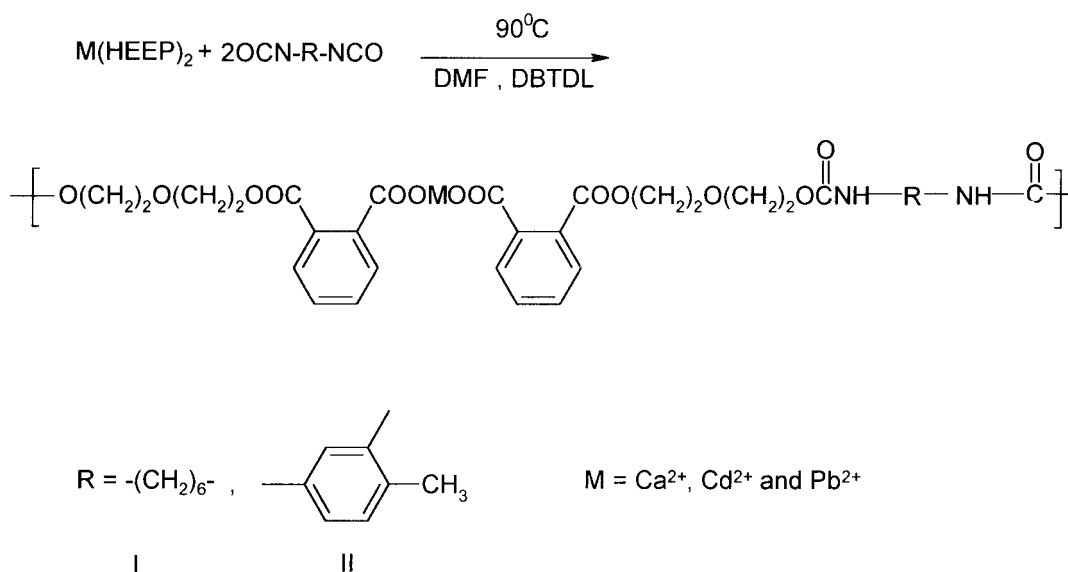
Polymer	I	II	III	IV	V	VI
R	I	I	I	II	II	II
M	Ca	Cd	Pb	Ca	Cd	Pb

Scheme 1 Synthesis of metal-containing PUs.

TABLE I
Synthesis, Viscosity, and Analytical Data of Metal-Containing PUs

Polymer no.	Polymer code	Yield (%)	η_{inh}	Repeating unit (formula)	Analytical data found Calculated (%)		
					C	H	M
I	Ca(HEEP)$_2$-HMDI	92	0.0764	CaC$_{32}$H$_{36}$N$_2$O$_{14}$	54.81 (53.93)	5.12 (5.09)	5.73 (5.62)
II	Cd(HEEP)$_2$-HMDI	89	0.0755	CdC$_{32}$H$_{36}$N$_2$O$_{14}$	49.05 (48.96)	4.73 (4.62)	14.40 (14.32)
III	Pb(HEEP)$_2$-HMDI	90	0.0795	PbC$_{32}$H$_{36}$N$_2$O$_{14}$	43.81 (43.69)	4.19 (4.13)	23.67 (23.55)
IV	Ca(HEEP)$_2$-TDI	90	0.0681	CaC$_{33}$H$_{32}$N$_2$O$_{14}$	55.12 (55.00)	4.52 (4.48)	5.63 (5.56)
V	Cd(HEEP)$_2$-TDI	86	0.0663	CdC$_{33}$H$_{32}$N$_2$O$_{14}$	50.10 (49.99)	4.13 (4.07)	14.25 (14.18)
VI	Pb(HEEP)$_2$-TDI	87	0.0675	PbC$_{33}$H$_{32}$N$_2$O$_{14}$	47.70 (44.65)	3.71 (3.63)	23.38 (23.34)

37°C for 24 h. After incubation the zones of inhibition were measured and were represented as (−), (+), (++), and (+++) depending upon the diameter and clarity.

Measurements

The IR spectra of the polymers were recorded in a Testscan Shimadzu FT-IR 8000 series spectrophotometer at room temperature with the KBr pellet method. The ^{1}H and ^{13}C NMR and DEPT spectra of the polymers were recorded in a JEOL GSX-400 MHz spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. The solid-state ^{13}C-CP-MAS-NMR spectra were obtained using a Bruker MSL 75 MHz spectrometer with adamantine as a chemical shift reference. The thermogravimetric analysis (TGA) was performed with a Mettler-3000 thermal analyzer using 2 mg of the sample at a heating rate of 20°C/min in air. The differential scanning calorimetric analysis was carried out using a DSC V4.OB Dupont 2100 model differential scanning calorimeter at a heating rate of 10°C/min under a nitrogen atmosphere. The X-ray diffractograms were recorded according to a powder method with a Philips PW 1710 diffractometer using CuK$_\alpha$ radiation. A Perkin-Elmer 2400 carbon-hydrogen analyzer was used for elemental analysis. The inherent viscosity η_{inh} of the polymers in DMSO was determined using an Ubbelohde-viscometer at 40°C. The flow time for the solvent as well as the polymer solution (1.0 g/dL) was determined. The solubility of the polymers was tested in various polar and nonpolar solvents by taking 10 mg of the polymers in 2 mL of different solvents in a closed test tube and set aside for 1 day.

RESULTS AND DISCUSSION

Synthesis of PUs

The M[HEEP]$_2$s were insoluble in in most of the organic solvents and the polymerization of these salts with diisocyanates had to be done in DMF or DMSO, which dissolved M[HEEP]$_2$ and the resulting polymers. The reaction of diisocyanates with diols cata-

lyzed by DBTDL takes place via the formation of ternary complex between the catalyst and the reagents.[36] In the synthesis of PUs, the mol ratio of diisocyanate/diol was taken as 1 : 1, so as to avoid side reactions such as the ring formation of dimeric and trimeric diisocyanates, branching, and crosslinking due to the formation of allophanate linkages. Any crosslinked product formed was filtered off after mixing the product with excess of DMF to dissolve the linear polymers. Subsequently, the dissolved linear polymers were reprecipitated by the addition of nonsolvent. The yields of the PUs were good in all cases. Using Ca[HEEP]$_2$, Cd[HEEP]$_2$, and Pb[HEEP]$_2$ six metal-containing PUs were prepared based on HMDI or TDI. They are coded as Ca[HEEP]$_2$–HMDI (**I**), Cd-[HEEP]$_2$–HMDI (**II**), Pb[HEEP]$_2$–HMDI (**III**), Ca-[HEEP]$_2$–TDI (**IV**), Cd[HEEP]$_2$–TDI (**V**), and Pb-[HEEP]$_2$–TDI (**VI**).

The PUs were soluble in aprotic polar solvents like DMSO, DMF, and DMAc, but insoluble in water, acetone, ethyl methyl ketone, ethyl acetate, toluene, benzene, xylene, dioxane, chloroform, carbon tetrachloride, carbon disulphide, n-hexane, and tetrahydrofuran. The elemental analysis data of PUs are given in Table I. The elemental analysis data reveals that the percentage values of carbon, hydrogen, and metal content are well within the range of calculated values.

Characterization of PUs

FTIR spectra

The peak at 3335–3328 cm^{-1} is due to N—H stretching. The carbonyl stretching frequency due to the urethane groups is seen at 1686–1640 cm^{-1}. The peaks between 1608–1550 cm^{-1} are attributed to the aromatic C=C stretching. The carboxylate ion gives two broad peaks between 1565–1545 and 1470–1420 cm^{-1}. The N—H bending modes are observed at 1628–1620 cm^{-1}. The C—H out of plane bending vibrations of aromatic system is seen at 770–748 cm^{-1}.

^{1}H NMR spectra

The ^{1}H NMR spectra of PUs (**I** and **IV**) are shown in Figure 2. The spectra show signals for N—H proton of
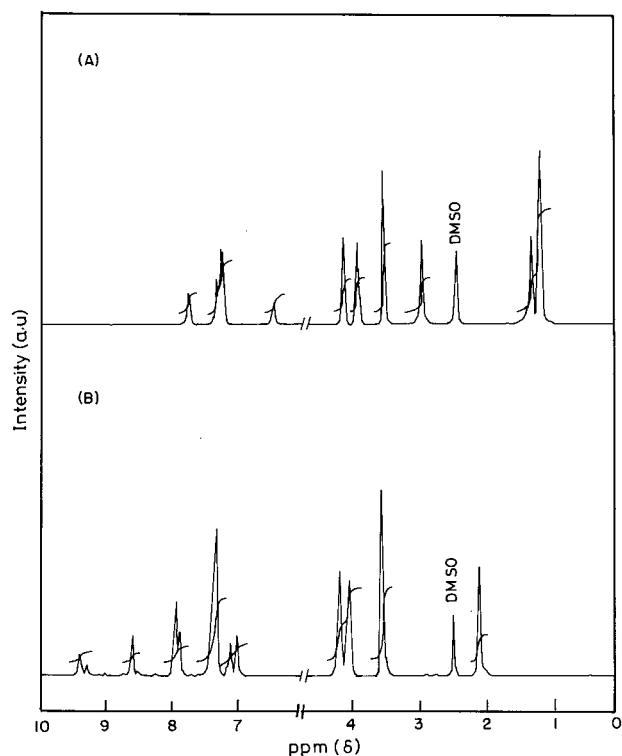
Figure 2 ¹H NMR spectra of PUs: (A) I and (B) IV.

the urethane group around 9.44–8.46 ppm for TDI-based PUs and at 6.56–6.52 ppm for HMDI-based PUs. The aromatic protons of the TDI-based PUs show resonance signals at 7.96–7.04 ppm, while the HMDI-based PUs show the resonance signals of aromatic protos at 7.74–7.32 ppm. The methyleneoxy groups attached to —COPh and —CONH show signals at 4.24–4.22 and 4.01–3.99 ppm, respectively. The methylene group attached to the —NH group of HMDI-based PUs shows a signal at 3.03–3.00 ppm. The peak at 3.56–3.54 ppm is due to —CH₂OCH₂— group. The peak at 2.13 ppm is due to the methyl groups attached to the aromatic rings. The other methylene groups are observed between 1.47 and 1.26 ppm.

¹³C NMR spectra

The ¹³C NMR spectra of PUs (**I** and **IV**) are shown in Figure 3 and 4. The peak at 170.76–170.64 ppm is due to the carbonyl carbon of the salt and that at 168.81–168.78 ppm is due to the carbonyl carbon of the ester group. The HMDI-based PUs show a peak at 156.12–156.05 ppm due to the carbonyl carbon of the urethane group while TDI- based PUs show the corresponding peaks at 154.15 and 153.48 ppm. The aromatic carbons of HMDI-based PUs show peaks at 136.68–125.74 ppm, while those of TDI- based PUs show peaks at 137.15–115.02 ppm. The peak at 68.71–68.06 ppm is due to the —CH₂OCH₂— group. The peaks between 64.32–64.12 and 63.54–63.31 ppm are due to the methylene carbons attached to —OCOPh and —OCONH, respectively. The signal due to the methylene group attached to the —NH group is overlapped with DMSO solvent peak. The other methylene carbons show peak at 32.25 and 26.62 ppm, respectively. The methyl car-
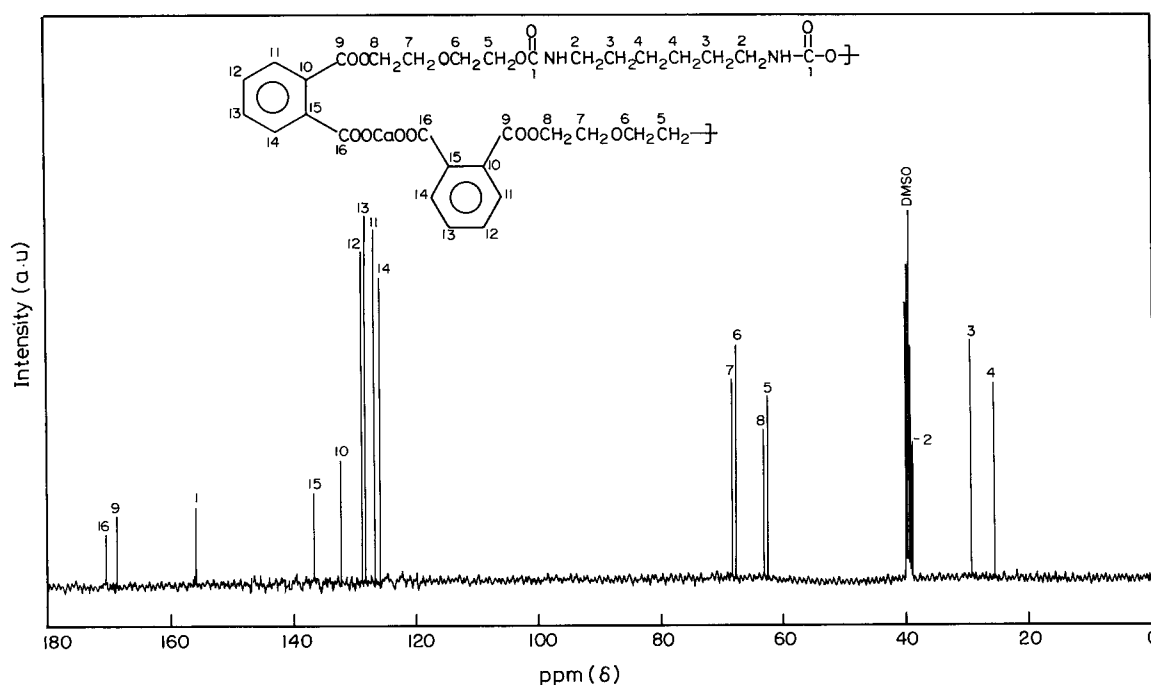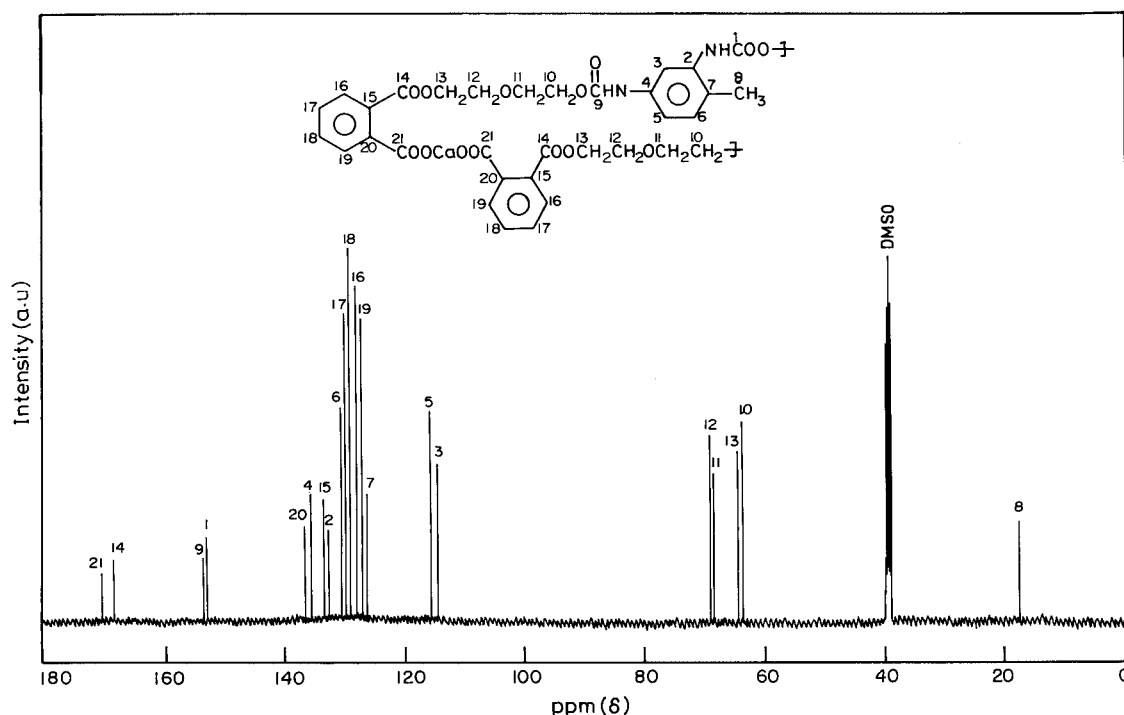

Figure 3 ¹³C NMR spectrum of Ca(HEEP)₂–HMDI (I).

Figure 4 ^{13}C NMR spectrum of Ca(HEEP)$_2$–TDI (IV).

bon attached to the aromatic ring of TDI-based PUs shows a signal at 17.32 ppm.

Solid-state ^{13}C-CP-MAS NMR spectra

Figure 5 shows the ^{13}C-CP-MAS NMR spectra of PUs **I** and **IV**. The broad peak at 177.17–177.12 ppm is due to the carbonyl carbon of the carboxylate and ester groups of TDI-based PU. The HMDI-based polymer shows the corresponding peak at 178.85–178.40 ppm. The carbonyl carbons of the urethane groups show peaks at 159.23 and 158.20 ppm for HMDI and TDI-based PUs, respectively. The aromatic carbons of the HMDI-based PUs show a broad peak between 138.53 and 134.10 ppm, while those of TDI-based PUs show the corresponding peak at 137.55–118.24 ppm. The methylenoxy groups attached to —COPh and —CONH groups show peaks at 60.16 and 60.23 ppm for HMDI and TDI-based PUs, respectively. The peak at 70.51–70.43 ppm is due to —CH$_2$OCH$_2$— group. The methylene carbon attached to the —NH group shows a peak at 44.15–44.10 ppm for HMDI-based PUs, but the corresponding peak is not observed in the ^{13}C NMR spectra of liquid samples as it is hidden with the DMSO peaks. The other methylene carbons of HMDI-based PUs show a broad peak around 28.29–28.22 ppm. The methyl carbon of TDI-based PUs shows a peak at 19.84 ppm.

Inherent viscosity

The inherent viscosity data are given in Table I. The inherent viscosities of metal-containing PUs are con-

siderably lower than those of metal-free analogs. This is generally the case for all metal-containing PUs and poly(urethane-urea)s.[20,21,23–25] The lowed viscosity may be attributed to the reduction in molecular weights of this PUs on dissolution due to the partial reversible dissociation of the metal-oxygen bonds. It should be noted that the HMDI-based PUs show higher inherent viscosities than the TDI-based PUs. This may be due to the fact that the chain lengths would be longer for the HMDI-based polymers than those for the TDI-based polymers for given molecular weights of the fragments.

Thermal properties

The TGA data of PUs are given in Table II. The initial decomposition temperatures (IDTs) of the PUs are in the range between 181 and 205°C, which are lower than those of blank PUs. However, their decomposition rates are less than those of blank PUs. This result indicates that the metal acts as a catalyst and facilitates the first stage decomposition but retards the rate of decomposition on further stages. All the PUs show multiple-stage decompositions. The TDI-based PUs show higher thermal stability than the HMDI-based PUs. This may be due to the stiff phenylene rings present in the main chains. The residual weights at 800°C correspond to metal oxides (CaO, CdO, and PbO) formed for the PUs based on Ca(HEEP)$_2$, Cd-(HEEP)$_2$, and Pb(HEEP)$_2$.

The glass transition temperature (T_g) data are given in Table II. The T_g values of the metal-containing PUs
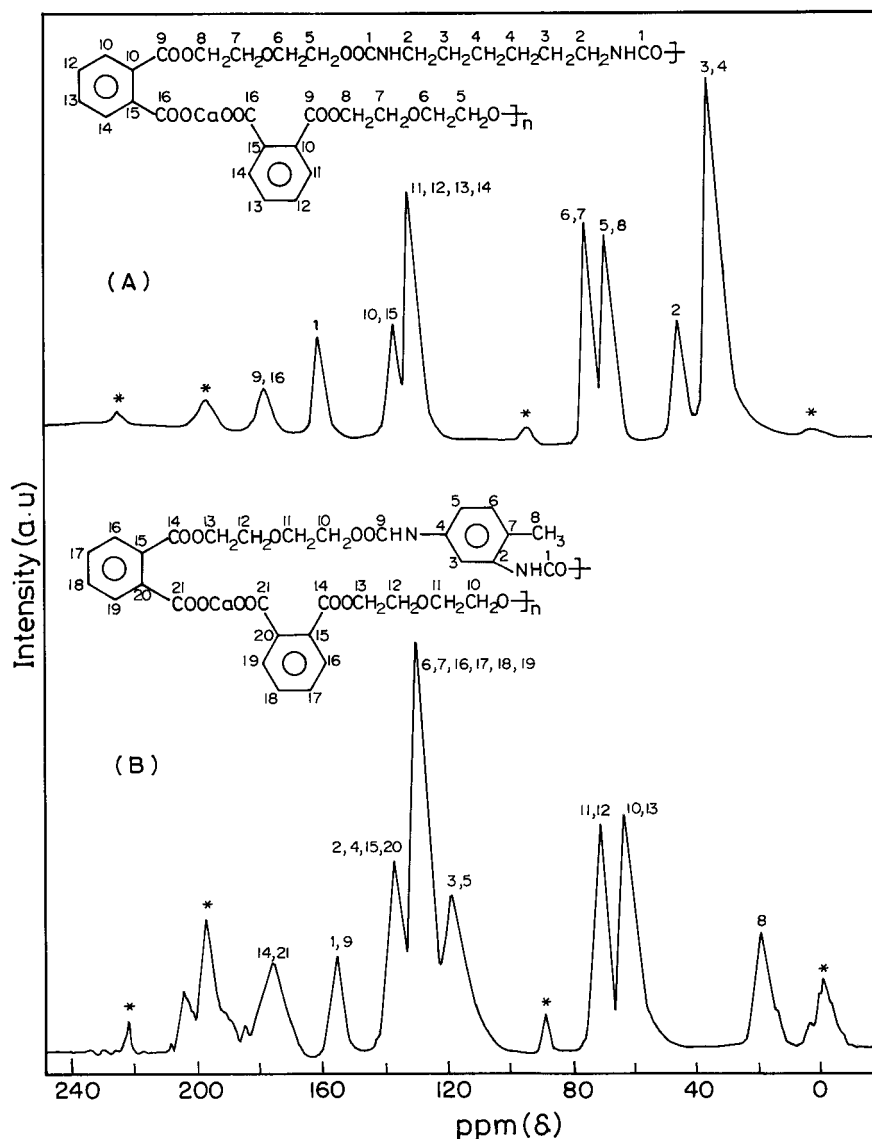
Figure 5 Solid-state ^{13}C-CP-MAS NMR spectra of PUs: (A) I and (B) IV.

range from 89.8 to 101.2°C. All the PUs showed single T_g values. This may be due to the absence of formation of mixture of homopolymer or a block copolymer. This may also be due to the absence of heterogeneous mixing of hard and soft segments in the polymer matrix. The T_g values of HMDI-based polymers are slightly lower than those for the corresponding TDI-based polymers. The T_gs of HMDI-based polymers are expected to be much less considering the flexibility due to the hexamethylene group.

X-ray diffraction

Figure 6 shows the XRD patterns of the PUs (**I, II, IV,** and **V**). The XRD patterns show that the HMDI-based PUs are partially crystalline, whereas TDI-based PUs are amorphous in nature. This may due to the folding of hexamethylene group present in the back-bone of the PUs. The crystalline nature of HMDI-based polymers was also confirmed by the solubility study of the PUs in DMSO. The TDI-based PUs was easily soluble in DMSO than the HMDI-based PUs.

TABLE II
Thermal Data of Metal-Containing PUs

Polymer no.	T_g (°C)	IDT (°C)	Weight loss at 800°C (%)
I	92.8	181	93.5
II	89.8	184	83.7
III	93.5	191	77.1
IV	99.7	198	91.5
V	95.8	193	83.3
VI	101.2	205	74.0

Antibacterial activity

The antibacterial activities of the metal-containing PUs were identified based on the zone of inhibition for bacterial growth around the wells. The results of activity were expressed as inactive, mild, moderate, and high. The results are summarized in Table III. The results reveal that all the compounds exhibited antibacterial activities to a certain extent.

Cd[HEEP]$_2$–TDI exhibited the highest antibacterial activities against *Pseudomonas fluorescence* (24 mm). Cd[HEEP]$_2$–HMDI, Pb[HEEP]$_2$–HMDI, and Pb-[HEEP]$_2$–TDI, exhibited moderate activities against *Pseudomonas fluorescence* (19 and 18 mm). The other PUs showed mild activities against the *Pseudomonas fluorescence*. Cd[HEEP]$_2$–HMDI and Cd[HEEP]$_2$–TDI showed the highest antibacterial activities against *E. coli* (28 and 26 mm). Pb[HEEP]$_2$–HMDI and Pb-[HEEP]$_2$–TDI, exhibited moderate activities against *E. coli* (17 and 19 mm). The other PUs showed mild activities against the *E. coli*.

Cd[HEEP]$_2$–HMDI, Pb[HEEP]$_2$–HMDI, Cd[HEEP]$_2$–TDI, and Pb[HEEP]$_2$–TDI showed the highest antibacterial activities against *Streptococcus* sp. (29, 27, 28, and 26 mm). Ca[HEEP]$_2$–HMDI showed mild activities against the *Streptococcus* sp. Ca[HEEP]$_2$–TDI showed inactivity against the *Streptococcus* sp. Cd[HEEP]$_2$–HMDI, Cd-[HEEP]$_2$–TDI, and Pb[HEEP]$_2$–TDI showed the highest antibacterial activities against *Salmonella* sp. (28, 29, and 26 mm). Pb[HEEP]$_2$–HMDI showed moderate activity against the *Salmonella* sp.(17 mm). The calcium-containing PUs showed mild activities against the *Salmonella* sp.
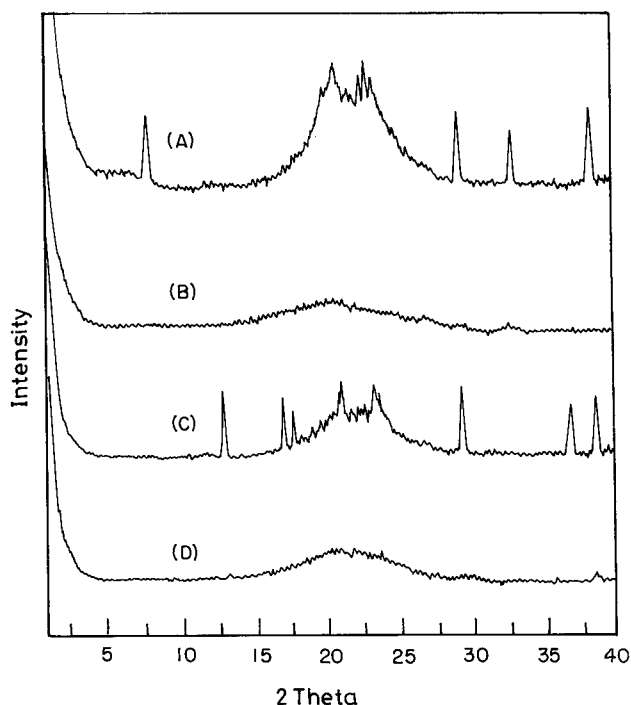
TABLE III
Antibacterial Activity Data of Metal-Containing PUs

Polymer no	*Pseudomonas flourescence*	*E. coli*	*Salmonella* sp.	*Streptococcus* sp.
I	+	+	+	+
II	++	+++	+++	+++
III	++	++	++	+++
IV	+	+	+	−
V	+++	+++	+++	+++
VI	++	++	+++	+++

− = Inactive (0–10 mm); + = mild active (10–15 mm); ++ = moderately active (16–20 mm); +++ = highly active (21–30 mm).

It has been shown that calcium ions do not exhibit any antibacterial activity.[18,23] However, the PUs containing calcium ions showed some antibacterial activities. This result reveals that their antibacterial activities may not be entirely due to the metal ions, and partially due to their isocyanate groups.

CONCLUSIONS

Metal-containing PUs were prepared by reacting 1 : 1 mixtures of M(HEEP)$_2$ and HMDI or TDI. The HMDI-based PUs showed higher inherent viscosities than TDI-based PUs. The TDI-based PUs showed higher thermal stabilities than HMDI-based PUs. The XRD pattern data showed that the HMDI-based PUs are partially crystalline and TDI-based PUs are amorphous in nature. The cadmium-containing PUs exhibited strong inhibitory effects on all the bacteria, but the PUs containing other metals exhibited different antibacterial activities depending on the bacterium. All the metal-containing PUs was found to exhibit antibacterial activities and may be used as antibacterial coatings.

Figure 6 XRD patterns of PUs: (A) I, (B) IV, (C) II, and (D) V.

References

1. Matsuda, H. J Polym Sci Part A Polym Chem Ed 1974, 12, 455.
2. Matsuda, H.; Kanaoka, K. J Appl Polym Sci 1985, 30, 1229.
3. Matsuda, H. J Appl Polym Sci 1979, 23, 2603.
4. Matsuda, H.; Takechi, S. J Polym Sci Polym Chem Ed 1990, 28, 1895.
5. Matsuda, H. J Appl Polym Sci 1978, 22, 2093.
6. Matsuda, H. J Polym Sci Part A Polym Chem Ed 1977, 15, 2239.
7. Zhenan, B; Yongming, C.; Luping, Y. Macromolecules 1994, 27, 4629.
8. Jong-Chen, L.; Akrnori, N.; Ikugoshi, T.; Takeshi, E. Macromolecules 1997, 30, 5205.
9. Matsuda, H. J Appl Polym Sci 1978, 22, 3371.
10. Matsuda, H. J Polym Sci Part A Polym Chem Ed 1974, 12, 469.
11. Matsuda, H. J Polym Sci Part A Polym Chem Ed 1974, 12, 2419.
12. Jayakumar, R.; Rajkumar, M.; Nagendran, R.; Nanjundan, S. J Macromol Sci Pure Appl Chem 2001, A35, 821.
13. Matsuda, H. J Macromol Sci Pure Appl Chem 1975, A10, 1143.
14. Matsuda, H. J Appl Polym Sci 1976, 20, 995.
15. Durairaj, B.; Venkata Rao, K. Polym Bull 1979, 1, 723.

16. Durairaj, B.; Venkata Rao, K. Eur Polym J 1980, 16, 941.
17. Jayakumar, R.; Arun Prasath, R.; Radhakrishnan, S.; Nanjundan, S. J Macromol Sci Pure Appl Chem 2002, A39, 853.
18. Jayakumar, R.; Rajkumar, M.; Nagendran, R.; Nanjundan, S. J Appl Polym Sci 2002, 85, 1194.
19. Rajalingam, P.; Radhakrishnan, G. Polymer 1992, 33, 2214.
20. Arun Prasath, R.; Nanjundan, S. Eur Polym J 1999, 35, 1939.
21. Arun Prasath, R.; Nanjundan, S. J Macromol Sci Pure Appl Chem 1998, A35, 821.
22. Arun Prasath, R.; Jayakumar, R.; Nanjundan, S. J Macromol Sci Pure Appl Chem 2000, A37, 469.
23. Jayakumar, R.; Lee, Y.-S.; Nanjundan, S. React Funct Polym 2003, 55, 267.
24. Jayakumar, R.; Lee, Y.-S.; Nanjundan, S. Int J Polym Anal, to appear.
25. Jayakumar, R.; Lee, Y.-S.; Nanjundan, S. J Appl Polym Sci, to appear.
26. Grasel, T. G.; Cooper, S. L. J Biomed Res 1989, 23, 311.
27. Okkema, A. Z.; Cooper, S. L. Biol Mater 1991, 12, 668.
28. Summers, O. A.; Silver, S. Annu Rev Microbiol 1978, 32, 637.
29. Mobley, H. L. T.; Rosen, B. P. Proc Natl Acad Sci USA 1982, 79, 6119.
30. Tyneck, Z.; Gos, Z.; Zajac, J. J Bacterial 1981, 147, 305.
31. Yoichi, S.; Kazuo, T.; Kazuo, H.; Toshio, T.; Michiro, Y.; Yochio, S. Jpn Kokai Tokkyo Koho JP01 1989, 115, 958.
32. Nishida, R. Jpn Kokai Tokkyo Koho JP09 1997, 911, 19.
33. Toshikai, S.; Inoue, S. S.; Yoshihis, Y.; Takagi, S.; Umiharu, T. Jpn Kokai Tokkyo Koho JP09 1997, 309, 952.
34. Jayakumar, R.; Rajkumar, M.; Nanjundan, S. Orient J Chem 2000, 16, 243.
35. Pelczar, M. J., Jr.; Chen, E. C. S.; Reid, R.D. Microbiology; New York: Tata McGraw Hill, 1990; p 488, vol. 5, 5th ed.
36. Liptova, T. E.; Bakalo, L.A.; Sivotinskaya, A. L. Vysokomal Soedin (RSSS) 1975, 17, 99.