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Solid-phase extraction and high-performance liquid chromatographic analysis of a toxic compound from γ-irradiated polyurethane

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ABSTRACT

Polyurethane (PU) is used in medical devices, but it has been reported that a carcinogen, 4,4′-diaminodiphenylmethane (MDA), is produced in PU when subjected to autoclave sterilization. MDA formation has also been reported in PU when sterilized by γ-irradiation. Analysis of serum MDA extracted from sterilized PUs was performed. Determination was by reversed-phase high-performance liquid chromatography using a mixture of an aqueous solution of ammonium acetate and acetonitrile as eluent. An ODS column and electrochemical detection were used. Prior to analysis, serum samples containing MDA were subjected to solid-phase extraction using C_{18}, phenyl or cyclohexyl reversed-phase columns. Elution was performed with alkaline methanol containing 1 *M* ammonia. A satisfactory recovery was attained. The efficiencies of solid-phase extraction and liquid–liquid extraction was compared.

INTRODUCTION

Polyurethane (PU) is widely used in medical devices due to its biocompatibility and biostability [1–4]. Two types are used for this purpose: thermoplastic and thermosetting PU. Fabrication of thermoplastic PU is identical with that of Pellethane 2363 [1–4].

We were interested in the leaching of 4,4′-diaminodiphenylmethane (MDA) from thermosetting PU when sterilized with γ-irradiation. Thermosetting PU is used as a potting material for the connection of blood dialysis fibres to device modules [5]. Thermosetting PU is fabricated by reaction of 4,4′-diphenylmethane diisocyanate (MDI) with partially saponified caster oil [5,6]. As a larger amount of MDI than castor oil is used, there is the potential for an excessive residue of unreacted MDI. MDI is both a toxic and a reactive compound [1–6].

Medical devices require sterilization, therefore sterilization procedures should not be detrimental to the device. In spite of the biocompatibility and biostability of PU [1–6], it has been reported that toxic MDI is residual in PU [1–6]. MDA is reportedly produced by hydrolysis of residual MDI during degradation of PU by heat, by irradiation, by chemical or enzymatic reactions [7–11]. MDA is reportedly toxic [12], mutagenic [13], teratogenic [14] and carcinogenic [15,16].

We considered it important to evaluate the degree of MDA production in sterilized medical devices and the subsequent migration of MDA into human blood. For this purpose, analysis of serum MDA was studied. A liquid–liquid extraction for serum MDA has been reported previously [17–19]. In these cases, the MDA recovery yields were low and complex processes were required [17–19], therefore a solid-phase extraction (SPE) was studied. As MDA is unstable when heated [20], reversed-phase high-performance liquid chromatography (HPLC) was considered to be more appropriate.

EXPERIMENTAL

Materials

Thermoplastic PU was fabricated as described for Pellethane 2363 (Dow Chemical) [1–4]. Thermosetting PU potting material was fabricated by reac-

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ting MDI with partially saponified castor oil [5,6]. An excess of MDI over castor oil was used, thus leaving residual MDI. MDI is readily converted to MDA in an aqueous environment [21]. This sample was identical with sample B in a previous study [22].

Other chemicals were of extra grade supplied by Wako (Tokyo, Japan). Serum was obtained from Kanto Kagaku (Tokyo, Japan).

HPLC analysis

A conventional end-capped ODS column (insufficiently end-capped), ODS 120T-1251, from Toso (Tokyo, Japan) was used with an aqueous eluent consisting of 50 m*M* ammonium acetate–acetonitrile (1:3) at a flow-rate of 1.2 ml/min. Detection was carried out simultaneously using electrochemical detection (ED) (900 mV application) and UV detection. The ED apparatus used was a VMD 501 supplied by Yanagimoto (Kyoto, Japan) with a glassy carbon working electrode and an Ag/AgCl reference electrode. UV detection was carried out at 250 nm, the wavelength of maximum absorption of MDA, with a Model SPD-2A spectrophotometer supplied by Shimadzu (Kyoto, Japan).

Solid-phase extraction procedure

Bond Elut® columns (C_1, C_2, C_8, C_{18}, phenyl, cyclohexyl and silica and a SCX strong cation-exchange column) were supplied by Analytichem (Harbor City, CA, USA).

Reversed-phase columns and the silica column were conditioned using 1 ml of methanol followed by 1 ml of water. Serum (1 ml containing MDA at a concentration of 0.1–10 ppm) was applied to the conditioned column, which was rinsed with 1 ml of water. The column-trapped MDA was eluted with 250 μl of methanol containing 1 *M* ammonia.

The Bond Elut SCX was conditioned with 2 ml of 1 *M* HCl and washed with 2 ml of methanol and thereafter with 2 ml of water. Serum (1 ml containing MDA at a concentration of 0.1–10 ppm) was adjusted to pH 2.5. The serum was deproteinized and centrifuged. The supernatant was applied to the conditioned SCX column. The column-trapped MDA was rinsed with 1 ml of 0.01 *M* HCl and eluted with 1.5 ml of methanol containing 1 *M* ammonia.

Conditioning and elution in SPE were carried out using a Model AP-115 AN vacuum pump supplied by Iwaki (Tokyo, Japan).

RESULTS AND DISCUSSION

HPLC analysis

MDA was detectable by ED due to the presence of two primary aromatic amino groups. When using an insufficiently end-capped ODS column such as ODS 120T-1251, it was necessary to add 50 m*M* ammonium acetate to attain a common ion effect for rapid elution and to prevent MDA peak tailing. For reproducible and sensitive detection by ED, a salt concentration above 50 m*M* is advisable.

MDA was detectable by ED above 500 mV. The response increased (Fig. 1) and the selectivity decreased with increasing voltage. As voltages above 1000 mV application deteriorates the glassy carbon working electrode, 900 mV is more appropriate. The UV absorption spectrum of MDA is presented in Fig. 1. MDA was detected at a maximum wavelength of 250 nm.

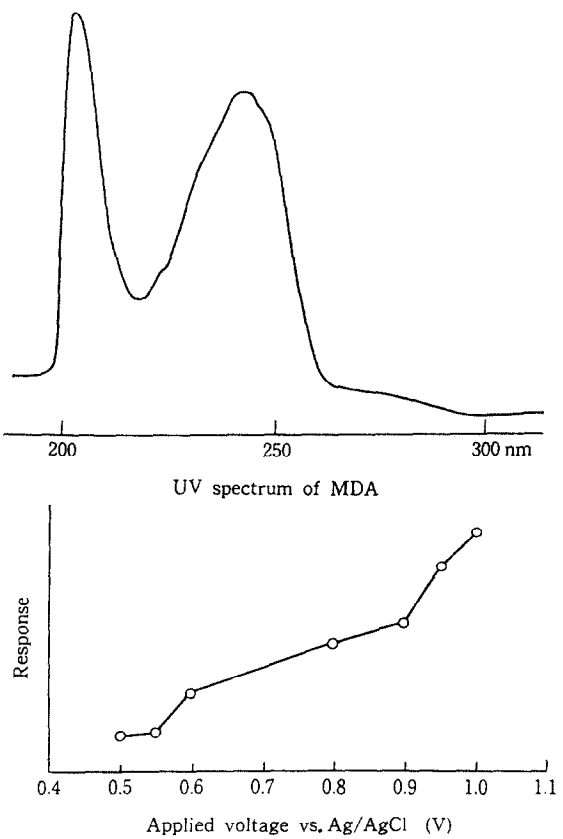

Fig. 1. (Top) UV spectrum of MDA and (bottom) relationship between applied voltage and response of MDA.

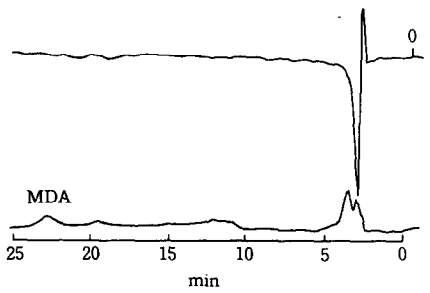

Fig. 2. HPLC of methanol extract of irradiated PU. In the lower chromatogram 0.48 ppm of MDA was detected by ED. No MDA was detected by UV (250 nm) in the upper chromatogram.

As shown in Fig. 2, there is a significant difference between the selectivity and sensitivity of UV and ED in MDA detection. UV detection showed a greater baseline fluctuation due to impurities in the ammonium acetate eluent. ED is clearly superior, being 50 times more sensitive. The detection limits of ED and UV detection are 3 and 150 μg/l, respectively.

Liquid–liquid extraction of serum MDA

A liquid–liquid extraction has been reported [17–19]. In order to suppress ionization of MDA, serum alkalized and extracted repeatedly with hydrophobic organic solvents such as heptane, diethyl ether or benzene [17–19]. These methods are complex procedures, requiring repeated extraction. The recovery yield is reported to be 70–80%, which is unsatisfactory [17–19].

When one part of alkaline serum was extracted twice with seven parts of chloroform–methanol (3:1), satisfactory recovery of serum MDA was achieved [21]. We have recently developed a more efficient method, in which two parts of acetonitrile are added to one part of serum [22]. In both instances, the extracts were vacuum evaporated in a water-bath maintained at 50°C. Temperature is a critical factor; above 50°C MDA degrades and an unsatisfactory recovery is obtained. The acetonitrile procedure requires a single extraction and shows a satisfactory recovery [average recovery yield 98% ($n = 5$), coefficient of variation (C.V.) = 1.6%] [22]. As liquid—liquid extraction requires condensation and a large volume of solvent, we elected to use SPE.

Solid-phase extraction of serum MDA

Bond Elut columns (C_1, C_2, C_8, C_{18}, phenyl, cyclohexyl, silica and SCX) were tested. The recovery yields are averages for five specimens. In all instances the C.V. is less than 1.4%.

The recovery yields of serum MDA from the C_1, C_2, C_8 and silica columns are 56%, 75%, 90% and 12%, respectively. Residual MDA was determined in the drain, indicating that MDA was insufficiently retained on the column. The recovery yields from the C_{18}, phenyl and cyclohexyl columns are 100%. When following this procedure, the concentration of recovered MDA from the C_{18}, phenyl and cyclohexyl columns is five times the original concentration without condensation (Fig. 3).

The lower recovery yield for the C_1, C_2, C_8 and silica columns and the higher recovery yields for the C_{18}, phenyl and cyclohexyl columns indicate that the predominant factors for retaining MDA on reversed-phase columns are Van der Waals binding and π-π binding between benzene rings. The binding of MDA to silanol in reversed-phase columns is not significant due to the lower recovery yield of serum MDA from the silica column. This is thought to be due to the interference of serum water with the combination of MDA to silanol. The recovery yield increased with increasing hydrophobicity from the C_1 to C_8 column, indicating that Van der Waals binding is involved in the binding factor.

A satisfactory recovery yield was attained using the SCX column, but conditioning of SCX is complex, requiring deproteinization and centrifugation of serum prior to application.

When eluting MDA from reversed-phase columns, methanol resulted in a 6% MDA recovery (Fig. 3); 10% was recovered by elution using 10 m*M* ammonium acetate–methanol (1:1) (Fig. 3). MDA was satisfactorily recovered by using strongly alkaline methanol containing 1 *M* ammonia (Fig. 3). Both methanol and acidified methanol resulted in an unsuccessful recovery of serum MDA. The lower recovery when using an acidified methanol cannot be explained, but acidification is thought to be depressed in alkaline serum.

When the chromatograms of serum obtained using C_8, C_{18}, phenyl and cyclohexyl columns were compared, the fast elution peaks proved to be retained more on the phenyl than on the other columns (Fig. 4). The recovery yield remained satis-

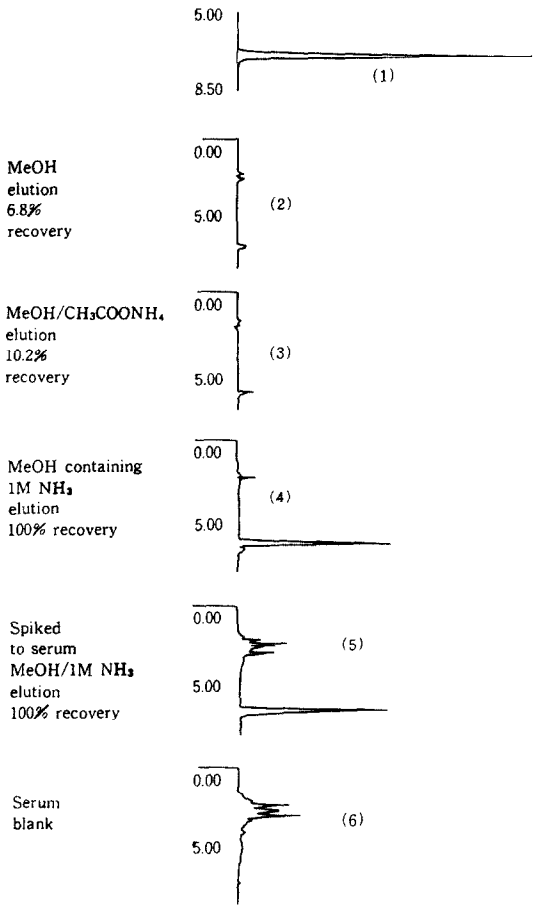

Fig. 3. HPLC of serum containing MDA. Serum MDA was recovered by SPE using a C_{18} column combined with various eluents. HPLC conditions: C_{18} column; eluent, methanol (MeOH)–10 m*M* aqueous ammonium acetate (1:1); flow-rate, 1.2 ml/min; detection, UV (254 nm); 10 μl of sample applied to the column. The MDA peak has a retention time of 6.95 min. (1) 105 ng per 10 μl of MDA standard solution. (2) 100 μl of 105 ng per 10 μl of MDA aqueous solution were applied to C_{18} resin (100 mg of resin and 120 μl void volume), eluted with 250 μl of methanol); 200 μl were collected and applied to HPLC. (3) The same procedure as in (2) except for the eluent: methanol–10 m*M* aqueous ammonium acetate (1:1), identical with HPLC eluent. (4) The same procedure as in (2) except for the eluent: methanol containing 1 *M* NH_3. (5) 50 μl of 21 μg/ml of MDA were added to 1 ml of serum to prepare the serum sample containing MDA at a concentration of 1 μg/ml. This was applied to C_{18} resin, eluted with 250 μl of methanol containing 1 *M* NH_3. 200 μl were collected and applied to HPLC. (6) Serum blank.

factory, confirming the sufficient binding capacity to retain MDA and other serum admixtures.

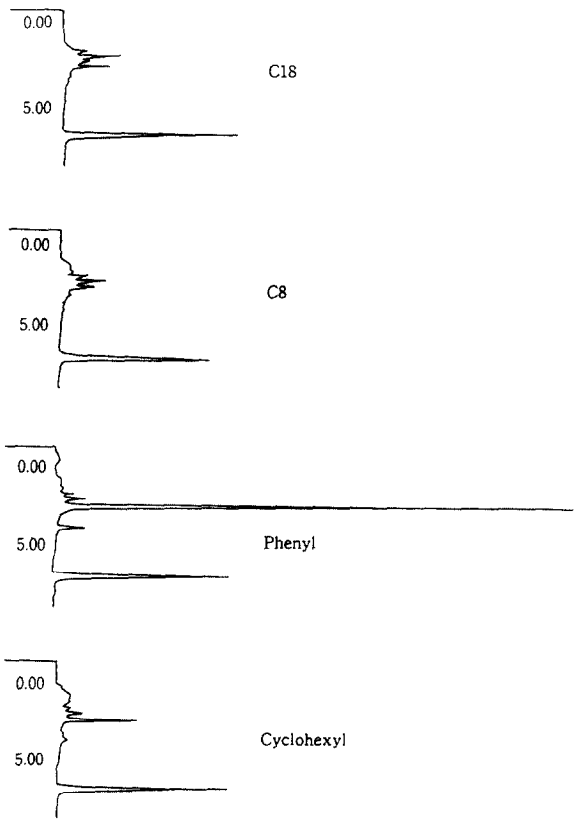

Fig. 4. Solid-phase extraction of serum MDA using C_8, C_{18}, phenyl and cyclohexyl columns. A 50-μl volume of a 21 μg/ml concentration of MDA was added to 1 ml of serum to prepare the serum sample containing MDA at a concentration of 1 μg/ml. This was applied to C_8, C_{18}, phenyl and cyclohexyl columns and eluted with 250 μl of methanol containing 1 *M* NH_3. Conditions are the same as in Fig. 3.

Determination of MDA in potting material sterilized by autoclaving or gamma irradiation

There was no indication of MDA formation in thermosetting PU potting material sterilized by autoclaving at 121°C for 60 min.

Potting material sterilized by 10 Mrad γ-irradiation produced MDA at a level of a few ppm. MDA formation increased with increasing irradiation dose according to a quadratic equation. At 2.5 Mrad, the irradiation level officially approved, MDA was formed at the concentration of less than 1 ppm.

The HPLC chromatogram of serum extract from irradiated potting material indicates that serum ex-

tracts both hydrophilic and hydrophobic compounds including MDA. Detection was by ED. As these extracts indicate mutagenicity, further study is required.

The risk factor of MDA formed at 10 Mrad irradiation (a few ppm) was estimated using ref. 16. In our opinion, the result is "not significant" as the estimated cancer-causing risk factor is 0.29 (29 in 100 persons) when intaking 1 mg/kg body mass of MDA per day. The MDA elution level is far less than the risk factor level.

The elution of compounds other than MDA from irradiated potting material is greater. They are mutagens, therefore other compounds must be evaluated as they are thought to migrate to patients. It is necessary to estimate the risk factor to patients exposed to these toxic compounds. For this purpose, the chemical structures and the biological characteristics of the exact mutagens in the extract should be further clarified.

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