Technical Note

Highly Efficient Absorbents for Removing ^{32}P -Labeled Nucleotides from Experimental Waste[†]

Miyoko NAKANO, Kazuo IWATANI, Hideto KURA, Chie KOHCHI
and Osamu YAMAMOTO

Radioisotope Center, Hiroshima University
1-4-2, Kagamiyama, Higashi-Hiroshima-shi, Hiroshima Pref. 739, Japan

Received November 7, 1996

Three kinds of charcoal (LH2C20/48, WH5C8/32 and X-7100-3), three kinds of ion exchange resin (DE52, MB5113J and RG501/X8), and one metal chelater (MC75) were tested for their ability to remove [α - ^{32}P] dCTP as a standard RI using a small column system. The most efficient absorber was DE52. However, DE52 was not suitable for practical treatment of the waste because flow rate was slow. LH2C20/48, WH5C8/32 and MB5113J also showed high absorption ability and their absorption efficiency was not affected by a pH range from 5 to 9 of the solution. On the basis of this result, a practical trial of the radioisotope waste treatment was also made. It was concluded that among the tested absorbents MB5113J was the most applicable for removing ^{32}P -labeled nucleotides from experimental waste.

Key Words: radioisotope waste treatment, removal of phosphorus-32-labeled nucleotide, ion exchange resin, charcoal

1. Introduction

Unsealed radioisotopes (RI) are used widely in biological or chemical experiments and medical treatments¹⁾. In RI laboratories, used RI are usually discarded into the drainage after dilution below the level prescribed by law²⁾. However, in order to maintain a clean environment, it is important to remove as much RI as possible from experimental or clinical wastes before draining. Here we describe a basic test of the effectiveness of various absorbents to remove the RI compounds and a practical trial to examine the factors affecting absorption efficiency.

2. Materials and Methods

2.1 Standard RI solution

A standard solution of [α - ^{32}P] dCTP (110 TBq/mmol, Amersham, England) in a TES-buffered (10^{-2} mol/dm³ Tris, 10^{-3} mol/dm³ EDTA, and 5×10^{-2} mol/dm³ NaCl) aq. solution (4×10^3 Bq/cm³) was prepared.

2.2 Absorbents

Three kinds of charcoal, LH2C20/48, WH5C8/32, and X-7100-3, were supplied by Takeda Yakuhin Co., Japan^{3), 4)}. An anion exchange resin, DE52, was purchased from Whatman, Germany. MB5113J and RG501/X8, which are anion/cation exchange resins, were obtained respectively from Sumitomo Chemical Co., Japan and Bio-Rad, U.S.A. A metal chelater, MC75, was also

[†] 実験廃液からの ^{32}P -標識ヌクレオチドの高効率吸着除去剤。中野美代子, 岩谷和夫, 久良秀人, 河内千恵, 山本 修: 広島大学アイソトープ総合センター, 739 広島県東広島市鏡山 1-4-2。

supplied by Sumitomo Chemical Co., Japan.

2.3 Measurements

The absorbent (100 to 1 500 mg) was packed in a disposable glass pipette (MARUEMU, $\Phi 8$ mm $\times$ 230 mm) plugged with cotton in the tip (Fig. 1). One cm^3 of the RI standard solution was applied to the column and the column was washed with deionized water three times. The solution remaining in the column was pushed out with a silicone nipple⁵⁾. All substances eluted through the column were collected in a vial and the radioactivity was measured with a liquid scintillation counter (Aloka LSC-3050)⁶⁾.

In another experiment, the absorbent (25 g) was packed in a 100 cm^3 syringe (Fig. 1). This column was used to elute actual experimental RI

Fig. 1 Flow column systems for analysis of absorbing efficiency.

waste containing ^{32}P compound (250 cm^3) followed by washing with 50 cm^3 of deionized water. The solution remaining in the column was pushed out with a plunger. Radioactivity in the eluate was measured as described above. ^{32}P absorption efficiency of the various test materials was calculated as follows:

$$^{32}\text{P} \text{ absorbing efficiency (\%)} = 100 \times \left[1 - \frac{^{32}\text{P} \text{ radioactivity in the eluate}}{^{32}\text{P} \text{ radioactivity applied}} \right]$$

3. Results

3.1 RI removal efficiency

Seven kinds of absorbents were examined for their ability to absorb $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ which is one of the most frequently used RI compounds in biological experiments. The results are shown in Fig. 2. When 1 cm^3 of the RI standard solution (pH 7) was eluted, at 100 mg of absorbent, DE52 (anion exchange resin) and LH2C20/48 (charcoal) were more efficient than other absorbents: the former absorbed almost 100% at 100 mg and the latter almost 100% at 500 mg. However, DE 52 is not suitable for practical application, because high pressure is necessary to elute the liquid. The order of absorption efficiency for the other materials was WH5C8/32 (charcoal) > MB5113J (anion/cation exchange resin) > RG501/X8 (anion/cation exchange resin) > X-7100-3(charcoal). When a larger amount of absorbent was used, the removal efficiency was almost 100% (at 1 000 mg for WH5C8/32 and at 1 250 mg for MB 5113J). For RG501/X8 and X-7100-3, the efficiency did not reach 80% even at 1 500 mg. MC 75 (metal chelater) had no absorbing ability. Therefore, the most useful absorbent is LH2C20/48. Other applicable absorbents are WH5C8/32 and MB5113J when used for the standard RI solution.

Fig. 2 Relationship between absorption efficiency and absorbent weight. Each resin was packed in a 1 ml disposable micropipette tip. One ml of RI standard solution (pH 7) was poured into the resins and the Cerenkov radiation of the eluted solution was counted by a liquid scintillation counter. ^{32}P removal efficiencies were calculated as described above (in Materials and Methods).

Table 1 Absorption efficiency of LH2C20/48, WH5C8/32 and MB5113J at pH 5 and pH 9 compared with that at pH 7 (100%)

Absorber	LH2C20/48	WH5C8/32	MB5113J
pH 7	100%	100%	100%
pH 5	99.96 $\pm$ 0.02%	99.94 $\pm$ 0.13%	99.77 $\pm$ 0.70%
pH 9	99.81 $\pm$ 0.01%	97.57 $\pm$ 3.89%	99.36 $\pm$ 0.69%

3.2 Effect of pH

The absorption efficiencies of three absorbents (LH2C20/48, WH5C8/32 and MB5113J) were compared at pH 5, 7 and 9 (Table 1). There was no significant difference in absorption over this range.

3.3 Effect of impurities

On the basis of the results of 3.1 and 3.2, three absorbents (LH2C20/48, WH5C8/32 and MB5113J) were tested to determine which is the most applicable for practical experimental waste (A – E in Table 2) including RI, salt, detergent, buffer and/or chelater.

In trials A and B, the three absorbents were equally efficient in absorbing RI. In trials C, D and E, MB5113J was the best among three absorbents tested. These results suggested that the absorption efficiency is dependent on the chemical structure of the RI compound and/or on the existence of impurities in the waste^{7,8)}.

4. Discussion

Elimination or separation of radioisotopes by elution through absorbents packed in columns has been reported^{7,8)}. We also studied highly efficient absorbents for the purpose of removing as much radioisotope as possible from experimental or

Table 2 Treatment of ^{32}P experimental waste* with absorbents

No.	^{32}P experimental waste			pH	Absorption efficiency (%)		
	^{32}P compound	(Bq/cm ³)	Adulterant		LH2C20/48	WH5C8/32	MB5113J
A	$[\alpha\text{-}^{32}\text{P}]\text{dCTP}$	(5.0×10^2)	NaCl, SDS, citric acid	8	91.4 ± 0.4	82.1 ± 5.2	98.5 ± 0.1
B	$[\alpha\text{-}^{32}\text{P}]\text{dCTP}$	(5.8×10^2)	Reagent, SDS, others	7	89.3 ± 0.4	83.6 ± 0.3	84.2 ± 0.2
C	$[\gamma\text{-}^{32}\text{P}]\text{ATP}$	(4.8×10^2)	NaCl, SDS, citric acid	6	66.9 ± 0.4	71.0 ± 0.2	92.5 ± 0.7
D	$[\gamma\text{-}^{32}\text{P}]\text{ATP}$	(5.1×10^2)	25% acetic acid	3	50.8 ± 0.3	48.6 ± 0.3	99.5 ± 0.1
E	$[\alpha\text{-}^{32}\text{P}]\text{GTP}$	(5.2×10^2)	MgCl ₂ , EDTA, Tris	6	39.2 ± 0.8	39.5 ± 0.3	97.1 ± 0.1

* Volume of waste used was 10 times that of absorbent (25g).

clinical wastes before draining.

The absorption efficiency of dCTP was different among the tested charcoal absorbents such as LH2C20/48, WH5C8/32 and X-7100-3. LH2C20/48 and WH5C8/32 were made of coconut shells while X-7100-3 was of coal. High efficiency of LH2C20/48 and WH5C8/32 is most probably due to the volume of small holes as the volume of these two absorbents are larger than the other absorbent (X-7100-3). Furthermore the higher efficiency of LH2C20/48 than WH5C8/32 could be due to the treatment for modifying small opening holes.

It has been expected that DE52 (cellulose compound), which is an anion exchange resin would be very highly efficient because of the anionic nature of CTP. However, a very low elution rate due to the very small size of the particles prevented the availability of CTP for the elution treatment. MB5113J is a styrene-DVB copolymer which has the equivalent capacity in anion and cation exchange. Although the efficiency of MB 5113J was a little lower than that of DE52, it was much higher than that of RG501/X8 as an anion-cation exchange resin. Therefore, it can be concluded that MB5113J is a good absorbent of nucleic acid compound radioisotopes.

In the case of the waste containing some non-ionic compound radioisotopes and hydrophobic compound radioisotopes, after prefiltration, elutions first through a column of LH2C20/48 and then through a column of MB5113J would be effective for the elimination of radioisotopes from the waste. However, further study is necessary for confirmation of their removal capacity for other kinds of radioisotope and also for other kinds of waste.

Acknowledgements

We are indebted to Dr. S. Shigeta for her kind provision of samples, and to Amersham Co., Takeda Yakuhin Co. and Sumitomo Chemical Co. for their donation of some reagents. We also thank to Dr. H. Hasai for his encouragement and to Dr. Y. Ohyama and Dr. Y. Ogata for their helpful discussions. We would like to express our sincere thanks to Dr. P.A. Broady of Canterbury University for correcting the English text and important advices. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 06915030).

References

- 1) Cho, T. : ^{33}P -Nucleotides in molecular biology applications, *Radioisotopes*, 44, 267–274 (1995)
 - 2) Nuclear Safety Technology Center and Science and Technology Agency Nuclear Safety Bureau : “Law of Concerning Prevention from Radiation Hazards Due to Radio-Isotopes, etc.”, Daiichi-Houki Press, Tokyo (1994)
 - 3) Japanese Industrial Standard Committee : “Japanese Industrial Standard, JIS K1470”, Japanese Industrial Standards Association, Tokyo (1967)
 - 4) Japanese Industrial Standard Committee : “Japanese Industrial Standard, JIS K1474”, Japanese Industrial Standards Association, Tokyo (1991)
 - 5) The Ministry of Education in Japan : “The Training and Practical Data for Teaching Staffs in Radioisotope Center of National University”, Press of the Ministry of Education, Tokyo (1992)
 - 6) The Environmental RI Committee and the RI Measurement Group in RI Center of Hiroshima University : “Working Group Report — the Methods for Measurements of RI in Experimental Draining Water, RI Center of Hiroshima University”, Higashi-Hiroshima (1992)
 - 7) Satoh, I., Matsusaka, N., Kobayashi, H. and Nishimura, Y. : Availability of zeolite as an eliminant for the incorporated radionuclides (3). Studies on ^{137}Cs , *Radioisotopes*, 43, 468–473 (1994)
 - 8) Uchida, S., Tagami, K. and Fujikawa, T. : A fundamental study for the chemical separation of technetium by TEVA·Spec resin, *ibid.*, 45, 784–787 (1996)
-