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Combinatorial evaluation of the chiral discrimination of permethylated carbohydrates using fast-atom bombardment mass spectrometry

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Abstract

The chiral discrimination abilities of several variously permethylated carbohydrates toward various amino acid 2-propyl esters were combinatorially evaluated from the relative peak intensity of the 1:1 diastereomeric complex ions with the deuterium-labeled L-amino acid 2-propyl ester protonated ion and with the unlabeled D-amino acid 2-propyl ester protonated ions in FAB mass spectrometry. The chiral discrimination abilities evaluated using FAB mass spectrometry approximately corresponded to the ratio of the association constants (K_R/K_S) toward each enantiomer in the solution. Therefore, this evaluation method is very useful for the screening of the chiral discrimination abilities of carbohydrates and their derivatives. © 2001 Published by Elsevier Science Ltd.

Keywords: Permethylated carbohydrates; Chiral discrimination; FAB mass spectrometry; Combinatorial evaluation

1. Introduction

Carbohydrates such as amyloses, cyclomaltooligosaccharides (cyclodextrins) and their derivatives are used around the world as a chiral stationary phases (CSP) in gas or liquid chromatography.[1,2] Carbohydrates are also used as chiral selectors in capillary electrophoresis.[3] The chiral discrimination of carbohydrates and their derivatives is a well-known fact.[4] However, the chiral discrimination abilities of various carbohydrates based on a 1:1 complexation with chiral compounds have not been systematically evaluated until now, because a facile evaluation method of the chiral discrimination abilities has not been fully established.[5]

We propose a facile evaluation method of determining chiral discrimination ability using the FAB mass spectrometry (MS)/enantiomer labeled (EL) guest method. By this method a 1:1 mixture of deuterium-labeled/unlabeled enantiomer guests is used and then evaluated the chiral discrimination ability of various carbohydrate derivatives and chiral compounds.[6]

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In this method the FAB mass spectra of three component samples in solution [3-nitrobenzyl alcohol (NBA) matrix] of a chiral host (H) such as carbohydrate derivatives and a 1:1 mixture of the S-enantiomer labeled with deuterium (G^{+}_{S-dn}) and unlabelled R-enantiomer (G^{+}_{R}) were measured at room temperature. The relative peak intensity $[I(H+G_R)^+/I(H+G_{S-dn})^+ = I_R/I_{S-dn}]$ values of the two diastereomeric 1:1 host–guest complex ions differing in molecular weight ($\Delta MW = n$, n: the number of deuteriums) corresponded to the chiral discrimination abilities. Thus, the chiral discrimination abilities can be easily evaluated in a short time from a single mass spectral chart.

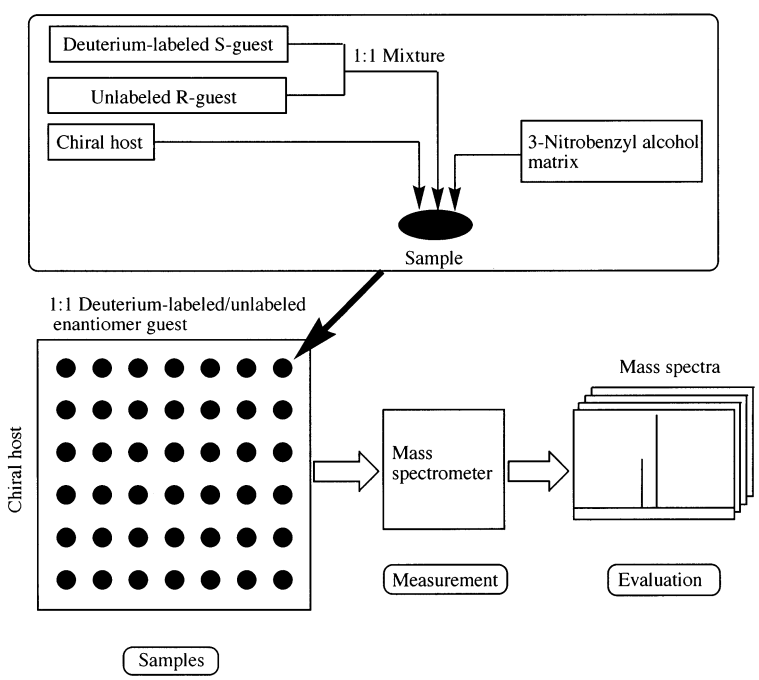
In this note we report that the chiral discrimination abilities of various permethylated carbohydrates toward various amino acid 2-propyl ester hydrochlorides that were combinatorially evaluated using the facile and quick FABMS/EL guest method.

2. Results and discussion

FABMS/EL guest method.—All samples were prepared by mixing a permethylated carbohydrate host solution and a 1:1 deuterium-labeled/unlabeled amino acid ester hydrochloride solution with a matrix as shown in the experimental section. The concentration ratio of the final samples is $[H]:[G^{+}_{S-dn}]:[G^{+}_{R}] = 1:3.3:3.3$. The spectra of the samples were measured at room temperature (Scheme 1). The relative peak intensity (I_R/I_{S-dn}) values of the observed diastereomeric complex ions are summarized in Table 1. For $I_R/I_{S-dn} > 1$, there is R-selectivity, for $I_R/I_{S-dn} < 1$, there is S-selectivity, and for $I_R/I_{S-dn} = 1$, there is no selectivity.[4] The typical FAB mass spectra are shown in Fig. 1.

In all the measured FAB mass spectra where the two diastereomeric complex ion peaks of the permethylated carbohydrates with the deuterium-labeled/unlabeled protonated amino acid 2-propyl ester ions were observed on the higher mass side, the chiral discrimination abilities of the all samples could be successfully evaluated. This fact shows that the FABMS/EL guest method is very effective for various carbohydrate structures (cyclic or acyclic structure, number of monosaccharide units, *etc.*).

Correlation between structure of carbohydrates and chiral discrimination ability.—Some of the details of the correlation between the structure of the carbohydrates and the chiral



Scheme 1. Combinatrial evaluation of chiral discrimination of permethylated carbohydrates using FAB mass spectrometry.

Table 1
Chiral discrimination ability (I_R/I_{S-dn} value) of permethylated carbohydrates toward amino acid 2-propyl ester hydrochlorides using FAB mass spectrometry

Permethylated carbohydrates	I_R/I_{S-dn} value								
	Ala	Val	Tle	Trp	Phe	Pgly	Pro	Ser	Met
Cyclic oligosaccharides									
Cycloinulohexaose	0.94	1.28	1.18	1.38	1.00	0.99	1.08	1.01	1.04
Cycloinuloheptaose	0.94	0.88	1.00	1.29	1.01	0.76	1.16	1.18	0.95
Cyclomaltohexaose	0.99	1.00	0.95	1.29	1.02	0.94	1.07	0.95	0.91
Cyclomaltoheptaose	1.06	1.00	0.94	1.23	1.01	0.91	1.07	1.15	0.91
Cyclomaltooctaose	0.90	1.00	0.93	1.17	1.00	0.89	1.14	0.99	0.92
Homo-oligosaccharides									
β-Maltose	1.02	0.96	0.95	1.06	1.06	0.98	1.16	1.07	1.07
β-Maltotriose	1.07	0.97	0.95	1.12	1.05	0.91	1.07	1.10	1.12
β-Maltotetraose	1.05	1.01	0.99	1.13	1.02	0.91	1.08	1.03	1.08
β-Maltopentaose	1.03	0.98	0.95	1.15	1.01	0.86	1.07	1.00	1.08
β-Cellobiose	0.99	1.00	0.97	0.62	0.97	0.90	0.94	1.02	1.04
β-Cellotriose	0.97	0.97	0.98	0.57	1.01	0.91	1.09	1.03	1.00
β-Cellotetraose	0.94	0.98	0.95	0.59	0.98	0.89	1.08	1.03	0.97
β-Cellopentaose	0.94	0.93	1.05	0.49	0.96	0.89	1.06	1.03	0.98
β-Laminarabiose	1.02	0.97	0.92	0.98	1.05	0.99	1.07	1.05	1.00
β-Laminarariose	1.05	1.07	0.98	0.88	1.03	1.01	1.09	1.07	1.12
β-Laminaratetraose	1.03	1.01	0.99	0.78	1.02	0.98	1.06	1.04	1.07
β-Laminarapentaose	1.04	0.97	0.91	0.79	0.96	0.96	1.16	1.06	1.01
Methyl 3,6-di-*O*-α-D-mannopyranosyl α-D-mannopyranoside	0.96	0.91	0.86	0.97	0.90	0.86	0.99	0.99	0.92
6-*O*-(3,6-di-*O*-α-D-Mannopyranosyl-α-D-mannopyranosyl)-3-*O*-α-D-mannopyranosyl α-D-mannose	0.96	0.90	0.85	0.98	0.93	0.83	1.02	1.01	0.91
Heterooligosaccharides									
1^F-Fructonystose	0.45	0.14	0.33	0.56	0.18	0.26	1.23	0.73	0.28
1-*O*-β-Inulotriosyl α-L-sorbopyranose	0.80	0.51	0.40	0.77	0.67	0.72	1.16	0.79	0.83
β-Inulotriosyl α-D-mannopyranoside	1.09	0.49	0.98	0.84	0.98	1.20	1.06	0.85	1.28
Methyl 6-O-β-inulotriosyl α-D-glucopyranoside	0.60	0.84	0.40	1.25	0.64	0.79	1.16	0.80	0.79
Raffinose	1.01	1.04	1.10	1.05	1.00	0.90	1.19	1.08	1.06
Stachyose	1.08	1.09	1.08	1.02	0.99	0.94	1.20	1.08	1.09
Melezitose	1.05	1.00	0.99	0.98	1.03	0.98	1.18	1.06	1.04
Monosaccharides									
β-Glucopyranoside	1.00	1.01	0.99	1.01	1.03	0.96	1.05	1.04	0.98

The accuracy of all data was within ± 0.03. Some data were reported in the following references: ref. 7a for parts of cyclic oligosaccharides; ref. 7b for parts of fructo-oligosaccharides; ref 7c for parts of gluco-oligosaccharides. Pgly = Phenylglycine, Tle = *tert*-Leucine. $I_R/I_{S-dn} > 1$, R-selectivity; $I_R/I_{S-dn} < 1$, S-selectivity; $I_R/I_{S-dn} = 1$, no selectivity.

discrimination ability have already been discussed in our other report.[7] For the permethylated cyclic oligosaccharides, a remarkable chiral discrimination was not observed due to the highly symmetric structures.[7a] For the permethylated homo gluco-oligosaccharides, the cello-oligosaccharides (dimer–pentamer) showed a relatively higher chiral discrimination ability toward 2-propyl tryptophanate (S-preference). The reason for this was assumed to be that, when comparing the S-enantiomer with the R-enantiomer, the π electrons of the indole moiety more strongly interact with the permethylated cello-oligosaccharides.[7c] For the permethylated mano-oligosaccharides, the number of monosaccharide units had no effect on the chiral discrimination ability toward the given guests. Permethylated fructo-oligosaccharides, which are produced by translation and hydrolysis from inulin, showed the most remarkable chiral discrimination ability of the permethylated carbohydrates presently stud-

ied. Among them, the chiral discrimination ability of the permethylated 1^F-fructo-nystose was very high toward the given guests except for 2-propyl prolinate. For example, the I_R/I_{S-dn} value for Val-O-iPr$^+$ was 0.14 (S-selectivity). One of the structural features in the fructo-oligosaccharides is the main chain which consists of oxyethylene units. During the complexation with the protonated amino acid ester, the ammonium ion moiety of the guest would be tightly fixed at the oxyethylene chain *via* the charge-dipole electrostatic interaction, and the degree of the steric interaction (repulsion) between the hosts and the other moiety of the guests would come to largely depend on the difference of the chirality of the guests. It was assumed that the difference of the interaction is a significant factor in the chiral discrimination.[7b] In other permethylated hetero-oligosaccharides such as raffinose, stachyose, and melizitose, which have no oxyethylene chains in the molecule, the chiral

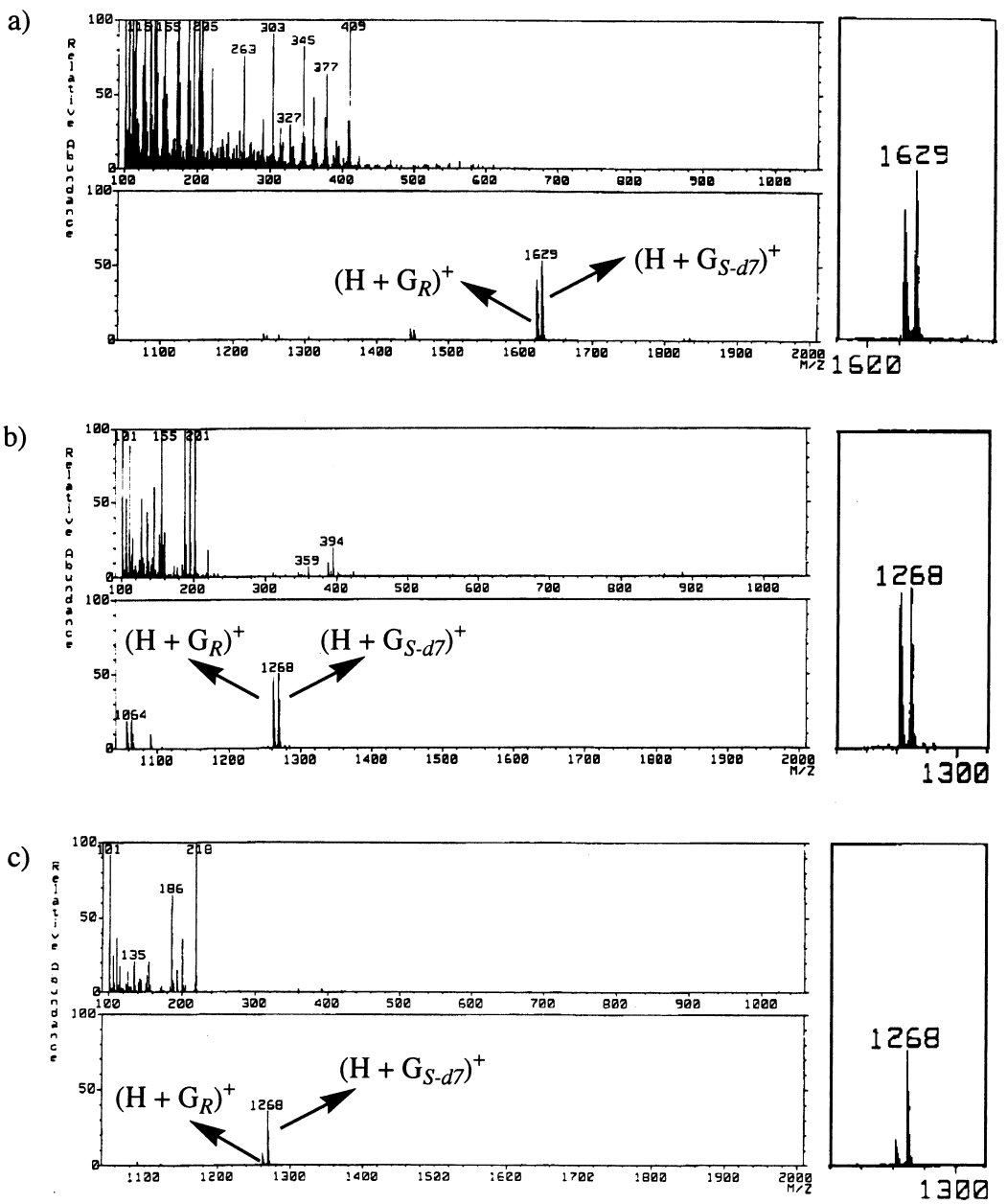


Fig. 1. Typical mass spectra the FABMS/EL guest method (a) Host: permethylated cycloinuloheptaose; guest: 2-propyl phenylglycinate, (b) host:permethylated α-laminaripentaose, guest: 2-propyl phenylglycinate, (c) permethylated 1-fructo-nystose; guest, 2-propyl phenylglycinate.

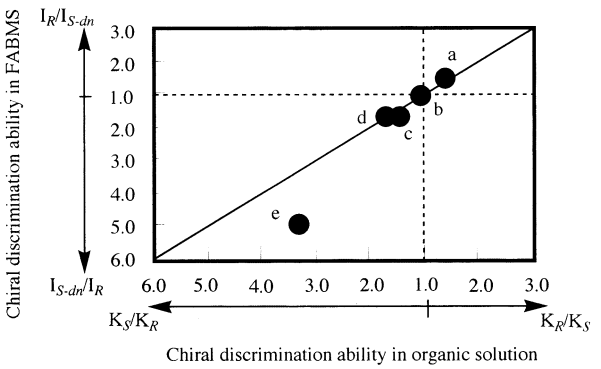


Fig. 2. Association constants ratio (K_R/K_S) in organic solution (chloroform) *vs.* relative peak intensity (I_R/I_{S-dn}) in FAB mass spectrometry (NBA matrix). (a) Permethylated cycloinulohexaose, 2-propyl tryptophanate, (ref. 7a); (b) permethylated α-cyclodextrin, phenylglycine derivative (ref. 8); (c) permethylated 6-O-αglucopyranoside, 2-propyl phenylalaninate, (ref. 7b); (d) permethylated 1^F-fructonystose, 2-propyl tryptophanate, (ref. 7b); (e) permethylated fructonystose, 2-phenylalaninate, (ref. 7b).

discrimination abilities were only slightly observed.

Correlation to association constants.—The 1:1 association constants (K_R and K_S) of the permethylated carbohydrates with some amino acid derivatives in solution such as chloroform or chloroform–dichloromethane were determined at 298 K. [7b,8] The chiral discrimination abilities (K_R/K_S) calculated from the association constants were plotted versus the I_R/I_{S-dn} values using the FABMS/EL guest method (Fig. 2). They are in good agreement with each other. Although a only few association constants of the permethylated carbohydrates were determined, their linear relationship suggests that the FABMS/EL guest method is a useful screening method to evaluate not only the enantioselectivity but also the magnitude of the chiral discrimination ability. Herein, different solvents were used in K_R/K_S (chloroform) and in I_R/I_{S-dn} (NBA).

Complexation properties depend on solvent effect. Therefore, the binding ability *i.e.*, the association constants in solution, drastically changes depending on kind of the solvent. However, as the chiral discrimination ability is represented as the ratio value of the association constants or peak intensity, a large part of the solvent effects on the diastereomeric complexation would be leveled off. Further, the given solvents such as chloroform and NBA, which are aprotic and low-polarity solvents, may show little difference of the solvent effects on the ratio values. Indeed, it was reported that the chiral discrimination ability (K_R/K_S) of a chiral crown ether derivative toward organic ammonium ions was hardly different in organic solvent from that in the gas phase (no solvent).[9,10]

Thus, the I_R/I_{S-dn} values were approximately regarded as the K_R/K_S values. However, we would like to pay attention to the fact that the I_R/I_{S-dn} values depend on the concentration of the host and the guest in the matrix. As the initial concentration ratio, $([G_R^+]_0 + [G_{S-dn}^+]_0)/[H]_0$, of the host and the guests decreased, the I_R/I_{S-dn} values are close to unity. Inversely, as the ratio is increased, the values are close to the K_R/K_S values. For the concentration ratio under the present sampling (concentration) conditions $[([G_{S-dn}^+] + [G_R^+])/[H] = 6.67]$ or larger ratio, the chiral discrimination abilities are approximately evaluated independently of the magnitude of the association constants, and the I_R/I_{S-dn} values are reasonably converted into the difference in the free energy ($-\Delta\Delta G_{enan}$) as in our previous reports.[6a] Although the K_R/K_S values change depending on the solvents (solvent effect), the changes are very small.[11]

In summary, the chiral discrimination abilities of the present permethylated carbohydrates toward amino acid ester hydrochlorides were combinatorially evaluated by the FABMS/EL guest method, and the I_R/I_{S-dn} values by FABMS showed good agreement with the ratio of the association constants (K_R/K_S). Thus, the chiral discrimination abilities (corresponding to $\Delta\Delta G_{enan}$) and the enantioselectivity of the permethylated carbohydrates were easily and speedily determined using our method. This method can automatically be performed with a frit or continuous-flow FAB mass spectrometer connected with an automatic sample mixer and injector.

Hence, the FABMS/EL guest method could be widely used as a screening method for the chiral discrimination ability of various carbohydrate derivatives in order to develop new CSP's for chromatography[2,12] and new chiral selectors for capillary electrophoresis.[3,13]

3. Experimental

Materials.—The present permethylated carbohydrates were prepared from free carbohydrates (hydroxy type) by the Hakomori method.[14] In the free carbohydrates, cyclofructans, laminara-oligosaccharides, 1-*O*-β-inulotriosyl α-L-sorbopyranose, β-inulotriosyl α-D-mannopyranoside, and methyl 6-*O*-β-inulotriosyl α-D-glucopyranoside synthesized using sugar-translation enzymes were used.[15,16] For the other free carbohydrates, commercial products were used (cyclodextrins, 1-kestose, nystose, 1^F-fructonystose, raffinose, glucose, Wako Chemicals Co.; malto-oligosaccharides, Hayashibara Biochemical Laboratories, Inc.; cello-oligosaccharides, Sigma Chemical Co.; isomalto-oligosaccharides, Seikagaku Co.; mannotriose, Toronto Research Chemicals, Inc.; mannopentaose, Dextra Laboratories; stachyose, Pfanstiehl Laboratories Inc.; melezitose, Tokyo Kasei). The anomers of the permethylated carbohydrates were separated and purified by medium-pressure liquid chromatography (Silica Gel 60, E. Merck; solvent, 5:5:1 *n*-hexane–ethyl acetate–methanol from the anomeric mixtures.

The present amino acid 2-propyl ester hydrochlorides were prepared from the esterification of the amino acids (commercial products, Sigma Chemical Co, Wako Chemicals Co., Tokyo Kasei, and Aldrich Chemical Co,) with 2-propanol with an acid catalyst.[17] L-Amino acids (S-enantiomers) were reacted with a deuterium-labeled 2-propanol (d_7, 99 + atom% D, Aldrich Chemical Co.), and D-amino acids (R-enantiomers) were reacted with 2-propanol (Wako Chemicals Co.). L-Tryptophan was esterified with a deuterium-labeled 2-propanol-d_6 [$(CD_3)_2CHOH$, 99.5 atom% D, CDN Isotopes, Canada], which has no active protons in order to protect the substitution of the protons in the indole moiety under acid conditions.

FAB mass spectrometry.—The FAB mass spectra (positive-ion mode) were measured with a JEOL SX-102 mass spectrometer operating at an accelerating voltage of 10 kV with a mass range of m/z 100–2400. The instrument was equipped with a standard JEOL FAB source and an ion gun. Xenon was used as the atom beam with an emission current of 10 mA and an acceleration of 3 kV. The ion source pressure was typically *ca.* $1–2 \times 10^{-5}$ Torr. The spectra were obtained with a magnet scan rate of 10 s/scan (to m/z 2400), and the data were processed with a JEOL JMA-DA 6000 data processing system. Calibration was carried out with CsI.

A sample solution was prepared by mixing a guest solution, a host solution, and a matrix (NBA, Aldrich Chemical Co.). The FAB mass spectra were measured at room temperature with a deposit of a 1 μL aliquot of the mixed solution that was left overnight to homogenize. The three solutions were as follows: (1) 10 μL of a 1.33 M MeOH solution of a 1:1 mixture of unlabelled R- and labeled S-amino acid ester salts ($[G_R^+] = 0.67$ M and $[G_{S-dn}^+] = 0.67$ M), (2) 5 μL of a 0.20 M $CHCl_3$ solution of a given permethylated carbohydrate, and (3) 15 μL of the NBA matrix. In this experiment, a large amount of sample was used in order to prepare the solutions with more exact concentrations. In practical cases, the measurement of the FAB mass spectrum required only about 1 μL of NBA solution. The accuracy of the 1:1 equivalent concentration of the R- and S-enantiomers was confirmed by whether or not the relative intensity (I_R/I_{S-dn}) values with 18-crown-6 (Tokyo Kasei), which is an achiral host, were experimentally obtained as unity within 1.00 ± 0.03. The averages of the I_R/I_{S-dn} values in 10th, 20th, 30th, and 40th scans were applied in Table 1. The accuracy of the I_R/I_{S-dn} values was ± 0.03.

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