Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 621-627 (1999)

Analysis of concomitant ^{18}O in ^{13}C-urea

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Summary

The ^{13}C-urea breath test by infra-red absorption or mass spectra has been extensively used to detect *Helicobacter pylori* infection. However, commercially available ^{13}C-urea contains higher ^{18}O, compared to natural urea. It is evidently important for this diagnosis to investigate the ^{18}O-isotope effect on infra-red absorption or mass spectra, based on the quantitative analysis of ^{18}O in ^{13}C-urea. For this purpose, a new analytical method using mass spectroscopy has been established to determine ^{18}O abundance in urea.

Key words: Analysis, ^{18}O Enrichment, Breath Test, ^{13}C-Urea, Mass Spectrometry

Introduction

The ^{13}C-urea breath test has been widely used to diagnose *Helicobacter pylori (H.pylori)* infection, closely related to gastritis, gastric ulcers and gastric cancer. This unique diagnosis exploits two facts that *H. pylori* is a potent urease producer and that ^{13}C-carbon dioxide derived from ^{13}C-urea can be easily detected in the breath. We also have developed the urea breath test using ^{13}C-urea tablet which can prevent a release of ^{13}C-urea in oro-pharynx, and state-of-the-art non-dispersive infrared spectrometer with Golay cell.

CCC 0362-4803/99/070621-07$17.50
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Received 18 September 1998

In general ^{13}C-urea is synthesized from ^{13}C-carbon monoxide in which not only ^{13}C but also ^{18}O are enriched in the cryogenic distillation process. Preliminary analysis of commercial ^{13}C-urea has made it clear to contain over 99 atom% ^{13}C and approximately 10 atom% ^{18}O. For the urea breath test using ^{13}C-urea containing higher ^{18}O occur the isotopic shift in the absorption bands of infrared spectra. For mass spectra the contribution of ^{18}O-isotope species to peak intensity of ^{13}C-carbon dioxide must be also taken into account. These spectral changes might result in the wrong diagnosis for *H.pylori* infection. Therefore it is important to investigate the ^{18}O-isotope effect on infra-red absorption or mass spectra(1). For this purpose, we needed to establish the analytical method of ^{18}O abundance in ^{13}C-urea. Whereas Kajiwara and his collaborators(2, 3) estimated ^{18}O abundance in commercial ^{13}C-urea, using ^{13}C-urea which was synthesized from ^{13}C-potassium cyanide having no oxygen atom. As the result, they found out that ^{18}O atom% in commercial ^{13}C-urea reached to about fifty times of natural abundance.

In this report, an alternative method of ^{18}O analysis in ^{13}C, ^{18}O-urea is described in detail.

Materials and Methods

^{13}C-urea(Lot No.MTI-10034-S2), which was purchased from MassTrace (Somerville, MA, USA), was used in this experiment. Two samples of ^{13}C-urea(Lot No.9611and 9612) by Good Manufacturing Practice(GMP) were also supplied from Kyowa Hakko Kogyo(Tokyo, Japan) to assay ^{13}C and ^{18}O abundance for the quality control. Reagent grade ^{12}C-urea and $HgCl_2$ were purchased from Nacalai Tesque(Kyoto, Japan). Also silver plates ($30 \times 4 \times 0.07$ mm) and quartz tubes consisting of inner tube (diameter: 6 mm) and outer tube(diameter: 9 mm)were obtained from Kyowa Pure Chemicals (Tokyo, Japan).

An analytical method of ^{18}O content of urea was developed using a Hitachi mass spectrometer Model RMI-2(Hitachi, Tokyo, Japan).

About 5 g each of ^{12}C-urea(A) and ^{13}C-urea(B) were weighed and dried to a constant weight at 60 ℃ over 40 h. For the validation and calibration study, A and B were accurately weighed to prepare seven samples with different isotope content as shown in Table 1. A and B were dissolved in 5 ml of deionized water. The solution was dried up at 60 ℃ for more than 40 h until its weight shows no change.

Reaction tubes, silver plates and other tools for analysis were heated at 540 ℃ for 2 h. About 20 mg of urea sample(Table 1) was transferred into the inner tube and inserted it into the outer tube. Next a silver plate with about 100 mg $HgCl_2$ was put into inner tube. Reaction tube was sealed at 1.3×10^{-3}

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Table 1; Isotope abundance of seven samples

Sample No.	Weight(mg)		Abundance(atom%)*	
	A	B	^{13}C	^{18}O
1	559.0	0	1.09	0.21
2	417.8	129.8	24.3	3.10
3	257.4	270.5	51.3	6.36
4	126.1	380.2	74.7	9.23
5	115.6	463.8	79.5	9.82
6	59.1	437.3	87.4	10.8
7	0	655.0	99.1	12.2

*observed.

Pa and heated at 430 ℃ for 4 h. After cooling it at room temperature, carbon dioxide formed was purified in vacuum using liquid nitrogen and dry ice-methanol to finally trap in a glass tube. Both ^{13}C and ^{18}O abundance in carbon dioxide were analyzed using the mass spectra in the m/z range 44 to 49 .

Analytical background

In the ^{13}C-urea breath test for *H.pylori* infected patients, twelve isotope species of carbon dioxide are derived from the urea containing ^{12}C, ^{13}C, ^{16}O, ^{17}O and ^{18}O in their breath(Table 2)(4).

Table 2; Isotope species of carbon dioxide and their contribution ratio to peak intensity in mass spectra

m/z	Species	^{12}C, ^{18}O -urea	^{13}C, ^{18}O -urea
44	(1) $^{12}C^{16}O^{16}O$	0.8012～0.9853	0.0081～0.01
45	(2) $^{13}C^{16}O^{16}O$	0.0081～0.00995	0.801～0.985
	(3) $^{12}C^{16}O^{17}O$	3.56×10^{-4}～3.95×10^{-4}	3.60×10^{-6}～3.99×10^{-6}
46	(4) $^{12}C^{16}O^{18}O$	0.0018～0.099	1.80×10^{-5}～9.98×10^{-4}
	(5) $^{13}C^{16}O^{17}O$	3.6×10^{-5}～3.99×10^{-4}	3.56×10^{-4}～3.95×10^{-4}
	(6) $^{12}C^{17}O^{17}O$	1.58×10^{-7}	1.60×10^{-9}
47	(7) $^{13}C^{16}O^{18}O$	1.80×10^{-5}～9.98×10^{-4}	0.0018～0.099
	(8) $^{12}C^{17}O^{18}O$	7.92×10^{-7}～3.36×10^{-5}	8×10^{-9}～4×10^{-7}
	(9) $^{13}C^{17}O^{17}O$	1.60×10^{-9}	1.58×10^{-7}
48	(10) $^{12}C^{18}O^{18}O$	3.96×10^{-6}～0.0099	4×10^{-8}～10^{-4}
	(11) $^{13}C^{17}O^{18}O$	8×10^{-9}～4×10^{-7}	7.90×10^{-7}～3.96×10^{-5}
49	(12) $^{13}C^{18}O^{18}O$	4×10^{-8}～10^{-4}	0.0099～3.96×10^{-8}

^{13}C atom% was calculated from m/z 45／m/z 44 and natural abundance of ^{17}O according to Saino(4).

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If ^{13}C and ^{18}O in ^{13}C-urea are highly abundant, the ^{18}O atom% can be evaluated by

$$^{18}\mathrm{O\ atom\%} = \frac{P46 + P47 + 2P48 + 2P49}{2(P44 + P45 + P46 + P47 + P48 + P49)} \times 100 \qquad (1)$$

where P46, P47, P48, and P49 represent the intensity of peaks to which m/z 46, 47, 48 and 49 correspond, respectively.

To investigate the effect of ^{18}O change in natural ^{12}C-urea on mass spectra, it is assumed that carbon is naturally abundant and ^{18}O changes from 0.2 to 10 atom% as follows(3):

^{12}C = 99 atom%
^{13}C = 1.0 atom%
^{16}O = 89.96～99.76 atom%
^{17}O = 0.04 atom%
^{18}O = 0.2～10 atom%

For this case, the contribution of P(3), P(5), P(6), P(7), P(8), P(9), P(11) and P(12) are less than 10^{-3} and negligible in Eq.(3) as given in Table 2.

In order to further investigate the effect of ^{18}O change in commercial ^{13}C-urea on mass spectra, ^{13}C and ^{18}O are assumed to be 99 and in the range from 0.2 to 10 atom%, respectively. The contribution of each isotope species to peak intensity was estimated, based on the following data(Table 2):

^{12}C = 1.0 atom%
^{13}C = 99 atom%
^{16}O = 99.76～89.96 atom%
^{17}O = 0.04 atom%
^{18}O = 0.2～10 atom%

As shown in Table 2, the contribution of P(3), P(4), P(5), P(6), P(8), P(9), P(10) and P(11) to the estimation of ^{18}O atom% in commercial ^{13}C-urea is less than 10^{-3} and can be neglected.

As summarized in Table 2, P(1), P(2), P(4), P(7), P(10) and P(12) should be taken into account in the calculation of ^{18}O atom%. In conclusion Eq.(1) can be generalized by

$$^{18}\mathrm{O\ atom\%} = \frac{P(4) + P(7) + 2P(10) + 2P(12)}{2[P(1) + P(2) + P(4) + P(7) + P(10) + P(12)]} \times 100 \qquad (2)$$

$$= \frac{P46 + P47 + 2P48 + 2P49}{2(P44 + P45 + P46 + P47 + P48 + P49)} \times 100 \qquad (3)$$

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Results and Discussion

Calibration

Regression analysis for intermediate precision data led to the excellent linear relationships between theoretical and observed values for ^{13}C and ^{18}O content of urea(Figure 1). It should be noted that the calibration can be made only in the ranges 1.09 to 99.1 atom% ^{13}C and 0.21 to 12.2 atom% ^{18}O.

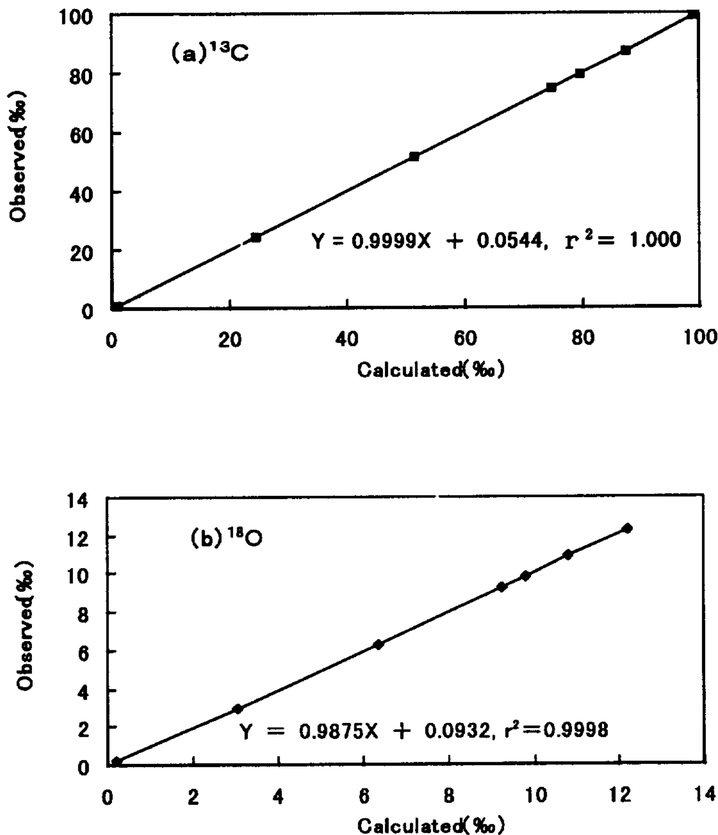

Figure 1; Calibration curves for ^{13}C and ^{18}O content in urea

Validation study

For the repeatability, three replicate measurements for three different samples, as presented in Table 3, were made on the same day.

For the intermediate precision, three replicate measurements for seven sample(Table 1) were made on three different days as shown in Table 4.

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Table 3; Repeatability results for the determination of ^{13}C and ^{18}O atom% in urea

Sample No.	^{13}C			^{18}O		
	Observed*1 (atom%)	R.S.D.*2 (%)	Accuracy*3 (%)	Observed*1 (atom%)	R.S.D.*2 (%)	Accuracy*4 (%)
1	99.2±0.159	0.16	100.1	12.2±0.024	0.20	100.0
2	51.5±0.149	0.29	100.4	6.30±0.009	0.15	99.06
3	24.1±0.066	0.27	99.18	2.95±0.012	0.42	95.16

Table 4; Results of intermediate precision for the estimation of ^{13}C and ^{18}O atom% in urea

Sample No.	^{13}C			^{18}O		
	Observed*1 (atom%)	R.S.D.*2 (%)	Accuracy*3 (%)	Observed*1 (atom%)	R.S.D.*2 (%)	Accuracy*4 (%)
1	1.09±0.009	0.81	99.96	0.21±0.002	1.09	100.16
2	24.2±0.097	0.40	99.47	2.94±0.012	0.41	94.87
3	51.2±0.30	0.61	99.78	6.26±0.043	0.69	98.36
4	74.8±0.147	0.20	100.12	9.23±0.026	0.28	99.95
5	79.5±0.084	0.11	100.08	9.84±0.030	0.31	100.25
6	87.2±0.070	0.080	99.80	10.9±0.025	0.23	100.57
7	99.0±0.051	0.052	99.92	12.3±0.059	0.48	101.02

*1 Mean±S.D.

*2 Relative standard deviation

*3 Mean accuracy(%) = (^{13}C atom%$_{observed}$/^{13}C atom%$_{theoretical}$) × 100

*4 Mean accuracy(%) = (^{18}O atom%$_{observed}$/ ^{18}O atom%$_{theoretical}$) × 100 .

In Table 4, sample 2 shows clearly the lower accuracy compared to other samples. It should be ascribed to the weighing error in sample preparation. It can be concluded that both accuracy and precision are sufficient to determine ^{13}C and ^{18}O atom% of urea. Therefore this analytical method makes it possible to estimate ^{13}C and ^{18}O atom% of commercial urea.

Determination of ^{13}C and ^{18}O atom% in ^{13}C-urea samples

Two GMP samples(Lot.9611 and 9612) were analyzed as summarized in Table 5.

Table 5; Analytical results of two GMP samples

Lot No.	^{13}C (atom%)	^{18}O(atom%)
9611	99.0	8.86
9612	99.1	11.6

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Acknowledgment

The authors would like to thank Mr.Takesi Matsuzaki, Mr.Masami Mori, Ms.Rie Sato and Mr.Kunimi Inoue for their assistance and discussion in this work.

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