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Short Communication

A simple method to determine bioethanol content in gasoline using two-step extraction and liquid scintillation counting

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ABSTRACT

A simple method for determining bioethanol content in gasoline containing bioethanol (denoted as E-gasoline in this study) is urgently required. Liquid scintillation counting (LSC) was employed based on the principle that ^{14}C exists in bioethanol but not in synthetic ethanol. Bioethanol was extracted in two steps by water from E-gasoline containing 3% (E3) or 10% (E10) bioethanol. The ^{14}C radioactivity was measured by LSC and converted to the amount of bioethanol. The bioethanol content in E-gasoline was determined precisely from the partition coefficient in the extraction and the amount of bioethanol in the water phases: 2.98 ± 0.10% for E3 and 10.0 ± 0.1% for E10 (means ± SD; $n = 3$). It appears that this method can be used to determine bioethanol content in E-gasoline quickly and easily.

1. Introduction

Recently, much attention has been paid to reducing CO_2 emissions to avoid further increases in global warming by greenhouse effects (Bojic and Mourdoukoutas, 2000; Chase et al., 2001; Apak, 2007), although further information is needed to fully understand the complex mechanisms involved. Under the Kyoto Protocol, the governments of some developed countries have committed to reducing their greenhouse gas emissions. In general, it has been accepted that CO_2 derived from fossil fuels is rapidly increasing and is the dominant human-influenced greenhouse gas (Albritton and Meira Filho, 2001). Biofuel-blended gasoline has the potential to significantly reduce these emissions. Biofuels are fuels with biological origins (i.e., biomass), and their burning results in no net release of CO_2 in the atmosphere. Therefore, their use is regarded as "carbon neutral."

Bioethanol is produced by alcoholic fermentation of renewable sources of biomass such as crops. It is the most promising biofuel because of its productivity and safety. The use of gasoline containing 3–10 vol.% bioethanol (denoted as E-gasoline in this study) is being promoted around the world. However, bioethanol is currently much more expensive than gasoline (Demirbas, 2007); therefore, some countries have imposed lowered taxes on E-gasoline consumption to stimulate its use. To facilitate the rise in demand, a simple method is urgently required for determining bioethanol content in E-gasoline.

Bioethanol and fossil ethanol are chemically identical and not distinguishable by classical analytical methods, such as chromatography or spectroscopy. The only difference is in the radioactivity derived from ^{14}C (the half life of 5730 y); bioethanol has ^{14}C radioactivity originating from atmospheric CO_2, whereas the radioactivity of fossil fuels has completely decayed over millions of years. Liquid scintillation counting (LSC) can be used to measure the ^{14}C radioactivity. Fuel samples have been directly measured (Dijs et al., 2006), but fuel dyes are added to gasoline, potentially disrupting quantitative measurement by means of LSC by quenching effects. We have recently reported that clear ethanol can be separated from dyed E-gasoline by water extraction (Saito and Nakamura, 2007a). Furthermore, we have reported that two-step extraction enables us to determine bioethanol content by means of LSC (Saito and Nakamura, 2007b). This paper describes the modification of the extraction protocol and the accuracy and reproducibility of bioethanol content determination in E-gasoline.

2. Methods

2.1. Reagents and materials

Gasoline was purchased from a gas station in Japan (Idemitsu Kosan Co. Ltd., Tokyo, Japan). Ethanol (high-performance liquid chromatography grade) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as "bioethanol." Methanol was also purchased from the same manufacturer. A scintillation cocktail was purchased from Nakalai Tesque (Clear-sol II; Kyoto, Japan). Polypropylene (PP) bottles with a tapered bottom were

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purchased from BD Bioscience (225 mL conical centrifuge tube; Tokyo, Japan).

2.2. Preparation of E-gasoline

Two types of E-gasoline (E3 and E10) were prepared. Bioethanol and gasoline were blended by the weight ratios of 3:97 and 10:90, respectively, aliquoted in the PP bottles (100 g), and left at room temperature (18 °C) for 3 h before the extraction of bioethanol.

2.3. Extraction of bioethanol from E-gasoline

Water (3 mL) was added to each bottle containing 100 g of E3 ($n = 3$), shaken for 1 min, and left to stand for 10 min, resulting in the separation of the upper oil phase from the lower water phase. The water phase was collected using a pipette having a PP tip and set in an LSC glass vial. It was then diluted with the scintillation cocktail to an approximate volume of 15 mL (first extraction). An additional water extraction was performed for the residual oil phase (second extraction). The water phase was collected and LSC samples were prepared in manners similar to those in the first extraction. Some LSC samples showed turbidity, causing gradual phase separation. Such samples were diluted with the same volume of the scintillation cocktail.

In the case of the extraction from E10 ($n = 3$), 10 mL of water was added to each bottle containing 100 g of E10. The procedure followed to prepare the LSC sample was the same as for E3, except that the water phases obtained were not fully used; an aliquot (4 g) of each water phase was fractionated and diluted with the scintillation cocktail.

The direct method was also investigated and compared with the proposed method. E3 (5 g) was directly diluted with the scintillation cocktail ($n = 3$) and subjected to LSC measurement.

2.4. Preparation of bioethanol standard and background solutions for LSC

To prepare the bioethanol standard solution for the proposed method, bioethanol (0.5, 1, 2, or 3 g) and water (3 g) were blended in a glass vial for LSC measurements, followed by the addition of the scintillation cocktail to an approximate volume of 15 mL. The background solution was prepared by adding the scintillation cocktail to a mixture of methanol (3 g) and water (2 g). To prepare the bioethanol standard solution for the direct method, bioethanol (0.0952, 0.261, 0.5, or 1 g) and water (3 g) were blended in the glass vial and diluted with the scintillation cocktail as described above.

2.5. LSC measurements

LSC was measured using Tri–Carb 3180 TR/SL (PerkinElmer, Waltham, MA) in the ^{14}C normal counting mode. Counting for 50 min was repeated 10 times (total of 500 min) for each sample. The count per minute (CPM) of the background was subtracted from each CPM obtained from the samples and divided by the counting efficiencies. The disintegrations per minute (DPM) thus obtained were used as the ^{14}C radioactivity to determine the bioethanol content.

2.6. Theory for the determination of bioethanol content in E-gasoline

The amounts of bioethanol in the water phases for the first (A1) and second (A2) extractions are expressed by the following equations:

$$A1 = r \cdot C, \quad (1)$$

$$A2 = r \cdot (C - A1), \quad (2)$$

where r is the partition coefficient of bioethanol in the extraction and C is the initial amount of bioethanol in E-gasoline.

Eq. (3) is derived from (1) and (2):

$$C = A1 \cdot A1/(A1 - A2), \quad (3)$$

indicating that C can be calculated from A1 and A2. To convert DMP to A1 or A2, a standard curve was constructed from the bioethanol standard solutions.

2.7. Basic principle of bioethanol quantification by LSC

The proposed method for determining bioethanol content in E-gasoline consists of two-step extraction of bioethanol by water and subsequent LSC measurements of the water phases obtained. The basic principle of bioethanol quantification by LSC is as follows. Plants take up carbon from atmospheric CO_2 by photosynthesis. During this process, carbon isotopes such as ^{13}C and radioactive nuclide ^{14}C are incorporated into carbohydrates in plants as well as the major stable nuclide ^{12}C, where the concentration of these isotopes is almost identical to that in the atmosphere. It is assumed that the concentration of ^{14}C in the atmosphere is constant during the growth period of plants (within one year in many cases); the production of ^{14}C from ^{14}N by cosmic rays is in equilibrium with the radioactive decay of ^{14}C (half life of 5730 y). The carbohydrate components in plants that might grow over decades probably show slightly different ratios of $^{14}C/(^{12}C + ^{13}C)$ than are in the present atmosphere. However, this is not so crucial to the proposed method; in contrast to the ^{14}C in plants, the radioactivity of ^{14}C in fossil fuel from plants has completely decayed over millions of years.

3. Results and discussion

3.1. Bioethanol standard curve

Fig. 1a shows the standard curves constructed from the bioethanol standard solutions containing 3 g of water. DPM linearly increased with increasing amounts of bioethanol in the range of 0.5–3 g. In the extraction, water was added to E-gasoline so that the weight of the water was almost equal to that of bioethanol. As a result, DPM of the water phases was in the range of the standard curve.

3.2. Usefulness of polypropylene bottles for bioethanol extraction by water

The determination of bioethanol content requires quantitative LSC analyses of bioethanol, which are based on mixtures of water and a scintillation cocktail. In our preliminary studies for the determination of bioethanol content in E-gasoline, glass vessels were used for the extraction (Saito and Nakamura, 2007a,b). Glass has hydrophilic surfaces, promoting the adhesion of the water phases and resulting in incomplete separation from the residual oil phases. In this study, PP bottles were used to eliminate the adhesion of the water phase. The hydrophilic surfaces and tapered bottom allowed us to collect the water phase easily and completely.

3.3. Determination of bioethanol content in E-gasoline by LSC

The amounts of bioethanol in the water phases for the first and second extractions (A1 and A2) were determined from DPM using the standard curve described above. The bioethanol contents in E3 and E10 were determined from A1 and A2 using Eq. (3). The results are listed in Table 1. The bioethanol content was precisely determined for E3 and E10. The method comprises a simple extraction