Spectrophotometric Determination of Glucosamine and Its Analogous Amino Sugars with o-Hydroxyhydroquinonephthalein and Palladium(II)

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A simple and highly sensitive spectrophotometric method for the determination of glucosamine and its analogous amino sugars was established based on fading of the palladium(II)-o-hydroxyhydroquinonephthalein-hexadecyltrimethylammonium complex. In the determination of glucosamine, Beer’s law is obeyed in the range of 0.02 – 0.18 µg ml−1, with an effective molar absorptivity at 630 nm and the relative standard deviation being 8.4 × 10^−1 mol−1 cm−1 and 1.08% (n = 10). This method is about 70-times more sensitive than the Elson-Morgan method. The method was successfully applied to the assay of glucosamine in actual samples.

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Glucosamine, which is a component of proteoglycan, is found in and around the cells of the cartilage in people’s joints, and is currently used for the treatment of degenerative joint disease in small animals, and represents a novel approach for the treatment of degenerative conditions. Also, glucosamine is considered to be a dietary supplement, and as such the content uniformity as well as the glucosamine products are rarely determined. Methods for analyzing glucosamine include spectrophotometry,1 – 8 spectrofluorometry,9,10 liquid chromatography 11 – 13 and gas chromatography.14,15

On the other hand, glucosamine and its analogous amino sugars, such as mannosamine and galactosamine, have several donor atoms capable of metal complex formation and binding16,17 with copper(II), zinc(II), nickel(II), iron(III), cobalt(III) and so on. We have reported18 – 20 on simple and sensitive methods for various organic compounds based on the fading of a colored dye-metal complex. In the color reaction between o-hydroxyhydroquinonephthalein (QP) and palladium(II) (Pd(II)), we noticed that the color development of the QP-Pd(II) complex was interfered with small amounts of palladium(II), we noticed that the color development of a QP-Pd(II) colored complex. In the determination of glucosamine, Beer’s law is obeyed in the range of 0.02 – 0.18 µg ml−1, with an effective molar absorptivity at 630 nm and the relative standard deviation being 8.4 × 10^−1 mol−1 cm−1 and 1.08% (n = 10). This method is about 70-times more sensitive than the Elson-Morgan method. The method was successfully applied to the assay of glucosamine in actual samples.

Reagents and apparatus
A stock solution (1.0 × 10−2 M, 1 M = 1 mol dm−3) of D-glucosamine hydrochloride (Wako Pure Chem. Co. Ltd.) in water, and the working solution was prepared by suitable dilution of this stock solution as required. A solution (5.0 × 10−4 M) of palladium(II) was prepared from a stock solution (Wako Pure Chem. Co. Ltd., 1000 µg l−1) by dilution with water. A solution of QP, which had been synthesized according to a method described in the literature,21 was prepared in a 1.0 × 10−3 M methanol solution containing one drop of hydrochloric acid. A 1.0 × 10−2 M aqueous solution of hexadecyltrimethylammonium chloride (HTAC, Tokyo Kasei Kogyo Co.) was prepared by dissolving HTAC purified by recrystallization in water. A Shimadzu spectrophotometer (Model UV-160) with 1.0-cm matched silica cells was used for an absorbance measurement. The pH measurements were made with a Horiba (F-11) pH meter in combination with a calomel glass electrode.

Standard procedure for the determination of glucosamine
The following components were mixed in a 10-ml volumetric flask: a solution containing 0.2 – 1.8 µg of glucosamine, 0.6 ml of a 5.0 × 10−4 M Pd(II) solution, 1.5 ml of a 1.0 × 10−2 M HTAC solution, 2.5 ml of the buffer solution and 0.8 ml of a 5.0 × 10−4 M QP solution. The mixture was diluted to 10 ml with water, transferred into a test tube, mixed well and kept at 50°C for 30 min. After the solution had been cooled in water to room temperature, the difference in the absorbance (ΔA) between the resultant solution and a reagent blank solution prepared under the same conditions was measured at 630 nm against water.

Results and Discussion
Optimization of reaction variables
In order to examine the utility of a method for the
determination of glucosamine based on fading of a dye-metal colored complex, the color reactions between various dyes and metal ions were studied by comparing the situations in the presence of glucosamine. The effect of metal ions on the reaction system was investigated. Only palladium(II) was effective among various metal ions tested: palladium(II), copper(II), zinc(II), nickel(II), cobalt(II), iron(III), manganese(II) and molybdenum(VI). Next, the dyes arranged in the following order with respect to the sensitivity: Orange and 4-(2-pyridylazo)resorcinol. These dyes could be interfered very little. The results are summarized in Table 2.

Calibration curve

A calibration curve for glucosamine was constructed by the standard procedure. A good linear relationship was observed over 0.02 - 0.18 µg mL⁻¹ of glucosamine. The effective molar absorptivity (ε) was calculated from the slope of the calibration graph to be 8.4 x 10⁵ dm³ mol⁻¹ cm⁻¹. The relative standard deviation (RSD) for ten runs of 0.90 µg of glucosamine was 1.08%. The calibration curves for other amino sugars and related compounds were also constructed under the optimum conditions. As shown in Table 1, the sensitivities of mannosamine and galactosamine were somewhat low compared with that of glucosamine. The reaction between proteoglycans, such as hyaluronic acid and chondroitin sulfate, and the QP-Pd(II) solution were not studied in the present work. This method is about 70-times more sensitive than the Elson-Morgan method, 1,2 and gives excellent reproducibilities.

Interference of foreign substances

The interference from various foreign substances on the determination of glucosamine was examined. Inorganic ions, such as sodium, potassium, ammonium, calcium, magnesium, chloride, bromide, nitrate, sulfate, and phosphate, did not noticeably affect the accuracy of the determination of glucosamine, even when these ions were present in large excess amounts compared with that of glucosamine. A fairly large error appeared in the presence of copper(II), iron(III), zinc(II) and glucuronic acid. Organic substances, such as glucose, glycine, caffeine, urea, ascorbic acid and methylcellulose, interfered very little. The results are summarized in Table 2.

Application

The proposed method was applied to the determination of glucosamine in commercial dietary supplements (tablets). The contents of the tablets were accurately weighed and ground in a mortar to a fine powder. The requisite volume of powder was weighed, transferred into a 100-ml volumetric flask, diluted to the mark with water and filtered. An appropriate amount of the sample solution was taken and assayed according to the standard procedure. The accuracy of the proposed method was checked by a thorough replicate analysis of each spiked sample. Neat standards were prepared under the same conditions. The HTAC was the most effective in terms of sensitivity.

The color development in this reaction system did not occur instantaneously at room temperature. Thus, the effects of the incubation temperature and time were examined at 40, 50 and 60°C. A maximum and constant ΔA value was obtained by heating at 50°C for 30 min, followed by cooling in water to room temperature. The ΔA value remained constant for at least 3 h after the solution had been cooled to room temperature.

Figure 1 shows the absorption spectra of QP-Pd(II) in the presence or absence of glucosamine and QP solutions.

![Absorption spectra of QP-Pd(II) and QP solutions in the presence of glucosamine.](image)

<table>
<thead>
<tr>
<th>Substance</th>
<th>ε/dm³ mol⁻¹ cm⁻¹</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>8.4 x 10⁵</td>
<td>1.08</td>
</tr>
<tr>
<td>Mannosamine</td>
<td>5.7 x 10⁵</td>
<td>1.48</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>5.2 x 10⁵</td>
<td>1.36</td>
</tr>
<tr>
<td>UDP-N-Acetyl-glucosamine</td>
<td>4.0 x 10⁵</td>
<td>a,b</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.5 x 10⁵</td>
<td>a,b</td>
</tr>
</tbody>
</table>

a. Mean of 10 determinations.
b. Undetermined.
recoveries of added quantity were about 102 - 103%. This indicates that the proposed method gives accurate results. These results are given in Table 3.

Composition of the colored complex and reaction mechanism

In order to clarify the reaction mechanism, the composition of the colored complex was studied by Job’s method of continuous variation and the molar ratio method. The Pd(II)-to-QP ratio and the [Pd(II)]/[QP] ratio were 1:1 and 1:1, respectively. On the other hand, the glucosamine-to-Pd(II) ratio was 1:1 in the presence and absence of QP. It was found that the reaction rate between glucosamine and Pd(II) was much faster than between QP and Pd(II), by tracing the absorption spectrum of the Pd(II) solution. The experimental results imply that glucosamine, which has ligands containing oxygen and nitrogen atom as donor atoms, reacted with Pd(II) to give the complex.

In conclusion, a simple and highly sensitive spectrophotometric method for glucosamine and its analogous amino sugars was established with QP and Pd(II) in a HTAC micellar medium. This procedure is based on a decrease in the absorbance of the QP-Pd(II)-HTAC complex. The proposed method was much more sensitive than that of other spectrophotometric methods. The proposed method, owing to no need for solvent extraction, should be useful for a simple and highly sensitive determination of glucosamine in real samples.