# Determination of Trace Amounts of Nickel(II) with $\alpha$-(2-Benzimidazolyl)-$\alpha',\alpha''$-(*N*-5-nitro-2-pyridyl hydrazone) toluene in the Presence of Triton X-100 by Fluorescence Method

**Chan-Il Park,*  Hyun-Soo Kim,† and Ki-Won Cha†**

*Department of Chemistry, University of Inchon, Inchon 402-749, Korea*
*†Department of Chemistry, Inha University, Inchon 402-751, Korea*

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A method is described for the fluorimetric determination of nickel, based on the formation of Ni(II)-$\alpha$-(2-Benzimidazolyl)-$\alpha',\alpha''$-(N-5-Nitro-2-Pyridylhydrazone)-toluene complex in the presence of a non-ionic surfactant. The complex has practically no fluorescence in the absence of surfactant, but the addition of Triton X-100 makes possible the fluorimetric determination of low concentrations of Ni(II) as it enhances the fluorescence intensity of the complex by up to about 5-fold. This method is very sensitive and selective for the direct determination of nickel ion. The optimum conditions are a Triton X-100 concentration of 2.0 mL (5.0%, v/v) and pH 9.0 ± 0.2 (ammonium chloride-ammonia buffer). The fluorescence is measured at 337 nm of emission wavelength under 300 nm of excitation wavelength. The fluorescence intensity is a linear function of the concentration of Ni(II) in the range 5-70 ng/mL, and the detection limit is 2.0 ng/mL. The proposed method has been successfully applied to the determination of trace amounts of Ni(II) in food and human hair samples.

## Introduction

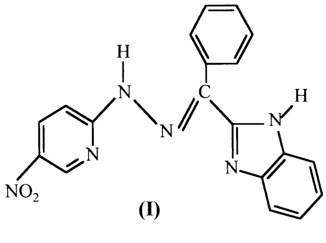
In recent years, surfactants have become of great interest because they provide a reaction medium in which they improved the sensitivity and selectivity of numerous reactions, and the metal complexes formed in micellar media are generally more stable than those formed in the absence of micelles.

The analytical method based on the photometric technique[1-10] is widely used, but surfactants are also being applied successfully in fluorimetry,[11-13] giving even higher sensitivity and lower detection limits.

Hydrazones (characterized by the grouping C=N-N, and related to Schiff's bases) are used as photometric and fluorimetric analytical reagents for the determination of metal ions.[14-20] Because of the inherent sensitivity of the fluorescence technique, fluorescence methods are being used increasingly in biochemical, medical and chemical research.

In a previous study,[21] the author synthesized $\alpha$-(2-Benzimidazolyl)-$\alpha',\alpha''$-(N-5-nitro-2-pyridylhydrazone) toluene [BINPHT, (I)], which showed a sensitive spectrophotometric reagent for Cu(II). In this paper, a sensitive fluorimetric method for the determination of Ni(II) with BINPHT is described.

In this paper, we have disclosed the merits of spectrofluorimetry in the study of the complex formed from BINPHT and Ni(II) ion under optimized optimized experimental conditions, and we have developed a fluorimetric method for the determination of Ni(II) in the presence of Triton X-100 as well. This procedure has been used to determine Ni(II) in food and human hair samples with satisfactory results.



(I)

## Experimental Section

The synthesis of BINPHT has been described previously.[21]

**Apparatus and reagent**. All fluorescence measurements were made with a Shimadzu RF-5301 Spectrofluorophotometer using 1-cm quartz cells. The slit width was set at 5 nm for excitation and 10 nm for emission monochromators. All pH measurements were made with a NOVA-310 pH Meter.

All materials and solvents were of analytical-grade and were provided by BDH Chemicals (UK). The working standard solution of nickel (10 ppm) was prepared by diluting Ni(II) AAS standard solution (1000 ppm) with distilled water. The following buffered solutions were prepared: hydrochloric acid-potassium hydrogen phthalate (pH 1~3); acetic acid-sodium acetate (pH 3~6); potassium dihydrogen phosphate-sodium hydroxide (pH 7); and ammonium chloride-ammonia (pH 8~9.5). They were used to adjust individual pH conditions. Surfactant solutions ($1\times10^{-2}$ M) were prepared by dissolving a suitable amount of surfactants (Cationic, Anionic, and Nonionic surfactants) in water with gentle heating.

$\alpha$-(2-Benzimidazolyl)-$\alpha',\alpha''$-(N-5-nitro-2-pyridylhydrazone) toluene ($1.0\times10^{-3}$ M) was prepared by dissolving 0.358 g of the reagent in 1000 mL of absolute methanol. BINPHT solution was allowed to settle for a long period of time in a brown bottle or in a dark place shielded from light.

**General procedure**. An appropriate amount of working standard nickel(II) solution was transferred to several 50 mL volumetric flasks. 5.0 mL of BINPHT solution, 5.0 mL of

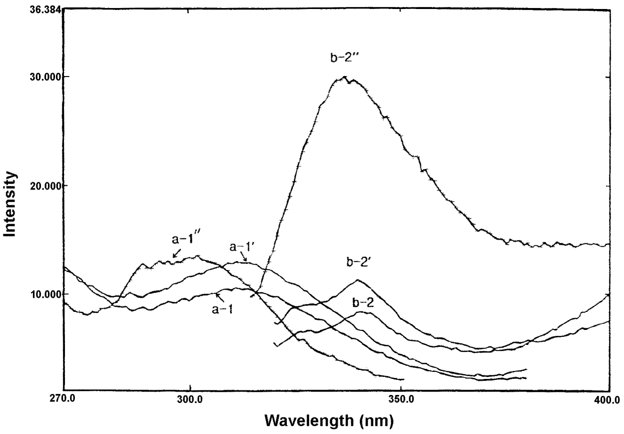


**Figure 1**. Fluorescence spectra. (a) Excitation and (b) Emissior spectra of BINPHT (1,2), Ni(II)-BINPHT (1',2') and Ni(II)-BINPHT-Triton X-100 (1'',2'') in 20% water and 80% methano medium. condition: 50 ng/mL Ni(II), 1.0× $10^{-3}$ M BINPHT, pH 9.0.

buffer solution (pH 9.0) and 2.0 mL of various surfactants were added to each 50 mL volumetric flasks, then diluted to the mark with methanol. After leaving undisturbed for about 10 min, the fluorescence intensity was measured at an emission wavelenght of 337 nm with an excitation of 300 nm.

The optimum concentration of methanol solution is recommended, which corresponds to 80% (v/v) methanol in the final mixing solution.

## Results and Discussion

**Fluorescence spectra**. Figure 1 shows the excitation and emission spectra of the BINPHT, Ni(II)-BINPHT and Ni(II)-BINPHT-Triton-X 100 systems (50 ng/mL of Ni(II) and pH 9.0). Fluorescence intensity in the presence of a nonionic surfactant such as Trition X-100 was 5 times higher than the intensity in the absence of it at 300 nm excitation and 337 nm emission wavelength. Hence, such wavelengths were used throughout the experiment. The reagent blank was found to exhibit a fluorescent maxima in the 339 nm of the emission wavelength but its intensity was very low.

**Optimum experimental conditions**. The system was optimized by varying each experimental condition in turn as the others were kept constant.

The pH of the medium had a great effect on the fluorescence intensity of the Ni(II)-BINPHT complex. The experimental results show that in a strongly alkaline or strongly acidic medium, the fluorescence intensities of the Ni(II)-BINPHT complexes are very low and that the optimum pH range for complex formation is between 8.5 and 9.5. Therefore, for all measurements of fluorescence intensity we selected a pH 9.0 buffer, which was prepared with 0.1 M ammonium salt.

In respect to concentration range, the fluorescence intensity of the systems increased with rising concentration of the reagents, reaching a maximum and remaining constant. The influence of the amount of BINPHT used on the fluorescence intensities of solution containing 50 ppb of Ni(II) was studied under the conditions established above. The fluorescence intensity increased as the amount of BINPHT was increased up to 4.0 mL (1.0× $10^{-4}$ M), remaining constant between 4.0-7.0 mL and decreasing slowly thereafter. Thus, 5.0 mL of BINPHT solution was selected to ensure a sufficient excess of the reagent throughout the experiment.

The effect of temperature on the fluorescence measurements was studied by heating the cell thermostatically between 10 °C and 60 °C. The fluorescence intensity was constant up to 32 °C, decreasing gradually thereafter. The temperature of the sample was kept at 25 °C (room temperature) throughout the experiment. After the fluorescence intensity of the systems reached the maximum, it remains constant for at least 80 min at room temperature. Continuous exposure for 3 hrs after first measuring the fluorescence intensity led to a reduction of 7.5% in fluorescence intensity.

Figure 2 shows that the fluorescence intensity of the Ni(II)-BINPHT-Triton X-100 system increases with increasing concentration of the methanol solution. The concentration of methanol is calculated based on the percentage concentration of pure methanol solvent (99.8%) contained in the sample solution. Figure 2 shows that the fluorescence intensity of the Ni(II)-BINPHT-Triton X-100 system increased in succession and that it increased remarkably at the 80% (v/v) methanol solvent in solution, however, further increases in methanol concentration did not result in comparable increases in fluoresecence intensity. Therefore, all experiments were carried out with methanol concentration of 80% in sample solution.

**Effect of surfactant on the fluorescence intensity of Ni(II)-BINPHT complex**. The effects of various surfactants were studied. The effects of cationic surfactants [cetylpyridinium chloride (CPC), cetyltrimethylammonium bromide (CTMAB), dodecyltrimethyl-ammonium bromide (DTMAB), myristyltrimethylammonium bromide (MTMAB)], nonionic surfactants [Brij 92, Brij 58, Brij 35, Triton X-100] and anionic surfactant [sodium dodecyl sulfate (SDS)] on the Ni(II)-BINPHT complexs were studied. As shown in Table

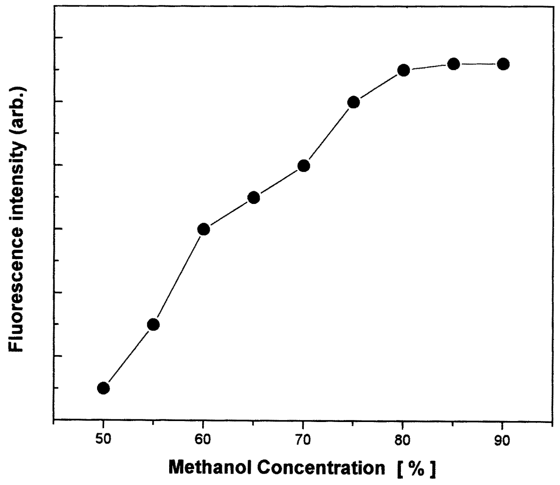


**Figure 2**. The effect of methanol concentration. Ni(II): 50 ng/mL BINPHT: 1.0 × $10^{-4}$ M, pH 9.0.

**Table 1**. Fluorescent characteristics of Ni(II)-BINPHT in the different surfactant. (Ni(II): 50 ng/mL, BINPHT: $1.0\times10^{-4}$ M, pH: 9.0)

| Surfactant | Concentration | $\lambda_{max}$ | Flu. Intensity | RFI |
|---|---|---|---|---|
| | [ M ] | (nm) | [Em.] | [Em] |
| None | – | 327 | 15.5 | 1.00 |
| CTMAB | $1\times10^{-3}$ | 336 | 11.6 | 0.75 |
| DTMAB | $1\times10^{-3}$ | 336 | 17.6 | 1.14 |
| MTMAB | $1\times10^{-3}$ | 336 | 17.1 | 1.10 |
| CPC | $1\times10^{-3}$ | 337 | 19.9 | 1.28 |
| Triton X-100 | $1\times10^{-3}$ | 335 | 32.2 | 2.08 |
| Brij 92 | $1\times10^{-3}$ | 335 | 20.1 | 1.30 |
| Brij 58 | $1\times10^{-3}$ | 331 | 7.2 | 0.46 |
| SDS | $1\times10^{-3}$ | 336 | 16.5 | 1.06 |

1, the nonionic surfactants led to an increase in fluorescence intensity, but in the anionic and cationic surfactants showed no positive effect or diminished the fluorescence intensity of the Ni(II)-BINPHT complex. As shown in Table 1, the use of the surfactant Triton X-100 as a nonionic surfactant was most effective in improving fluorescence intensity. This suggests that the Ni(II)-BINPHT complex interacts with the nonionic surfactant and forms the micellar medium in hydrophobic solvation of the chelate, improving solubility. The fluorescence intensity increased greatly with an increase in Triton X-100 concentration until 2.0-3.0 mL (5.0%, v/v), but the fluorescence intensity increased more slowly with further increases in Triton X-100. Therefore, 2.0 mL of Triton X-100 was selected for further investigations.

The molar ratio of the Ni(II)-BINPHT complex in the presence of $2.0\times10^{-3}$ M Triton X-100 was also determined using the continuous variation method. The maximum fluorescence intensity was obtained at 1 : 2 molar ratio of Ni(II) and BINPHT. That is, the molar ratio of Ni(II) and BINPHT in the Ni(II)-BINPHT complex with the Triton X-100 is 1 : 2. This means that bivalent Ni(II) ion bonds with two BINPHT molecules acting as a ligand to form the most stable chelate compound, which shows the maximum fluorescence intensity.

**Calibration range, sensitivity and precision**. Under the above operating conditions, there was a satisfactory linear relationship between fluorescence intensity and Ni(II) concentration in the range 5-70 ng/mL. The resultsare plotted in Figure 3. The detection limit was 2.0 ng/mL and the relative standard deviation (n=5) was 3.0% for determinations of 50 ng/mL Ni(II).

**Effect of foreign ions**. To assess the usefulness of this method, the effects of foreign ions, which often interfere the determination of Ni(II), were studied. The tolerance limits of some ions for the determination of 50 ng/mL Ni(II) by the above-mentioned procedure were studied, and the results are given in Table 2. The relative error was less than ± 5.0%. This shows that most of foreign ions examined did not greatly interfere, even when present in large amounts. However, Cu(II) and Fe(III) ions, which form strong compexes with BINPHT, caused severe interference. The most interference for all the systems was found at X concentration of copper and 2X concentration of iron for X concentration of nickel, but the interference could be removed by adding an excessive amount of sodium thiosulfate and potassium cyanide solution as a masking agent, respectively.

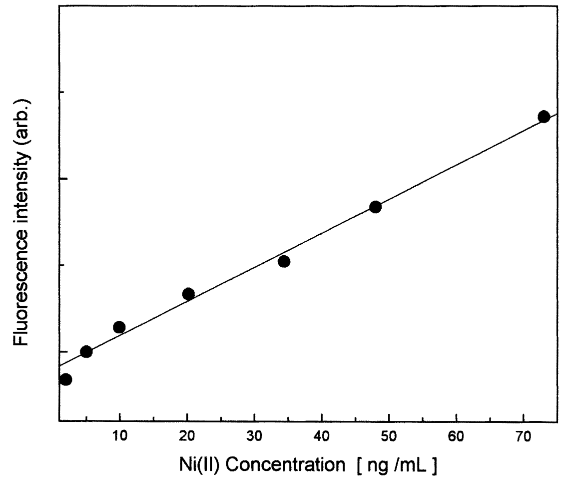


**Figure 3**. Calibration curve of Ni(II)-BINPHT-Triton X-100 a pH 9.0 using optimized procedure.

**Table 2**. Tolerance of foreign ions in the determination of 50 ng/mL Ni(II)

| Ions added | Tolerance ratio (W/W) |
|---|---|
| Na(I), $NO_3^-$ | 2500 |
| K(I), $Cl^-$ | 2000 |
| Citric acid | 1200 |
| Mg(II), $SO_4^{2-}$, Malonic acid | 500 |
| Pr(III), Cyanide | 200 |
| Mn(II), Cd(II), Citric acid | 100 |
| Zn(II), Co(II), Sn(II), Pb(II) | 80 |
| Pt(III), Zr(IV) | 20 |
| Pd(II), Au(III) | 10 |
| Mo(VI), Sm(III) | 7 |
| Sc(III), Cr(III) | 5 |
| Ge(IV) | 3 |
| Fe(III) | 2 |
| Cu(II) | 1 |

**Practical application**. The proposed method has been satisfactorily applied to the determination of Ni(II) in food and human hair samples.

**Determination of nickel in food and human hair samples**: The required amount of sample (5.0 g of the powdered coffee and 10 g of an orange fruit) was digested in a Kjeldahl flask with a $H_2SO_4$-$HNO_3$ mixture (1 : 3). The excess acid was then neutralized with concentrated sodium hydroxide, the solution was transferred to a 50 mL calibrated flask and diluted to marked volume with distilled water. The results are shown in Table 3. We used 1mL of this volume to perform the above-mentioned experimental procedure.

The proposed method was also applied to the analysis of hair samples. After the hair sample was washed with water, it was immersed in acetone for a day, then washed again

**Table 3**. Determination of Ni(II) in food and human hair samples

| Sample | This method* | | ICP |
|---|---|---|---|
| | Ni(II), Found (ppm) | RSD (%) | Ni(II), found (ppm) |
| powdered coffee (commercial brand) | 1.8 ± 0.1 | 2.4 | 1.7 |
| Orange | 1.0 ± 0.1 | 2.1 | 1.1 |
| Human hair I | 9.5 ± 0.1 | 3.5 | 9.7 |
| Human hair II | 9.1 ± 0.3 | 2.8 | 9.2 |

*Cu(II) and Fe(III) are masked by thiosulphate ($1.0 \times 10^{-3}$ M) and cyanide ($5.0 \times 10^{-4}$ M), respectively.

with water and dried under an infra-red lamp. After cooling, an appropriate amount of the sample was burned at low temperature then carbonized in a furnace at 700 °C for 30-60 min. After the residue was cooled to room temperature, it was extracted by heating in 1.0 mL of 9 M $H_2SO_4$. The solution was finally transferred to a 50 mL volumetric flask and diluted to the marked volume with water and analyzed in a procedure similar the one used for the food sample. The results are given in Table 3.

We scanned the background spectrum to investigate factors interfering with determining the Ni(II) in hair samples within the background solution itself. We found that the background spectrum was almost uniform without remarkably observed excitation and emission peaks. From the result of this background spectrum, we determined that the background solution gave rise to no serious trouble in analyzing the Ni(II) of samples.

Results from the proposed experimental method, the Ni(II) content was 1.8 ± 0.1 ppm (RSD 2.4%) for the powdered coffee, 1.0 ± 0.1 ppm (RSD 2.1%) for the orange fruit and 9.5 ± 0.1, 9.1 ± 0.3 ppm (RSD 3.5%, 2.8%, respectively) for the human hair. These values are in reasonable agreement with those determined by the ICP-AES.

## Conclusion

A simple, sensitive and selective spectrofluorimetric method for the determination of Ni(II) in methanol (80%, v/v) medium has been developed. In the presence of Triton X-100, Ni(II)-BINPHT complex has excitation and emission maxima at 300 nm and 337 nm, respectively. There was a linear relationship between Ni(II) concentration and fluorescence intensity over the range 570 ng/mL Ni(II). Among the different for experimental methods determing Ni(II), the instrumental system such as ICP or AAS may detect Ni(II) on the ng/mL level, but this calls for skilled operation. The proposed method can be used to determine Ni(II) on the 2.0 ng/mL level simply and selectively without using instrumental systems. Although further investigation may be necessary, this steady supports proposed methods for assay of trace amounts of Ni(II) ions in food and human hair samples.

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