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Spectrophotometric Investigation of the Pd(II)-Quercetin Complex in 50% Ethanol

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Summary. Composition and stability constant of the Pd(II)-quercetin complex were determined by suitable spectrophotometric methods and *pH*-metric measurements in 50% Ethanol. It was found that the Pd^{2+} ion and quercetin form a 1:1 complex in which Pd^{2+} is linked to quercetin through the carbonyl and the 3-hydroxyl group. The stability constant $\log\beta_1$ ranged from 6.05 at $pH = 5.00$ to 4.96 at $pH = 6.50$. The conditions for the spectrophotometric determination of quercetin by means of complex formation were investigated. *Beer*'s law was obeyed up to $5.00 \times 10^{-5}\,M$ quercetin.

Keywords. Complex; Quercetin; Palladium(II) chloride; Spectrophotometric methods.

Spektrophotometrische Untersuchung des Pd(II)-Quercetin-Komplexes in 50% Ethanol

Zusammenfassung. Zusammensetzung und Stabilitätskonstante des Pd(II)-Quercetin-Komplexes in 50% Ethanol wurden mittels geeigneter spektrophotometrischer Methoden und *pH*-metrischer Messungen bestimmt. Es wurde festgestellt, daß Pd^{2+} und Quercetin einen 1:1-Komplex bilden, in dem das Pd-Ion über seine Carbonyl- und 3-Hydroxylgruppe an Quercetin gebunden ist. Die Stabilitätskonstante $\log\beta_1$ bewegte sich im Bereich von 6.05 ($pH = 5.00$) bis 4.96 ($pH = 6.50$). Die Bedingungen für eine spektrophotometrische Bestimmung von Quercetin mittels Komplexierung wurden untersucht. Das *Beer*sche Gesetz wird bis zu $5.00 \times 10^{-5}\,M$ Quercetin befolgt.

Introduction

Quercetin belongs to the group of flavonoids, a very important class of phenolic compounds occurring in all parts of plants. Flavonoids have received increased attention during the last years because of their wide range of biological activities. They are present in various kinds of foods (fruits and vegetable), plant based beverages, and pharmaceutical preparations.

Quercetin ($C_{15}H_{10}O_7$) is a flavonoid of the flavonol type that contains five hydroxyl groups in positions 3, 5, 7, 3', and 4', and a carbonyl group in position 4; owing to these features, quercetin easily forms complexes with many metals.

Quercetin has been investigated spectrophotometrically using *via* interaction with metal ions [1–4] and has also been used as a reagent for molybdenum [5] and thorium [6] determination. On the other hand, $PdCl_2$ is widely used as a color forming reagent in spectrophotometric determinations of many drugs [7–9].

However, there are no data in the literature about Pd^{2+}-quercetin interaction. The aim of the present work is to investigated the Pd(II)-quercetin complex and to optimize this complexion reaction to develop a new and simple spectrophotometric method for quercetin determination using $PdCl_2$ as an analytical reagent.

Results and Discussion

Absorption spectra

The reaction of quercetin with Pd^{2+} ion was investigated at $pH = 6.2$ in hexamethylentetramin (*HMT*) buffer. The spectra were recorded from 350 to 500 nm, $PdCl_2$ having no significant absorbance in this region in the applied concentration. Quercetin shows a high absorbance value below 430 nm (Fig. 1, curve 2); and curve 1 denotes mixture of $PdCl_2$ and quercetin, whereas curve 3 is due to the formation of a complex between quercetin and Pd^{2+}. The complex has a yellow-orange color with an absorption maximum at 423.6 nm. Since the absorption of quercetin is negligible at wavelengths beyond 450 nm, all following measurements were performed at this wavelength against 50% ethanol.

Composition of the complex

The composition of the complex was determined by application of the method of continual variations of equimolar solutions [10] and by the molar ratios method [11]. For the first method, mixed solutions of $PdCl_2$ and quercetin with a total concentration of $c_o = 1.0 \times 10^{-3} M$ were used. The curve had a maximum at $x_{Pd} = 0.5$, denoting the formation of the Pd^{2+} : quercetin = 1 : 1 complex (Fig. 2). For the second method, solutions containing a constant concentration of $PdCl_2$ ($5.0 \times 10^{-4} M$ and varying quercetin concentrations (from 2.5×10^{-4} to $1.5 \times 10^{-3} M$) were used. A straight line $A = f(c_{querc}/c_{Pd})$ with an intercept at $c_{querc}/c_{Pd} = 1$ was obtained, thus proving the stoichiometric ratio of Pd^{2+} to quercetin to be 1:1 (Fig. 3).

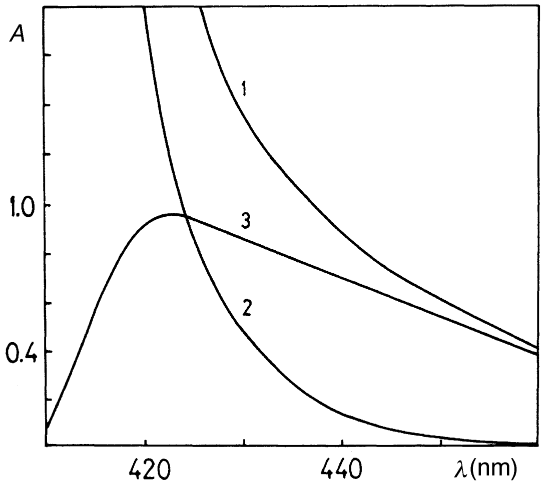

Fig. 1. Absorption spectra; curve 1: $2.50 \times 10^{-4} M$ $PdCl_2$ and $1.75 \times 10^{-3} M$ quercetin, blank: 50% ethanol; curve 2: $1.75 \times 10^{-3} M$ quercetin, blank: ethanol; curve 3: complex

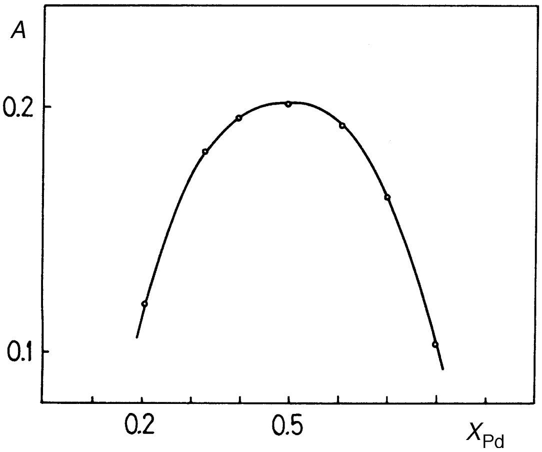

Fig. 2. Method of continual variations of equimolar solutions: $c_{Pd} = c_{querc} = 1.00 \times 10^{-3}\,M$, $\lambda = 450\,nm$

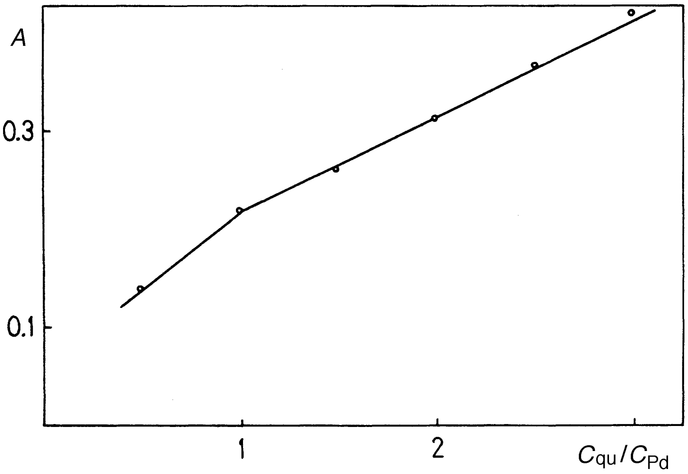

Fig. 3. Method of molar ratios: mixtures of $5.00 \times 10^{-4}\,M$ $PdCl_2$ and quercetin (from 2.50×10^{-4} to $1.50 \times 10^{-3}\,M$), $\lambda = 450\,nm$

Infrared spectra of quercetin and of the complex

Quercetin reacts in the dissociated form with metal ions forming complex compounds [1–3]. In flavonoids, increasing *pH* generally causes primarily dissociation of H^+ from the 3-hydroxyl group due to the formation of a strong hydrogen bond between the hydroxyl hydrogen and oxygen of the carbonyl group. Second, the hydrogen of the 5-hydroxyl group is affected; the last hydrogens to dissociate are those of the 3′- or 4′-hydroxyl groups. To find out the binding position of Pd^{2+} in *HMT*, IR spectra of quercetin and of the isolated complex were recorded. The IR spectrum of quercetin exhibits a strong band at $1660\,cm^{-1}$ due to the carbonyl group which is hydrogen bonded to the hydroxyl group in position 3 (free carbonyl groups have absorptions of about $50\,cm^{-1}$ towards higher wave numbers). In the IR spectrum of the complex, however, this band has vanished, and a new broad band at about $1680–1600\,cm^{-1}$ appears, indicating that complex formation of Pd^{2+} with quercetin takes place through the carbonyl and 3-hydroxyl

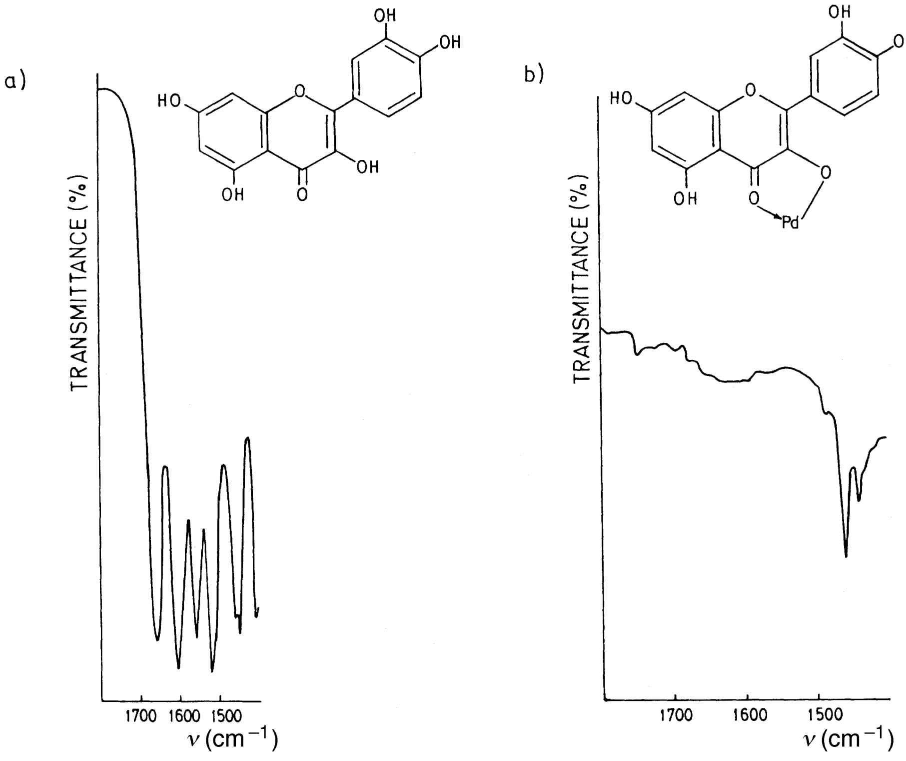

Fig. 4. IR spectra of a) quercetin and b) Pd(II)-quercetin complex

groups (Fig. 4). This corresponds with previous results of linking flavonoids with metal ions [12–13].

Effect of pH

The dependence of the absorption spectra of the Pd^{2+}-quercetin complex on *pH* was investigated in the *pH* range from 4.15 to 6.95 (Fig. 5, curves 1–4). The shapes of the absorption curves and the positions of the absorption maxima are independent of *pH*, indicating the formation of only one type of complex (with unique stoichiometry) at these *pH* values.

Stability constant of the complex

For the calculation of the stability constant of the complex, β_1, besides forthcoming equations, *Bjerrum*'s method [14] was partly used. For different *pH* values, the absorbances of a mixture containing $6.25 \times 10^{-5}\,M$ $PdCl_2$ and $1.25 \times 10^{-3}\,M$ quercetin (Fig. 6, curve 1) and of a solution containing $1.25 \times 10^{-3}\,M$ quercetin only (curve 2) were measured. Two curves were obtained, and from them the curve of the complex absorption $\Delta A = f(pH)$ was calculated (curve 3). The highest concentration of the complex was found at $pH = 6.80$, and it may be assumed that at that *pH* value the concentration of the complex is approximately equal to the

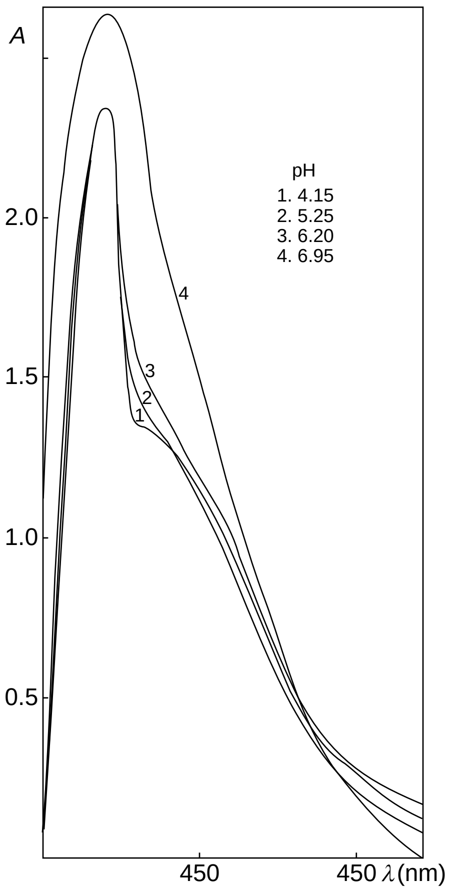

Fig. 5. Effect of *pH* on complex formation; $c_{\mathrm{Pd}} = 6.25 \times 10^{-5}\,M$, $c_{\mathrm{querc}} = 1.25 \times 10^{-3}\,M$

total $\mathrm{PdCl_2}$ concentration ($[Complex] \approx [\mathrm{Pd}^{2+}]_0$) in view of the fact that the concentration of quercetin in the solution is twenty times higher than that of $\mathrm{PdCl_2}$. Therefore, the molar absorptivity of the complex was calculated from the expression:

$$a = \frac{A_{\max}}{[\mathrm{Pd}^{2+}]_0}$$

The concentrations the complex, Pd^{2+}, and $\mathrm{C_{15}H_9O_7^-}$ ($[L^-]$) were calculated from the following equations:

$$Complex = \frac{A}{a},$$

$$[\mathrm{Pd}^{2+}]_0 = [\mathrm{Pd}^{2+}] + [Complex]$$

$$[\mathrm{H}L]_0 = [\mathrm{H}L] + [L^-] + [Complex]$$

$$k_{d_1} = \frac{[\mathrm{H}^+][L^-]}{[\mathrm{H}L]}$$

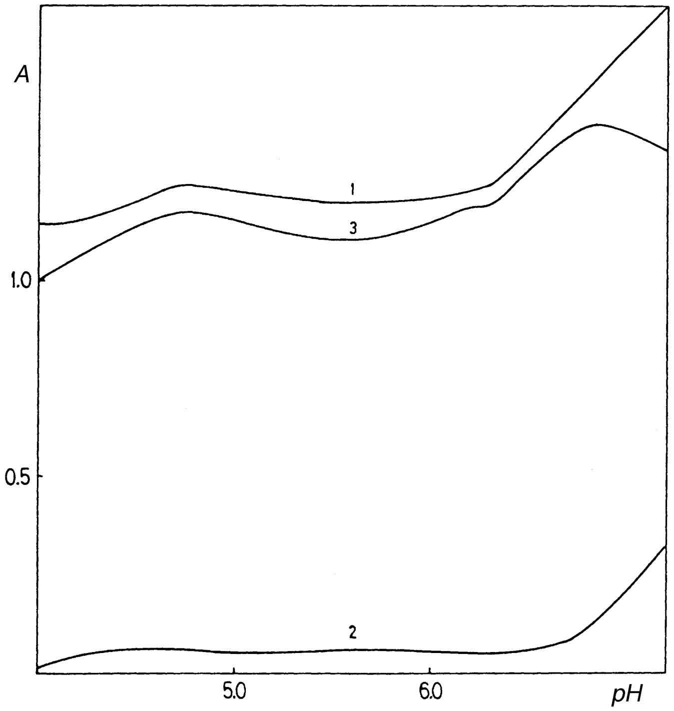

Fig. 6. Dependence of absorbance on *pH*; curve 1: mixture of $6.25 \times 10^{-5}\,M$ $PdCl_2$ and $1.25 \times 10^{-3}\,M$ quercetin, blank: 50% ethanol; curve 2: $1.25 \times 10^{-3}\,M$ quercetin, blank: ethanol; curve 3: $\Delta A = f(pH)$, $\lambda = 450\,nm$

Table 1. Stability constant of the Pd(II)-quercetin complex

pH	$[Pd^{2+}]$ (*M*)	[Complex] (*M*)	$[C_{15}H_9O_7^-]$ (M)	$\log \beta_1$
5	1.10×10^{-5}	5.13×10^{-5}	4.06×10^{-6}	6.05
5.5	1.34×10^{-5}	4.91×10^{-5}	1.28×10^{-5}	5.46
6	1.16×10^{-5}	5.09×10^{-5}	3.94×10^{-5}	5.04
6.5	5.34×10^{-6}	5.71×10^{-5}	1.16×10^{-4}	4.96

$a = 22400\,cm^{-1}\,mol^{-1}$

$k_{d_1} = 3.4 \times 10^{-8}$ is the first dissociation constant of quercetin in water-ethanolic solution [15]. The stability constant β_1 was calculated for four different *pH* values (Table 1).

$$\beta_1 = \frac{[Complex]}{[Pd^{2+}][L^-]}$$

Quantitative determination of quercetin

The formation of the complex between Pd^{2+} and quercetin permits the determination of microquantities of quercetin. Solutions with a constant $PdCl_2$ concentration ($1.00 \times 10^{-3}\,M$) and varying amounts of quercetin were prepared, and their absorbance was measured against $1.00 \times 10^{-3}\,M$ $PdCl_2$. A linear dependence of the absorbance on the concentration of quercetin was obtained for a concentration range of quercetin from 5.00×10^{-5} to $1.00 \times 10^{-3}\,M$. By the application of the method of least squares, the regression equation

Table 2. Spectrophotometric determination of quercetin

Taken (*M*)	Found (*M*)	SD	CV (%)
5.0×10^{-5}	5.38×10^{-5}	1.75×10^{-6}	3.25
5.0×10^{-4}	5.07×10^{-4}	1.16×10^{-5}	2.20
1.0×10^{-3}	1.02×10^{-3}	2.62×10^{-5}	2.56

$y = 288.173x + 0.00346$ was calculated with a correlation coefficient of $r = 0.99842$. The accuracy of the method was determined for three different quercetin concentrations (Table 2).

Materials and Methods

Apparatus

Spectrophotometric measurements were performed with a Beckman DU-650 spectrophotometer using a 1 cm quartz cell. For *pH*-metric measurements, a Radiometer pHM 28 *pH*-meter equipped with a saturated calomel-glass electrode was used. IR spectra (KBr pellets) were recorded on a 983 G Perkin Elmer spectrophotometer.

Reagents

The reagents used were: $PdCl_2$, ethanol, HCl, NaOH (all Merck); $NaNO_3$ (Mallinckrodt Chemica); quercetin (Fluka AG).

General procedure

All prepared solutions have been prepared in a mixture of water and ethanol in a 1:1 ratio, the solubility of both components ($PdCl_2$ and quercetin) having a maximum at this composition.

The palladium(II) chloride solution ($1.0 \times 10^{-2}\,M$) was prepared by dissolving $PdCl_2$ in water (to which 0.6 ml of concentrated hydrochloride acid had been added) by warming the mixture on a water bath. The solution was cooled and diluted to volume in a 250 ml volumetric flask.

Hexamethylentetramin buffer ($pH = 6.2$) was prepared by mixing 250 ml of 1 *M* hexamethylentetramin with 25 ml of 1 *M* HCl and diluted up to 500 ml.

All solutions were prepared in *HMT* buffer except for those used for the investigation of the *pH* effect on the complex absorption where pH was adjusted by addition of HCl and NaOH. The ionic strength of the final solutions was kept constant at 0.01 *M* by addition of $NaNO_3$.

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