HYDROTHERMAL SYNTHESIS AND DIELECTRIC PROPERTIES OF FERROELECTRIC SrBi₂Nb₂O₉ CERAMICS

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SrBi₂Nb₂O₉ (SBN) compound was synthesized by the hydrothermal method at 220°C for 8h. The dielectric characteristics of ferroelectric SBN ceramic in the 10 Hz – 13 MHz frequency range at various temperatures (300-950 K) have been studied. This study relates initially to measurements of dielectric constant (ε_r'), and the dielectric losses ($\tan\delta$) according to the temperature, as well as the determination of the Curie temperature of SBN. The nature of ferroelectric - paraelectric transition was then deduced. The bulk conductivity of SBN ceramic was performed at different temperatures and the activation energy was calculated.

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Introduction

Piezoelectric materials, used in devices working under severe thermal conditions, find applications increasingly. They occupy a very important place in the basic research as well as in electronics and micro-electronics industries. Indeed, such an important research is done nowadays in several fields where high temperature is a decisive parameter. One can quote the use as ultrasonic sensors functioning between 200 and 600 °C for the control in the course of service of equipment in chemical industry, metallurgical or in foundry. They can be also used as accelerometers for the fatigue inspection of the engines of plane or for the heat exchangers in the nuclear reactors. Among materials likely to agree with this type of realisation one finds those containing bismuth layer structure ferroelectric (BLSF) making it possible to reach the field of application until approximately 400 °C. These materials had sufficiently high Curie temperatures to be able to preserve piezoelectric properties[1].

The strontium bismuth niobate SrBi₂Nb₂O₉ (SBN) belongs to the Aurivillius phases of perovskite ferroelectrics with general chemical formula (Bi₂Aₘ₋₁BₘO₃ₘ₊₃) in which A is a large cation with 12-fold coordination such as (Na, K, Ca, Sr, Ba, Pb, Bi, Ln,…), B is a small cation suitable for 6-fold coordination such as (Fe, Ti, Nb, Mo, W,…) and m is an integer between 1 and 5. The structure consists of $(Bi_2O_2)^{2+}$ layers interleaved with perovskite-like $(A_{m-1}B_mO_{3m+1})^{2-}$ layers[2]. The basic crystal structure for m = 2, consisting of perovskite (NbO₆) octahedra separated at intervals by bismuth oxides $(Bi_2O_2)^{2+}$ planes[3].

SrBi₂Nb₂O₉ (SBN) is a ferroelectric material having a Curie temperature bordering 691 K.[4] The high Curie temperature makes it among the most promising materials useful for various applications like non-volatile memory (FeRAM), piezoelectric converters[5-6]. Many authors were interested in the preparation of SBN ceramics by the conventional solid-solid method[7-8]. Nowadays, there is a considerable interest for evaluation of new methods for the functional ceramics synthesis in order to carry out a better control of the physicochemical properties. The way of hydrothermal synthesis has a considerable technological potential due to obtaining nanomaterials at low temperatures of heat treatment[9-11].

In this work, SrBi₂Nb₂O₉ (SBN) complex solution was synthesized by the hydrothermal way using an autoclave introduced into a controlled furnace, by optimizing the temperature and the thermal processing time. Dielectric measurements were studied.

Material and methods

The SrNb₂Bi₂O₉ (SBN) complex solution was prepared by the hydrothermal way using an autoclave introduced into a controlled furnace. The product obtained was characterised by powder XRD for its phase purity. The XRD powder data was collected using a Bruker D8 Advance model diffractometer in the two theta range 20 – 70°. The resulting powders were added with 1 wt % of polyvinyl alcohol as a binder and compacted into disks (13 mm in diameter and 1 - 2 mm thick) at the pressure of 500 Kg/cm². The disks were supported on Al₂O₃ setters and sintered at 1100 °C for 2 h in air. Both sides of the ceramics were polished and sputtered with silver to form electrodes. A HP 4192A impedance gain phase analyser was used to measure the dielectric constant as a function of temperature on heating at frequencies of 10 Hz to 13 MHz. These measurements were taken by gradually increasing the temperature between 300 K and 950 K using a Pekly Hermann-Moritz model furnace.

The hydrothermal method is a new way of synthesis for ferroelectric materials. The principle of this method consists in introducing the reagents and a solvent into a vessel known as a digestion bomb. The bomb is then heated to increase the pressure. The starting chemicals were commercially available: Sr(NO₃)₂ (MERCK, 99%), Bi(CH₃COO)₃ (INTERCHIM), HCl (PANREAC), CH₃COOH (FLUKA, 99,5%), NaOH (ACROSS) and NbCl₅

(ALDRICH, 99,995%). Stoichiometric quantities of $Bi(CH_3COO)_3$ and $NbCl_5$ were dissolved respectively in CH_3COOH (glacial) and HCl (37%) to obtain transparent solutions. The later were mixed and then NaOH was added gradually in order to precipitate Nb and Bi basic hydroxides. The Nb basic hydroxide was washed several times with distilled water, to remove chloride anions. Starting from a $Sr(NO_3)_2$ solution, NaOH was added gradually, a white precipitate corresponding to the strontium hydroxide $Sr(OH)_2$ appears. These hydroxides were mixed and put under agitation for one hour. The final solution was poured in the presser container and then placed in a furnace for 2 - 8 h at 180-220 °C. The resulting powder was repeatedly washed and dried at 100 °C. The prepared powders were subjected to a heat treatment during 2 h at 400 °C to remove water of hydration.[12]

Results and discussion

In order to optimise the effect of time and temperature of hydrothermal treatment, we carried out several syntheses of SBN compositions by using various reaction times at temperatures which varied between 180 °C and 220 °C. Examination of DRX data confirmed that single phase samples had been obtained for SBN composition at 220 °C /8h. The observed diffraction data agreed well with reported powder data JCPDS- 89-8156. The X-rays pattern is given by Figure 1.
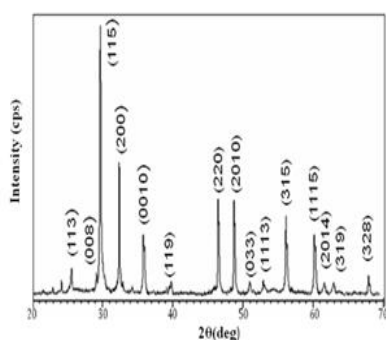
Figure 1. X-ray pattern of $SrBi_2Nb_2O_9$ (SBN) synthesized by hydrothermal reaction at 220°C/ 8h.

The electrical properties of the SBN ceramics were investigated by using a complex impedance spectroscopy technique in the frequency range covered (10 Hz to 13 MHz). In this technique, data could be analysed in terms of complex impedance (Z^*) and complex permittivity (ε_r^*). They are in turn related to each other as follows:

$$Z^* = \dfrac{1}{j\omega C_0 \varepsilon_r^*} = Z' + jZ'' \qquad (1)$$

where

ω is angular frequency ($2\pi f$),

C_0 is the vacuum capacitance of the present ample,

Z', Z'' are the real and imaginary parts of the complex impedance (Z^*).

The real (ε_r') and imaginary (ε_r'') parts of the complex dielectric constant (ε_r^*) were calculated from the following equations:

$$\varepsilon_r' = \dfrac{-Z''}{\omega C_0 \left(Z'^2 + Z''^2 \right)} \qquad (2)$$

$$\varepsilon_r'' = \dfrac{Z'}{\omega C_0 \left(Z'^2 + Z''^2 \right)} \qquad (3)$$

The dielectric factor dissipation tanδ, which represents the dielectric losses, is given by the following relation:

$$\tan \delta = \dfrac{\varepsilon_r''}{\varepsilon_r'} \qquad (4)$$

The bulk materials can be analysed by an equivalent circuit of one parallel resistance–capacitance (RC) element giving rise to arcs in complex plane. The impedance Z'' vs. Z' plots measured at (673, 723 and 773 K) and then at (823, 873 and 923 K) for $SrBi_2Nb_2O_9$ ceramic are shown in Figure 2. When the temperature increases, we note that the semicircles become smaller and shift towards lower Z' values. This phenomenon was also observed by Dhak et al .[13]
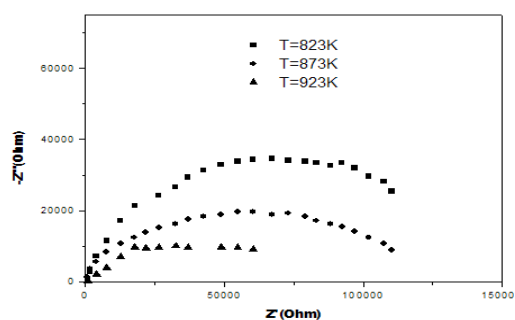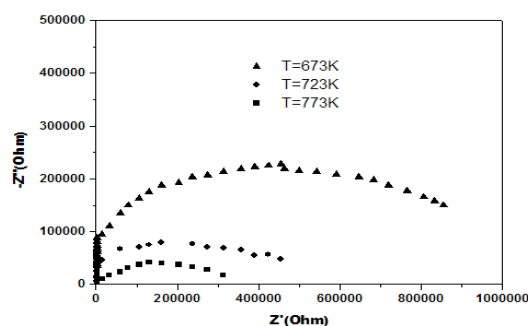
Figure 2. Complex impedance plots of SrBi$_2$Nb$_2$O$_9$ ceramic at different temperatures.

The temperature dependence of real ε_r' and tanδ of SBN ceramic are represented on Figures 3 and 4 respectively. The different frequencies used in this study are 1 KHz, 10 KHz and 1 MHz. Dispersion in frequencies is observed with maximum values of ε_r' at weak frequencies and with high temperatures (figure 3). This property appears to be a common feature in ferroelectrics materials associated with ionic type conductivity[8]. Moreover, for the various frequencies, the maximum of ε_r' occurs at 703 K. This temperature corresponds to the Curie temperature T$_C$ which indicates that the ferroelectric undergoes paraelectric transition. It should be noted that the dielectric constant ε_r' and the factor dissipation tanδ values at 10 kHz and 300 K are respectively, 148 and 0.010, while at T$_C$, these are 1500 and 0.35.
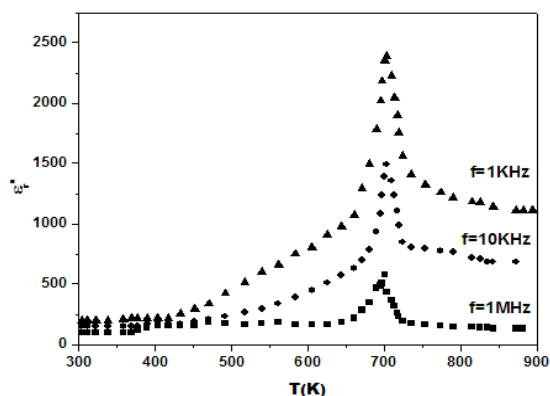
Figure 3. Temperature dependent dielectric constant of SrBi$_2$Nb$_2$O$_9$ ceramic measured at various frequencies.
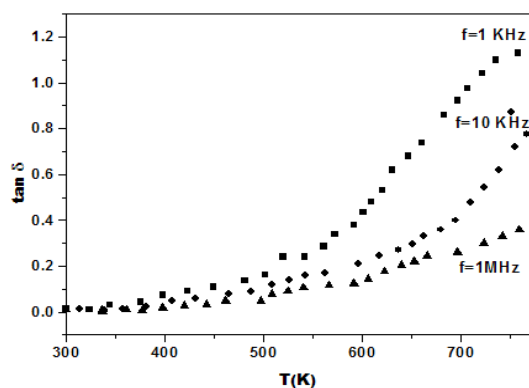

Figure 4. Temperature dependent tangent loss of SrBi$_2$Nb$_2$O$_9$ ceramic measured at various frequencies.

Into the bargain, the thermal evolution of the dielectric constant ε_r', has to T$_C$ a discontinuity which results in a maximum of ε_r'. In the paraelectric region, ε_r' follows a Curie - Weiss law of the following form:

$$\varepsilon_r' = \frac{C}{T - T_0} \qquad (5)$$

where C is the Curie constant.

With an aim to examine the nature of the ferroelectric paraelectric transition in SBN ceramic, the reciprocal dielectric constant $1/\varepsilon_r'$ is plotted at 1 kHz against temperature and is shown in Figure 5. The Curie - Weiss temperature (T_0 = 692 K) is obtained by linear extrapolation on the temperature axis and the value of the Curie - Weiss constant (C= 0.45 x 10^5 K) has been deduced from the slope. The T_0 value is less than T$_C$, this confirms that the nature of the ferroelectric - paraelectrique transition in SrBi$_2$Nb$_2$O$_9$ is of first order. These results are in agreement with those reported by Kajewski et al and Vankataraman et al.[8-14]
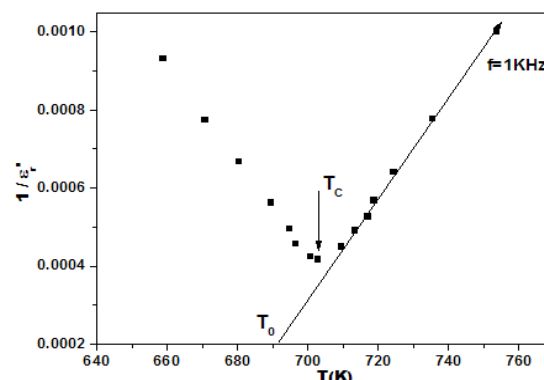
Figure 5. The inverse dielectric (1/ ε'$_r$) as a function of temperature at 1KHz for SBN ceramic.

The bulk conductivity σ of SBN ceramic was calculated starting from taken measurements and via the relation:

$$\sigma = \frac{e}{S \times R} \qquad (6)$$

where

     R is the resistance of the bulk (intersection of semicircle on the real-axis),

     e is the thickness and

     S is the area of the sample.

The curve ln(σ) according to the reciprocal temperature was plotted on Figure 6. The linearity of this curve is confirmed within the studied temperature range indicating that the conductivity of the ceramic followed the Arrhenius law:

$$\sigma = \sigma_0 \exp(-E_a / kT) \qquad (7)$$

where

     σ_o is a pre-exponential factor and

     E_a, represents the apparent activation energy for conduction process,

k is the Boltzmann constant

T is the absolute temperature.

The activation energies were deduced from the slope of the straight-line region. The value of activation energy is 0.924 eV, which corresponds to the activation energy to ionic conduction motion in perovskite materials and found to be in agreement with several reports [15-16]. It seems that the ionic nature of conductivity is related to the presence of defects and/or impurities in the network. In oxide ferroelectrics, doubly charged oxygen vacancies are the most mobile charges and play an important role in the conduction process [17]. The motion of oxygen vacancies is well known to give rise to activation energy of about 1 eV [18] in perovskite oxides at high temperature. Thus, the experimental activation energy value may be related to the
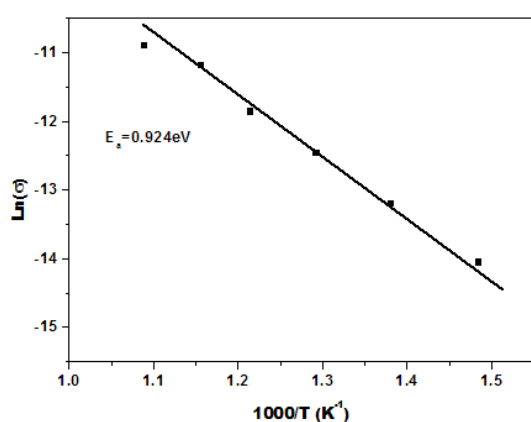
presence of oxygen ion vacancy concentration [8-19-20].

Figure 6. The inverse dielectric (1/ ε'$_r$) as a function of temperature at 1KHz for SBN ceramic.

Conclusions

Perovskite SrBi₂Nb₂O₉ (SBN) materials were prepared by hydrothermal method at different temperatures and times and conventionally sintered in air. X-ray diffractograms of the samples reveal the single phase layered perovskite structure formation. The dielectric and impedance behaviour of SBN ceramics were investigated over a wide temperature and frequency ranges. The Curie temperature was determined to be equal 703 K. The nature of the ferroelectric - paraelectric transition in SBN was confirmed to be of first order. Bulk conductivity of the samples was deduced at different temperatures through impedance investigations. The value of activation energy is   0.924 eV indicating the presence of ionic conductivity could be due to the mobility of the oxygen vacancies.

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AN OVERVIEW ON SYNTHETIC METHODS OF ISOAMYL BENZOATE

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Keywords: overview; synthetic study; isoamyl benzoate; catalysts

A few of synthetic methods of isoamyl benzoate using different catalysts such as sulfonic acid (p-toluene-sulfonic acid and aryl sulphonic acid), inorganic salt ($NH_4Fe(SO_4)_2 \cdot 12H_2O$, $NaHSO_4 \cdot H_2O$, $Fe_2(SO_4)_3 \cdot xH_2O$ and $FeCl_3 \cdot 6H_2O$), heteropolyacid ($H_4O_{40}W_{12}$ and $TiSiW_{12}O_{40}/TiO_2$) and solid super acid ($Ti(SO_4)_2/TiO_2$ and $Zr(SO_4)_2 \cdot 4H_2O$) have been discussed in the present paper. The yields of isoamyl benzoate have been improved by the addition of above catalysts. Due to simple process and low investment cost these methods are having an advantage over other conventional methods.

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INTRODUCTION

Isoamyl benzoate is not only a colourless liquid of fruit aroma but also one of the important chemical products.[1] Due to floral fragrance, it is widely used in different areas such as daily use chemical essence as a mobilizing agent for fragrance, type fragrance as a fixative for a white-tip clover or an orchid, food flavours as a mobilizing agent for apples, peaches, plums, almonds and cherries, etc.[2] It is also used as a solvent for fats or resins. Benzoic acid with concentrated sulphuric acid as a catalyst reacts with isoamyl alcohol to produce isoamyl benzoate, used in industry as manufacturing process. This method has a lot of disadvantages such as poor quality of products, serious equipment corrosion, complicated operating system and environmental pollution issues, etc. [3]

In the present paper, a new class of catalysts such as sulfonic acid (p-toluene-sulfonic acid and aryl sulphonic acid), inorganic salt ($NH_4Fe(SO_4)_2 \cdot 12H_2O$, $NaHSO_4 \cdot H_2O$, $Fe_2(SO_4)_3 \cdot xH_2O$ and $FeCl_3 \cdot 6H_2O$), heteropolyacid ($H_4O_{40}W_{12}$ and $TiSiW_{12}O_{40}/TiO_2$) and solid super acid ($Ti(SO_4)_2/TiO_2$ and $Zr(SO_4)_2 \cdot 4H_2O$) have been discussed.

DISCUSSION

Sulfonic acid as a catalyst to produce isoamyl benzoate

Ding Liangzhong[4] introduced the effect of the reaction conditions on the yield of isoamyl benzoate. Using p-toluene-sulfonic acid as a catalyst and benzoic acid and isoamyl alcohol as feedstocks produced isoamyl benzoate. The optimum conditions were the molar ratio of benzoic acid to isoamyl alcohol (1.0:3.0), the reaction time (2.5 hours) and the amount of p-toluene-sulfonic acid (1.25 g) respectively. The maximum yield and purity of isoamyl benzoate were 88.3% and 94.4%, respectively. The experimental results showed that p-toluene-sulfonic acid had good catalytic performance and low prices and was easily available. Its equipment corrosion and environmental pollution of p-toluene-sulfonic acid were less than that of concentrated sulphuric acid. Furthermore, p-toluene-sulfonic acid was one of the better catalysts and occupied the place of concentrated sulphuric acid.

Li Xiuyu[5] described the synthetic method of isoamyl benzoate and studied that the different conditions had an effect on the yield of isoamyl benzoate. Aryl sulphonic acid (ASA) and benzoic acid and isoamyl alcohol used as a catalyst and feedstocks respectively generated isoamyl benzoate. The experimental results represented that the best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of ASA were 2.0 hours, 1.0:2.0 and 0.73 g, respectively. The maximum yield of isoamyl benzoate was 98.35%. ASA used as a catalyst had good catalytic performance also.

Inorganic salt as a catalyst to generate isoamyl benzoate

Yu Shanxin[6] described the synthetic method of isoamyl benzoate by using $NH_4Fe(SO_4)_2 \cdot 12H_2O$ as a catalyst. The effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol, the amount of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ had been discussed. The best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol, the amount of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ were 4 hours, 1.0:2.0 and 4.0 g, respectively. The maximum yield of isoamyl benzoate was 82.3%.

Guang Shibin[7] explained why $NaHSO_4 \cdot H_2O$ as a catalyst took the place of concentrated sulfuric acid to generate isoamyl benzoate. The effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol, the amount of $NaHSO_4 \cdot H_2O$ had been discussed. The experimental results represented that the best conditions were the reaction time, the molar ratio of benzoic acid to isoamyl alcohol, the amount of $NaHSO_4 \cdot H_2O$ were 3 hours, 1.0:6.0:5.0 and 1.50 g, respectively. The maximum yield of isoamyl benzoate was 94.8%. Furthermore, $NaHSO_4 \cdot H_2O$ is very cheap, stable and insoluble in organic acids and organic alcohol, so it maybe is a good choice as a catalyst to produce isoamyl benzoate.

Liu Xinhe[8] described the synthetic method of isoamyl benzoate by using $Fe_2(SO_4)_3 \cdot xH_2O$ as a catalyst. The effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $Fe_2(SO_4)_3 \cdot xH_2O$ had been discussed. The best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $Fe_2(SO_4)_3 \cdot xH_2O$ were 3.0 hours, 1.0:3.0 and 0.49 g, respectively. The maximum yield and purity of isoamyl benzoate were 92.7% and 98.5%, respectively.

Zhang Hong [9] introduced the synthetic principle of isoamyl benzoate and the effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $FeCl_3 \cdot 6H_2O$ on the yield of isoamyl benzoate. The best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $FeCl_3 \cdot 6H_2O$ were 2.5 hours, 1.0:3.0 and 1.0 g, respectively. The maximum yield of isoamyl benzoate was 90.3%.

Heteropolyacid as a catalyst to produce isoamyl benzoate

Zhou Wenkai [10] introduced the preparation of $H_4O_{40}W_{12}$ and the effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $H_4O_{40}W_{12}$ on the yield of isoamyl benzoate. The best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $H_4O_{40}W_{12}$ were 1.5 hours, 1.0:1.5 and 0.18 g, respectively. The maximum yield of isoamyl benzoate was 78.0%.

Yang Shuijin [11] used $TiSiW_{12}O_{40}/TiO_2$ as a catalyst to produce isoamyl benzonate. The effect of the reaction conditions such as the reaction time, the reaction temperature, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $TiSiW_{12}O_{40}/TiO_2$ had been discussed. The best conditions were the reaction time, the reaction temperature, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $TiSiW_{12}O_{40}/TiO_2$ were 2.5 hours, 140 - 148°C, 1.0:4.0 and 5.93 g, respectively. The maximum yield of isoamyl benzonate was 83.3%.

Solid super acid as a catalyst to generate isoamyl benzoate

Zhang Fujuan [3] used $Ti(SO_4)_2/TiO_2$ as a catalyst to generate isoamyl benzoate. The effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $Ti(SO_4)_2/TiO_2$ had been discussed. The best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $Ti(SO_4)_2/TiO_2$ were 1.5 hours, 1.0:4.0 and 2.5 g, respectively. The maximum yield of isoamyl benzoate was 96.6%.

Zhang Fuxing [12] used $Zr(SO_4)_2 \cdot 4H_2O$ as a catalyst and benzoic acid and isoamyl alcohol as feedstocks to produce isoamyl benzoate. The effect of the reaction conditions such as the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $Zr(SO_4)_2 \cdot 4H_2O$ on the yield of isoamyl benzoate had been discussed. The best conditions were that the reaction time, the molar ratio of benzoic acid to isoamyl alcohol and the amount of $Zr(SO_4)_2 \cdot 4H_2O$ were 2.5 hours, 1.0:2.5 and 0.4 g, respectively. The highest yield of isoamyl benzoate was 96.3%.

CONCLUSION

Based on the above discussion and review, aryl sulphonic acid (ASA) is one of the best catalysts. It is used to produce the highest yields of isoamyl benzoate (98.35%). On the other hand, $H_4O_{40}W_{12}$ is one of the worst catalysts and its maximum yield of isoamyl benzoate only reaches 78.0%.

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THE IMPORTANCE OF SERUM SELENIUM CONCENTRATION MEASUREMENTS IN PATIENTS WITH MILD THYROID-ASSOCIATED ORBITOPATHY RECEIVING ADJUVANT SELENIUM SUPPLEMENTATION – A PILOT STUDY

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The essential trace element selenium (Se) exerts complex effects on the endocrine and immune systems, partly due to its antioxidant capacity. The importance of an optimal Se intake has been established in autoimmune thyroiditis. A recently published study reported beneficial results in Se supplemented patients with mild Graves' orbitopathy. Eight female patients with mild Graves' orbitopathy and three female patients with mild orbitopathy based on Hashimoto thyreoiditis participated in the study. The status of the orbita was registered at the beginning of the study and one-six months thereafter both according to the scores recommended by the American Thyroid Association (ATA) and clinical activity scores (CAS). The patients were treated according to their thyroid hormone status, their antibody results and their CAS. Moreover, they received adjuvant 100 or 200 µg Se as sodium-selenite. Serum Se concentrations were measured before Se supplementation was implemented and 1-6 months after the beginning of treatment.. ATA scores improved in 10 patients and worsened slightly in one female patient. CAS improved in all study participants. Mean Se concentrations were 75.08 ± 11.55 µg L^{-1} before supplementation was started and 91.35 ± 17.37 µg L^{-1} one-eight months later (p<0.05). At the beginning of the study, the majority of patients exhibited elevated antibody levels. After conventional treatment and adjuvant Se supplementation, antibody levels decreased or reached the normal range (except in the case of one patient).

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INTRODUCTION

Graves' orbitopathy is an autoimmune inflammatory disorder affecting the orbit around the eye, in particular the connective tissue and the external eye muscles. It is part of a systemic process caused by autoantibodies. It accompanies Graves' disease in about 10-30% of cases and is about 2-3 times more frequent in women than in men. The disease is characterized by upper eyelid retraction, swelling, redness, and bulging eyes. About 10% of patients do not have Graves' disease, but do have autoantibodies. Cigarette smoking raises the incidence significantly.[1,2]

Graves' orbitopathy can present in mild, moderately severe and active forms. The activity of the disease can be assessed by using the criteria of the American Thyroid Association (ATA) and/or the Clinical Activity Score (CAS). CAS may range from 1 in a mild case to maximum 10 in the most active form[3]. There are effective treatments available for the moderately severe and active forms, including therapy with glucocorticoids and orbital irradiation, or both[4,5]. Unfortunately, these therapies have many side effects and are avoided in mild forms of the disease which may often resolve spontaneously. However, many patients with mild Graves' orbitopathy have a substantial decrease in their quality of life.[6] Therefore, it would be advantageous to treat patients with mild Graves' orbitopathy with an affordable, well-tolerated and widely available agent.

Selenium, an essential trace element with antioxidant and immunomodulatory properties, has been recently shown by the European Group on Graves' Orbitopathy to significantly improve quality of life, reduce ocular involvement and slow progression of the disease in patients with mild Graves' orbitopathy in a 6-month study.[7] However, serum selenium concentrations were not measured in those 54 patients that were given sodium selenite. Our study, in contrary, focused on both the course of the disease in patients with mild Graves' orbitopathy and on the measurement of their serum selenium levels at the beginning of and during treatment.

METHODS

Eleven patients with thyroid-associated orbitopathy (eight female patients with mild Graves' orbitopathy and three female patients with mild orbitopathy based on autoimmune thyreoiditis) participated in the study. All study participants were recruited at the Polyclinic of the Hospitaller Brothers of St. John of God in Buda, Budapest, Hungary.

After the Ethics Committee had approved the study, informed consent was obtained from all study participants in writing before collection of blood samples. Approximately 10 ml of blood was collected from each individual after 12 hours of fasting. Blood was taken in metal-free glass tubes free of anticoagulant, using the standard venipuncture technique. Blood samples were allowed to clot and then samples were immediately centrifuged for 10 min at 2400

rpm. Serum was separated into metal-free plastic tubes and stored frozen at –80 ºC until analysis, on average for 2-3 months.

The status of the orbit was registered at the beginning of the study and one-six months thereafter both according to the scores recommended by ATA and CAS. The patients were treated conventionally according to their thyroid hormone status, their antibody results and their CAS. Moreover, they received adjuvant 100 or 200 μg Se as sodium-selenite. Serum Se concentrations were measured before Se supplementation was implemented and 1-6 months after the beginning of treatment. Clinical characteristics of all study participants including their smoking status are shown in Table 1.

Table 1. Clinical characteristics of study participants

	Study participants (n=11)
Age mean ± SD (years)	44.6 ± 15.2
Diagnosis: Graves' disease	8
Diagnosis: Hashimoto thyroiditis	3
Smokers	6
Length of disease (years)	0-10

Laboratory parameters were determined on a Hitachi 917 instrument. Thyroid-stimulating hormone (TSH) was determined by a method based on chemoluminescence.

Free thyroxin (fT4), free triiodothyronine (fT$_3$), thyrotropin receptor antibodies, anti-thyroglobulin antibodies (anti-Tg), and anti-thyroid peroxidase antibodies (anti-TPO) were measured using ECLIA.

Sample preparation for selenium determination was carried out in duplicate using the nitric acid - perchloric acid – sulphuric acid digestion procedure recommended by Hershey et al[8]. After digestion, selenium (VI) was reduced to selenium (IV) with hydrochloric acid. Serum selenium concentration was determined by atomic absorption spectrometry following hydride generation on a Solaar M5 AA Spectrometer, Thermo Elemental equipment. The analytical method was verified by analysis of a human reference serum.

Statistical analysis was performed using the Microsoft Excel program. Results were considered significant if p<0.05.

RESULTS AND DISCUSSION

All participating subjects exhibited mild thyroid-associated orbitopathy. According to their ATA scores, the cornea and vision were not affected.in any of the patients. Clinical activity scores ranged from 2 in the mildest case to 5 in the most severe case.

Mean Se concentrations were 75.08±11.55μg/l before supplementation was started and 91.35±17.37μg/l one-eight months later (p<0.05) which indicates that patients adhered to therapy. Moreover, it can be concluded that out of the many commercially available selenium compounds a drug was chosen whose absorption was effective.

ATA and Clinical activity scores improved in all 11 patients although some patients were treated only for a relatively short time interval as their symptoms improved rapidly and they did not need constant surveillance.

At the beginning of the study 4 patients had hyperthyroidism according to their TSH-levels, one patient had an elevated TSH-value, while TSH-levels were normal in 6 patients. TSH levels normalized in 3 patients during treatment.

Initially, all patients exhibited elevated thyrotropin receptor antibodies, nine study participants had elevated anti-thyroglobulin antibody (anti-Tg) and seven patients had high anti-thyroid peroxidase antibody levels (anti-TPO). After receiving conventional treatment and adjuvant Se supplementation for 4 months on average, thyrotropin receptor antibody levels decreased in all patients, anti-thyroglobulin antibody and anti-thyroid peroxidase antibody levels decreased in 10 and 11 patients, respectively.

Selenium methionine has been applied with convincing results in patients with autoimmune thyroiditis for the past 10 years[9,10]. The exact mechanism of the benefit achieved with selenium is not entirely clarified. The antioxidant properties of selenium as part of the selenoenzyme glutathione peroxidase[11] should exert a profound effect. However, recently selenium was also reported to have a dose-dependent inhibitory effect on the expression of HLA-DR molecules of thyrocytes induced by interferon-γ which indicates that the beneficial effect of selenium on autoimmune mechanism might be due to very complex mechanisms[12].

Similarly to autoimmune thyroiditis, the beneficial results reported in selenium-supplemented patients with thyroid-associated orbitopathy are difficult to explain. It should be noted, however, that most treated patients to date came from areas in which selenium levels are known to be marginally decreased in the general population[7]. This is also the case in Hungarian healthy blood donors who have been shown to be selenium-deficient[13] and Hungarian pregnant women who were reported to have very low serum selenium concentrations[14]. However, the frequent measurement of serum selenium concentrations in Se-supplemented subjects is of utmost importance and should always be performed in order to prevent Se overdose.

In summary, serum selenium concentrations increased in study participants while ATA scores and Clinical Activity Scores improved. Patients tolerated selenium supplementation and their compliance was adequate. There were no registered side effects during the study.

This is the first Hungarian study administering adjuvant Se supplementation of patients with mild thyroid-associated orbitopathy. Based on our results, patients currently enrolled in the study will continue Se supplementation for another 6 months.

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EFFECT OF SO₄²⁻ CONCENTRATION ON Ni(II) EXTRACTIONS FROM AQUEOUS SOLUTION USING 1-PHENYL-3-METHYL-4-TRICHLOROACETYL PYRAZOL-5-ONE IN CHLOROFORM

A. Sangoremi[a] J. Godwin[b], and B. A. Uzoukwu[c]

The extraction of Ni^{2+} from aqueous solutions of various concentrations of SO_4^{2-} ions in buffered media using 1-phenyl-l,3-methyl4-trichloroacetyl-pyrazolone-5 (HTCP) has been investigated. The synergistic effect of butanol on the extraction of the metal was also examined. Ni^{2+} was quantitatively extracted from solutions by HTCP only at high pH values (5 - 8) over the pH range 0 – 8 studied. However, addition of butanol resulted in a synergistic extraction of Ni^{2+} up to 97.78% over the range of pH 5.00 – 8.00 from aqueous buffer media containing 0.1M SO_4^{2-} ion concentration. Increased SO_4^{2-} ion concentration resulted in a slight decrease in the extraction of Ni^{2+} ion due to masking of the metal by the SO_4^{2-} ion. In addition, overall extraction was shifted to higher pH values for extraction into chloroform solution of the ligand for Ni^{2+} using butanol. The log K_{ex} and $pH_{1/2}$ of the various extraction systems were determined.

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Introduction

Studies have shown 4-acyl pyrazolone derivatives to be excellent metal extracting agents for various type of metals, ranging from lanthanides[1,2], actinides[3,4] and other transition metals[5,6]. These studies have shown that these ligands have the capability of extracting these metal ions at pH values lower than 2 to neutral as ion pair or adduct complexes[8]. Most of the 4-acyl pyrazolone derivatives studied have $pK_a > 4$ and in the quest to evaluate the performance of 4-acyl derivatives with lower pKa,

Umetani and Freiser[8] successfully studied the quantitative extraction of some lanthanides with 1-phenyl-3-methyl-4-trifluoroacetyl-pyrazolone-5 with pKa < 3 at pH values close to 1. 1-phenyl-3-methyl-4-trichloroacetyl-pyrazolone-5 (HTCP) with pK_a 3.02 shown in Figure 1 has also been reported to have been successfully used in the extraction of a range of metal ions.[7,9,10] Almost all extraction studies with ligands have shown that various concentrations of different anions in the aqueous phase containing the metal ions play an important role in the percentage extraction of these metal ions[5,7,9,10,11,12], which can either be enhancing or masking the extraction of the metal ions. The effects have been utilized in optimizing extractions and separation of metal ions[13,14].In continuation of our studies on the extractions of metal ions with the ligand 1-phenyl-3-methyl-4-trichloroacetyl-pyrazolone-5 (HTCP), we report the effect of various concentrations of sulphate ions on the extraction of Ni(II) ion in different buffered aqueous media.

Materials and Methods

Reagents and Apparatus

1-Phenyl-3-methyl-4-trichloroacetyl-pyrazolone-5 (HTCP) was synthesized by methods reported earlier.[15] The ligand's purity after recrystallization from aqueous ethanol was established by elemental analysis for C, H and N; analysis of IR and NMR spectral data was performed at the Institute for Inorganic Chemistry Technology, University of Dresden, Germany.

Stock solutions of 0.05 M HTCP were prepared by dissolving appropriate amount of the ligand in $CHCl_3$. Stock solutions of 1.704×10^{-2} M (1,000 mg/L) of Ni(II) were prepared by dissolving appropriate mass of ammonium nickel(II) tetraoxosulphate(VI) hexahydrate ($NiSO_4.(NH_4)_2SO_4.6H_2O$) in 0.1 mL of 10 M HCl and making up to mark in a 50 ml volumetric flask with deionized water. Buffer solutions were prepared containing 0.01M, 0.1M and 1.0M SO_4^{2-} ions with 0.1M HCl/0.1 M NaCl (pH 0.1-2.9), 0.1 M acetic acid/0.1 M NaCl (pH 3.0-3.5), 0.1 M acetic acid/0.1 M Na-acetate (pH 3.6-5.6), and 0.1 M KH_2PO_4/0.1 M NaOH (pH 5.7-8.0). pH of the buffered solutions were determined with a Labtech Digital pH meter. All experiments were performed at ionic strength of 0.1 M ($NaClO_4$).

Extraction Procedure

2 mL aliquot of a buffer solution containing 8.52×10^{-4} M (50 mg/L) of Ni(II) ions and the desired pH and concentration of SO_4^{2-} ions was prepared in a 10 ml extraction container. An equal volume (2 mL) of 0.05 M HTCP in $CHCl_3$ solution or 1.9 ml of 0.05 M HTCP in $CHCl_3$ solution made up to 2 ml by addition of 0.1 ml butanol and the mixture shaken mechanically for 30 minutes at room temperature of about 32°C. A shaking time of 30 minutes was found suitable enough for attaining the equilibration. For HTCP variation, the organic phase contained HTCP in $CHCl_3$ ranging from 1.25×10^{-2} M – 4.375×10^{-2} M, while for Ni^{2+} variation, the aqueous phase contained Ni^{2+} concentration in the range 12.5 mg/L – 37.5

mg/L. The phases were allowed to settle and separated. Concentration of Ni(II) ion in aqueous phase was determined with a Buck Scientific Atomic Absorption Spectrophotometer (AAS) 205. Ni(II) ion concentration extracted into the organic phase was determined by the difference between the concentration of Ni(II) ion in aqueous phase before and after the extraction. Distribution ratio D was calculated as the ratio of metal ion concentration in the organic phase (C_o) to that in the aqueous phase (C). Thus D $= C_o/C$.
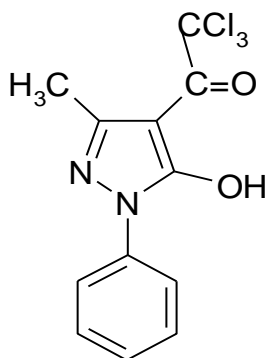
Figure 1: Structure of 1-phenyl-3-methyl-4-trichloroacetyl-pyrazol-5-one (HTCP)

Results and Discussion

Effect of sulphate ions and pH on the extraction of Ni^{2+} from buffered solutions.

The studies show that, while there was marginal extractions of Ni^{2+} from pH of 2.07 for 0.01M SO$_4^{2-}$, extractions at higher concentrations of SO$_4^{2-}$ started at higher pH values (4.53 for 0.1M and 5.53 for 1.0M). Figure 2 show that the percentage extraction of 97.96% achieved for 0.01M SO$_4^{2-}$ on the extraction of Ni^{2+} at pH 6.34 – 7.30 dropped gradually to 96.28% at pH 7.31 – 7.65 for 0.1 M SO$_4^{2-}$ to 94.47% at pH 7.38 – 7.78 for 1.0 M SO$_4^{2-}$.

Extraction data from Table 1 also show that the pH$_{1/2}$ shifted to higher pH values (5.20 ± 0.1)[0.01M] < 6.20 ± 0.1[0.1M] < 6.75 ± 0.1[1.0M] and log K$_{ex}$ decreased -9.37 ± 0.83)[0.01M] > -10.95 ± 0.48[0.1M] > -11.3 ± 0.56. [1.0M] as the concentration of SO$_4^{2-}$ increased. These findings are similar to results reported in other studies.[7,9,16]

Figure 2a -2c showed that a slope of 2 was obtained for all the three (SO$_4^{2-}$) concentrations 0.01M, 0.1M, 1.0M studied, indicating that 2 moles of protons were displaced during the extraction process by the interaction of Ni^{2+} with HTCP and combining these results with those from Figure 4 and 5, we can write the equation of reaction for the extraction process as follows;

$$Ni^{2+} + 2HTCP \longrightarrow Ni(TCP)_2 + 2H^+ \qquad (1)$$

The complex specie Ni(TCP)$_2$ extracted, shown in equation 1, is similar to what was reported earlier.[7,17,19]

The results imply that masking of Nickel II ion is enhanced as the sulphate (SO$_4^{2-}$) ion concentration of the solution increased. For 0.1M sulphate ion concentration, Ni^{2+}

was not extracted at pH range (0-4.22). Similarly, for 1M sulphate ion concentration Ni^{2+} was unextractable up to pH 5.04. The implication is that Nickel may have formed stable and unextractable sulphato-Nickel complexes[18] in solution as shown in equation 2:

$$Ni^{2+} + 2SO_4^{2-} \longrightarrow [Ni(SO_4)_2]^{2-} \qquad (2)$$

Thus, the results indicated that the extraction of Ni^{2+} is more favourable at higher pHvalues with decrease in sulphate ion concentration in the buffered solution.
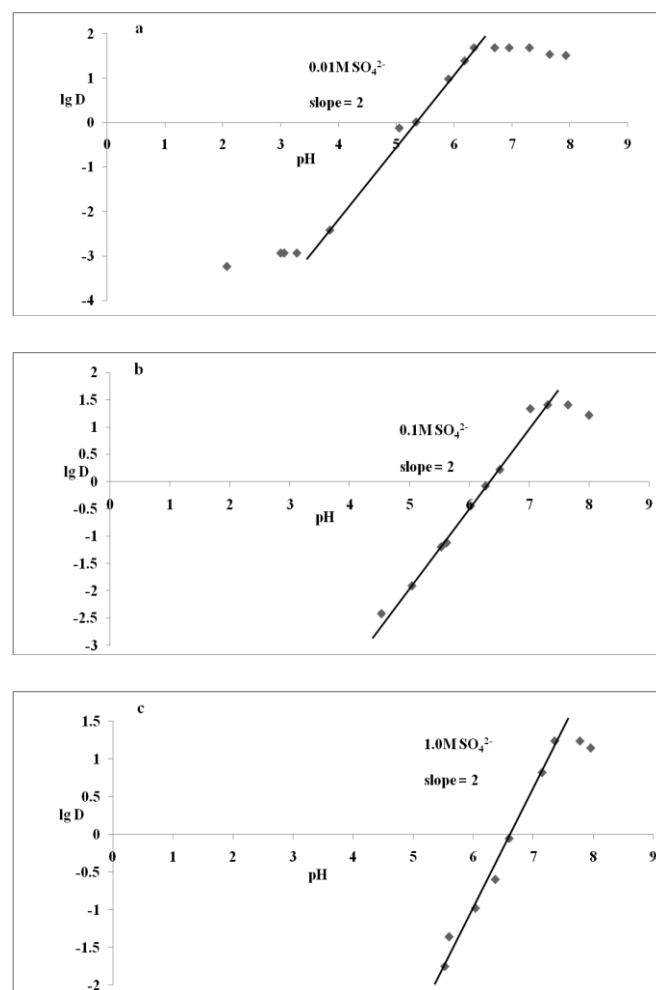
Figure 2: Extraction Plot of 50 mg/L Nickel (II) ion with Solution of 0.05M HTCP in CHCl$_3$ from Buffer Solutions containing (a) 0.01M SO$_4^{2-}$ (b) 0.1M SO$_4^{2-}$ (c) 1.0 M SO$_4^{2-}$ at 32^{0}C

Effect of Butanol as Synergist

This was studied in buffered solution containing 0.1M SO$_4^{2-}$ to see the effect of the synergist in the extractions.

Figure 3 shows that quantitative percentage extraction increased to 97.78% from 96.28% acheived for 0.1M SO$_4^{2-}$ without synergist. Table 1 also shows that the extraction parameters log K$_{ex}$ (HTCP/CHCl$_3$BuOH -7.54 ± 1.39 > HTCP/CHCl$_3$ -10.95 ± 0.48 and log D (HTCP/CHCl$_3$BuOH 1.63 ± 0.02 > HTCP/CHCl$_3$ 1.41 ± 0.01) were higher than those obtained without synergist and shift of the pH$_{1/2}$ value from 6.20 to 4.75 (a more acidic region) indicating that

extraction of Ni^{2+} at lower pH values is more effective in chloroform solutions containing butanol as a solvating agent as the distribution of Ni^{2+} into the organic HTCP/CHCl$_3$ was better. We also noted that, while there was no extractions from pH 0 – 4.22 in the absence of butanol as synergist, there was marginal extraction of Ni^{2+} from pH 2.07. Uzoukwu and Mbonu[7] obtained similar results from their studies on the effect of chloride ion (Cl$^-$) concentration in the extraction of Cu (II) and Ni (II) ions using 1-phenyl-3-methyl-4-trichloroacetyl pyrazolone-5. They attributed the improved distribution of Ni^{2+} in the presence of butanol to formation of adduct and proposed the extracted metal complex to be Ni(TCP)$_2$BuOH.
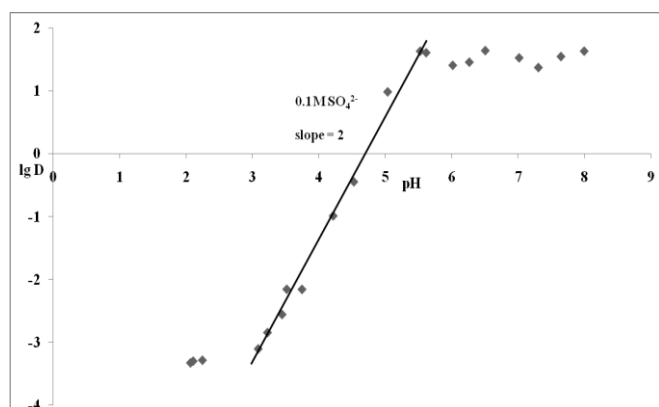

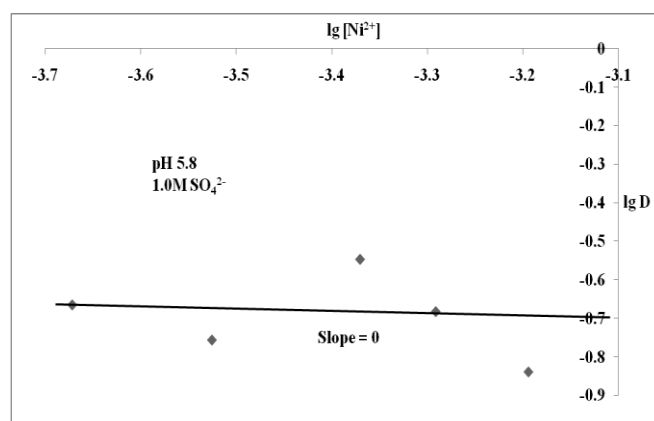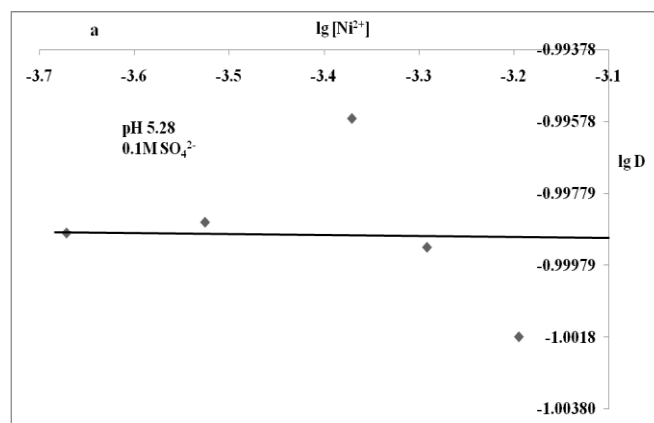**Figure 3:** Extraction Plot of 50 mg/*l* Nickel (II) ion with Solution of 0.05M HTCP in CHCl$_3$/BuOH from Buffer Solutions containing 0.1M SO$_4^{2-}$ at 32^{0}C
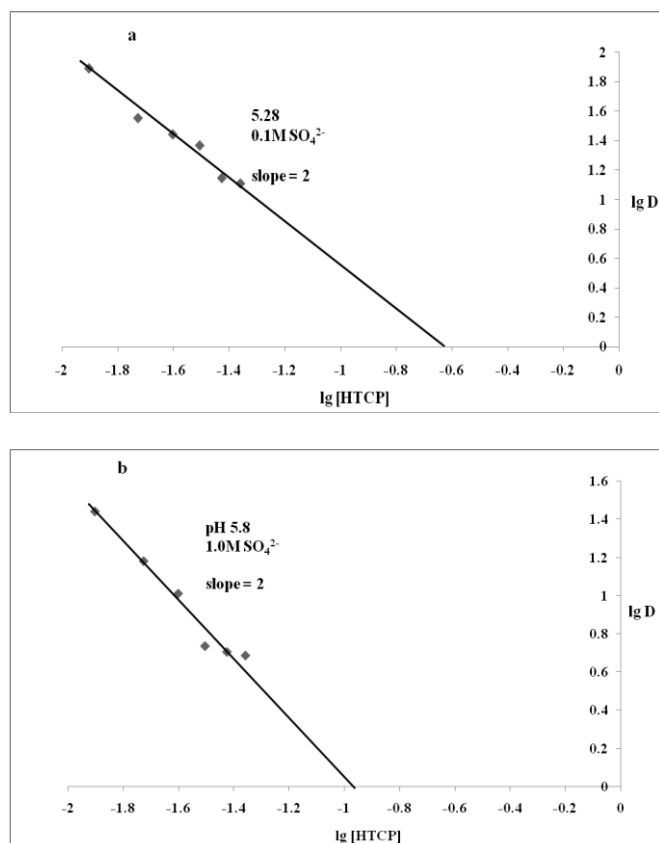
Figure 4: Ligand [HTCP] Variation at (a) pH 5.28 for solution containing 0.1M SO$_4^{2+}$ (b) pH 5.8 for solution containing 1.0M SO$_4^{2-}$ for extraction of 50 mg/*l* Nickel (II) ion at 32^{0}C

Figure 5: Metal [Ni^{2+}] Variation at (a) pH 5.28 containing 0.1M SO$_4^{2-}$ (b) pH 5.8 containing 1.0M SO$_4^{2-}$ for extraction of Nickel (II) ions into 0.05M HTCP in CHCl$_3$ containing 0.1M SO$_4^{2-}$ at 32^{0}C

Table 1. Extraction data for 8.52×10^{-4} M (50 mg/L) Ni^{2+} extraction in buffered aqueous media containing various concentrations of SO$_4^{2-}$ into organic phases containing 0.05 M HTCP/CHCl$_3$ and 0.05 M HTCP/CHCl$_3$/BuOH

Organic Phase HTCP/CHCl₃	SO$_4^{2-}$ Concentration in Aqueous Phase		
	0.01M	**0.1M**	**1.0M**
pH$_{1/2}$	5.20 ± 0.1	6.20 ± 0.1	6.75 ± 0.1
Log D	1.68 ± 0.01	1.41 ± 0.01	1.23 ± 0.01
Log K$_{ex}$	-9.37 ± 0.83	-10.95 ± 0.48	-11.37 ± 0.56
Organic Phase HTCP/CHCl₃ /BuOH	**0.01M**	**0.1M**	**1.0M**
pH$_{1/2}$	-	4.75 ± 0.1	-
Log D	-	1.63 ± 0.02	-
Log K$_{ex}$	-	-7.54 ± 1.39	-

Conclusion

The effect of various sulphate ion (SO$_4^{2-}$) concentrations on the extraction of Ni^{2+} using 0.05M HTCP/CHCl$_3$ studied show that the extraction of Ni^{2+} is masked slightly by anionic sulphate-Nickel complexes formed in solution at increasing sulphate ion concentration. Slope analysis showed that Ni^{2+} was extracted as Ni(TCP)$_2$. Extraction of Ni^{2+} was enhanced by the introduction of butanol as solvating agent due to formation of adduct Ni(TCP)$_2$BuOH..

The result also showed that the pH$_{1/2}$ value increased with increase in sulphate ion concentrations. This suggested that anionic sulphato complexes of Nickel II ion (Ni^{2+}) formed in solution have some effect on the extent of extraction of Nickel from the buffer media and this effect is more pronounced as the concentration of sulphate ions in solution increased.

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EVOLUTION OF BIO-ACTIVE SUBSTANCES IN ASPARAGUS AS AFFECTED BY DIFFERENT HARVEST TIMES

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Fresh asparagus is rich in protein, vitamins and minerals with high nutritional value. But nutritional content and composition are affected by the genotype and the harvest time. In our trials at the experimental farm Pallag of Debrecen University 3 asparagus varieties (*Cumulus, Gijnlim, Grolim*) were tested on brown humus sand soil in ridge cultivation. Solids, nutritional fibre, protein, sugar and vitamin-C contents were measured in the white spears at 3 different harvest times (8 May, 24 May, 6 June 2012). Mineral element tests included B, Ca, Cu, Fe, K, Mg, Na, P, S, Zn and sulphate sulphur. Our trials confirmed the strong negative correlation (r = -0.828) between the sugar and vitamin-C content of the spears. This investigation confirm that the variety with higher vitamin-C content has lower flavour materials. On the start of the harvest period we measured higher sugar and lower vitamin-C content in the spears. In our variety comparison trial the variety *Cumulus* had the highest solids (7.1 %), nutritional fibre (4.7 %), protein (2.81 %) and sugar (3.51 %) contents on the brown humus sand. Among the microelements the high Fe content (4.93 – 7.63 mg kg^{-1} fresh weight) deserves attention. Fe is variety dependent at different genotypes. The highest value was found in *Gijnlim* with 7.63 mg kg^{-1} on fresh weight basis. The sulphate-S content is responsible for the special taste and flavour of asparagus. In our trials *Cumulus* had the highest sulphate-S value (279 mg kg^{-1} fresh weight). *Cumulus* also excelled in Mg content (84.10 mg kg^{-1} fresh weight) and *Grolim* in Ca content (27,23 mg kg^{-1} fresh weight). The low Na content of the element composition is essentially favourable. It varied between 17.07 and 27.23 mg kg^{-1} on fresh weight basis in the tested 3 varieties.

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Introduction

In the development of illnesses and the preservation of health nutrition has a decisive part both from qualitative and quantitative points. In checking civilisation illnesses the consumption of certain vegetables with scientifically proved favourable bio-active effects, play a central part. Such a crop is the asparagus.

Asparagus has considerable vitamin-C contents (9-32 mg 100 g^{-1}) which play an important role in Fe incorporation and pholic acid and protein metabolism. The top of the spear contains the highest quantities which decrease towards the base[1]. Different factors such as cultivars, fertilisation and environmental conditions have influence on the quality of white asparagus[2]. Furthermore there are genotypical differences in spear quality such as spear diameter, head tightness and fibrousness[3,4,5]. But information about genotypical differences in the content of inorganic and organic compounds in asparagus spears are limited. It was therefore the aim of this study to determine quality parameters such as the mineral composition in spears of different genotypes.

The special taste of asparagus is caused by sulphur compounds[6]. Asparagus is remarkable for its low Na content and excellent nutrient element composition[7]. The evolution of the minerals is greatly affected by the genotype and the nutrient content and availability of the soil as also indicated by data giving 4500 µg g^{-1} Na quantities[1].

Spears have higher mineral element contents, especially Mn, Cr and K, at the top[8]. Consequently, broken bits of head can play an important part in processing, mostly in producing freshly pressed juice.

During processing mineral element contents are subjected to considerable changes. Lopez and co-workers[9] showed considerable losses in Fe and Mn during cleaning while quantities of Cu and Zn increased. This correlates with the peeling loss. Thicker spears had higher mineral element content and lower cleaning loss.

Nutritive elements – including minerals – are very important as asparagus plays a significant part in the mineral element supply of the human body[10].

The ion ratio of the human body can be characterized by $(Ca^{2+} + Na^+) / (Mg^{2+} + K^+)$ correlation with ~ 1.0 value under optimal conditions but values about 4.0 are often measured. This ratio is very low in vegetables which contributes to equalize the ion balance of the human body[11].

The trials aimed to determine the evolution of bioactive substances during 6-week harvest time at 3 sampling dates.

Experimental

In our trials at the experimental farm Pallag of Debrecen University 3 asparagus varieties (*Cumulus, Gijnlim, Grolim*) were tested on brown humus sand soil in ridge cultivation.

Soil analysis data are summed up in Table 1. Data show satisfactory P and K supplies. Soil compactness, Ca and N supply were suitable for asparagus production.

The trials aimed to determine the evolution of bioactive substances during a 6-week harvest time at 3 sampling dates. Laboratory measurements at 3 different dates (8 May, 24 May, 6 June 2012) included:

- solids content (%) - gravimetric method MSZ ISO 6496:2001

- nutritional fibre content (%) - enzymatic determination by Codex Hungaricus 3-2-2008/1

- protein (%) - Kjeldahl method MSZ 08-1783-6:1983

- sugar (%) - by Luff-Schorl MSZ 6830-26:1987

- vitamin-C (mg $100g^{-1}$ fresh weight) iodometric titration titrimetric method, MSZ ISO 6557-2:1991

- mineral elements (mg kg^{-1} fresh weight) - B, Ca, Cu, Fe, K, Mg, Na, P, S, Zn – Codex Hungaricus-08-1783:1985 by ICP, PERKIN ELMER Optima 3300 DV

Table 1. Soil analysis results

pH	6.25
pH(in distilled water)	6.88
K_A (compactness by Arany)	25
Water-soluble total salt content	0.013 %
Conductivity	384 μS
Calcium carbonate content	0.884 %
Humus content	1.05 %
KCl solution soluble nitrite and nitrate N-content	28.9 mg kg^{-1}
AL*-soluble P-content as P_2O_5	463 mg kg^{-1}
AL*-soluble K-content as K_2O	617 mg kg^{-1}

*AL – ammonium-lactate

Meteorological data of the location are shown in Fig. 1. The harvest periods were rich in natural precipitation (> 30 mm).
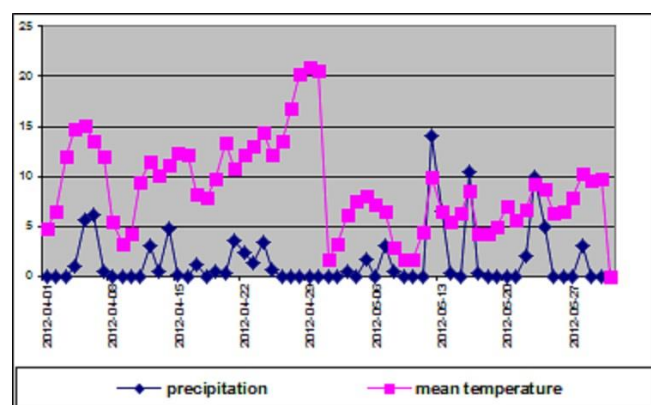
Figure 1. Precipitation (mm) and mean temperature (°C) distribution, Debrecen-Pallag 2012

Results and discussion

Solids, nutritional fibre, protein and sugar are important nutritive elements. Beside production conditions the genetical traits of the variety are also involved. Data can be seen in Fig. 2. showing 6-7 % variations in the solids content on the mean of harvest dates. The variety *Cumulus* had the highest values in solids as well as in nutritional fibre, protein and sugar. The statistically significant difference indicates the high quality of this genotype under our production conditions.
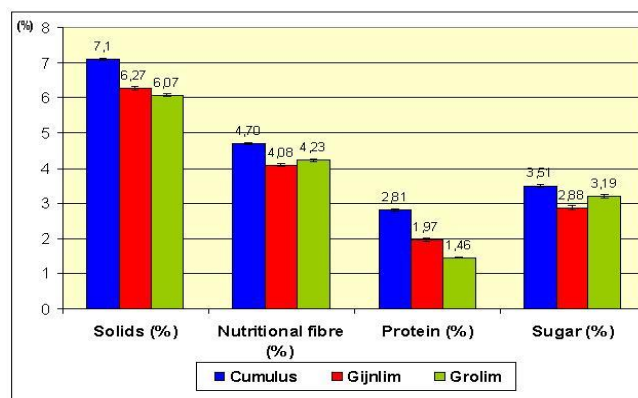
Figure 2. Evolution of nutrient elements in the varieties on the mean of harvest dates

In consumption vitamin-C and sugar play an important part as freshly pressed asparagus juice. In this case vitamin-C and the agreeable taste are desired features. In our experiment *Gijnlim* had the highest vitamin-C content (11.7 mg 100 g^{-1}) (Fig. 3.) but the lowest sugar content (2.88 %).

Correlation between sugar and vitamin-C of the spears is found in Fig. 4. On the mean of varieties data show a close reverse correlation between the two contents (r = -0.823) which means that varieties of higher vitamin-C content are pour in taste.
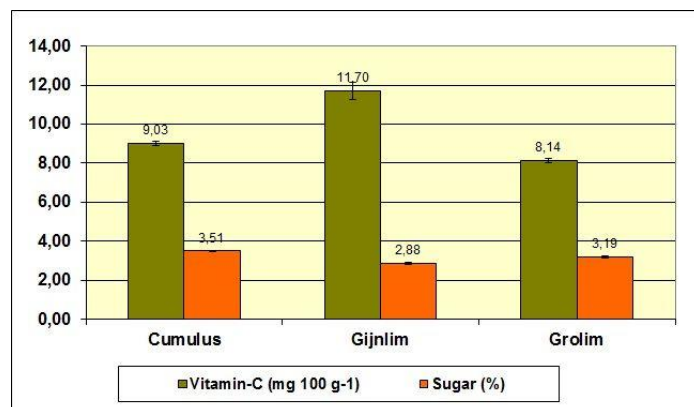
Figure 3. Vitamin-C sugar contents on the mean of sampling

Among the microelements Fe is especially important as it plays part in numerous biochemical processes. In our measurements (Fig. 5.), *Cumulus* appeared with 7.63 mg kg^{-1} (121.7 μg g^{-1} on dry matter basis) which is higher than the corresponding value given in literature (86.8-98.3 μg g^{-1})[12]. No considerable differences were found in Zn, Cu and B content of the varieties.
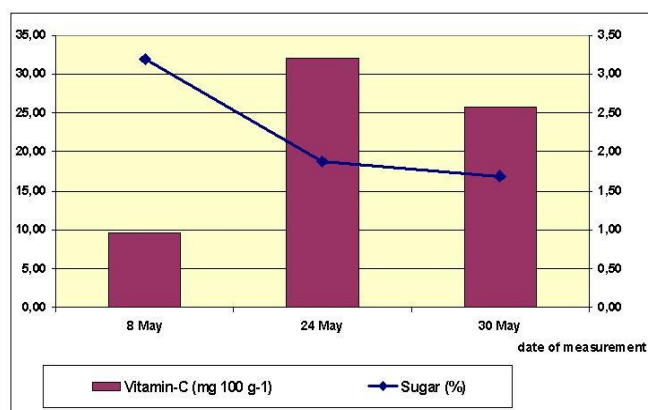
Figure 4. Correlation between vitamin-C and sugar contents

Figure 5. Microelement content of asparagus on the mean of harvests

Besides macroelements (K, P, S) Sulphate-S was also measured and shown in Fig. 6. K content of asparagus varied between 1344 – 1562 mg kg^{-1} depending on variety and location. Certain literary data give much higher values[5]. The sulphate-S content must be mentioned as it is responsible for the special asparagus taste and flavour. In our trials *Cumulus* had the highest sulphur-S content (279 mg kg^{-1}).
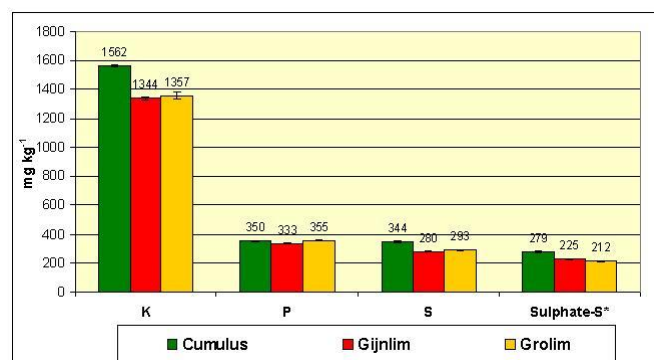
Figure 6. Macroelement content of asparagus on the mean of harvests

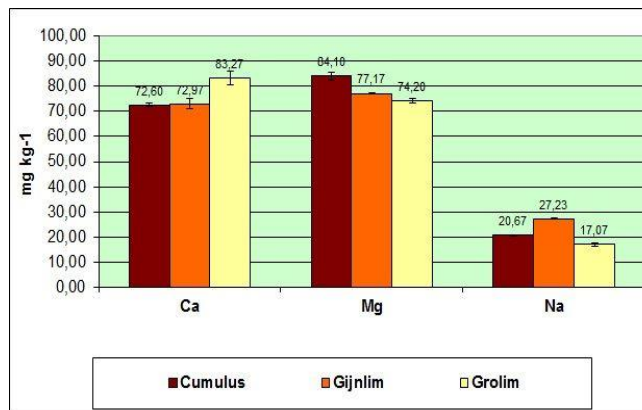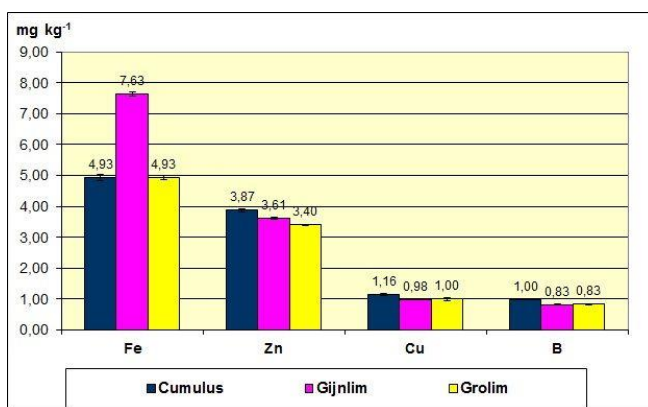Beside K, Ca, Mg and Na amounts are also involved in the ion-balance of the human body. Data are found in Fig. 7. *Cumulus* appeared in Mg content (84.10 mg kg^{-1}) and *Grolim* in Ca content (27.23 mg kg^{-1}). In the element composition of asparagus the low Na content is especially favourable. It varied between 17.07 and 27.23 mg kg^{-1} in the tested 3 varieties.

Figure 7. Ca, Mg and Na contents of asparagus varieties on the mean of harvests

Table 2 represents the mineral element contents of asparagus on the mean of 3 varieties and 3 harvest dates. The comparison containing our own measurements and literary data[13] indicate that the mineral element content of asparagus is considerably affected by the genotype, production methods and conditions which explain the important differences in K content.

Our measurements give value of 1420 mg kg^{-1} of K on the mean of varieties and samples while Rodler[13] found 2400 mg kg^{-1}. Even more spectacular is the difference in the case of Na. We measured nearly the tenth (21.66 mg kg^{-1}) of the value given by Rodler (200 mg kg^{-1}) and Moreno[1].

Table 2. Mineral element content (mg kg^{-1} fresh weight) of asparagus on the mean of varieties based on our own measurements and literary data

Mineral elements	our own measurements	Rodler[13]
B	0.89±0.10	-
Ca	76.28±6.06	70
Cu	1.05±0.10	1.75
Fe	5.83±1.56	-
K	1420±122	2400
Mg	78.49±5.08	70
Na	21.66±5.15	200
Zn	3.63±0.23	3.3
P	346.00±11.29	400
S	305.56±33.89	-
Sulphate-S	238.67±35.84	-

Consequently, the nutritional element contents of this excellent vegetable can only be evaluated by testing samples, that is, tabulated data do not give exact information of the nutrient element contents of asparagus in every case. Our investigations confirm that the mineral element content of spear showed differences between cultivars. To produce good quality product it is essential to choose proper variety for the given condition. In our growing conditions *Cumulus* proved to produce the best quality.

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AN OVERVIEW ON SYNTHETIC METHODS OF ALKYL CINNAMATES

Liu Shu[a]*and You Hongjun[b]

Keywords: overview; synthetic method; alkyl cinnamates; catalysts

Three synthetic methods of alkyl cinnamates using different catalysts such as heteropolyacids ($H_8SiW_{12}O_{42} \cdot XH_2O$) and solid superacids ($SO_4^{2-}$/$La_2O_3$-$ZrO_2$-HZSM-5 and SO_4^{2-}/TiO_2) have been reviewed in the present paper. Effects of the reaction conditions such as the microwave heating power and times, the amount of the catalyst, the molar ratio of cinnamic acid to alcohols and the reaction time on the yields of alkyl cinnamates have also been discussed. The yields of alkyl cinnamates are improved by the addition of the abovementioned catalysts. Due to low investment costs and simple process these methods are having the advantages on other processes.

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Introduction

Alkyl cinnamates are not only important organic intermediates but also widely used in different areas such as perfume essence, soap and flavouring essence due to pronouncing fruit or flower aromas.[1] Cinnamic acid under the condition of a catalyst reacts with alcohols to produce alkyl cinnamates. Alkyl cinnamates reviewed here consist of methyl cinnamate, ethyl cinnamate, n-propyl cinnamate, n-butyl cinnamate, isobutyl cinnamate, n-pentyl cinnamate and isopentyl cinnamate, etc.[2] There are two types of methods (the classical method and the microwave heating method) for manufacturing of alkyl cinnamates in industrial scale. The classical method means that cinnamic acid reacts with methanol or ethanol in the presence a catalyst to produce methyl cinnamate or ethyl cinnamate, respectively. This method has a lot of disadvantages such as consuming long time to finish the reaction, using benzene or toluene as a dehydrating agent, requiring a lot of catalysts and getting low yield of alkyl cinnamates, etc. Another method is the microwave heating method. Although the microwave heating method can decrease the reaction time and increase the yield of alkyl cinnamates, the unit is very complicated and it is very difficult to increase the output of alkyl cinnamates.[3]

In the present paper, three types of catalysts such as heteropoly acids ($H_8SiW_{12}O_{42} \cdot XH_2O$) and solid super acids (SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and SO_4^{2-}/TiO_2) have been discussed. Effects of the reaction conditions such as the microwave heating power, the microwave heating times, the amount of the catalyst, the molar ratio of cinnamic acid to alcohols and the reaction time on the yields of alkyl cinnamates have also been discussed. Furthermore, the optimal reaction conditions have also been pointed out.

DISCUSSION

$H_8SiW_{12}O_{42} \cdot XH_2O$ as a catalyst to produce ethyl cinnamate

Li Jing [4] introduced and described a synthetic method of ethyl cinnamate and studied the effect of the reaction conditions (the microwave heating power and heating times) on the yield of ethyl cinnamate. Using $H_8SiW_{12}O_{42} \cdot XH_2O$ as a catalyst and cinnamic acid and ethanol as feedstocks produced ethyl cinnamate. The optimal microwave heating power and microwave heating times were 340 W and 20 minutes, respectively. It was observed that the microwave heating time, the amount of the catalyst, the molar ratio of cinnamic acid to ethanol were 20 minutes, 2.93% of cinnamic acid weight (g) and 1.0:7.0, respectively. Table 1 showed the relationship between the microwave heating power and the yields of ethyl cinnamate.

The yield of ethyl cinnamate increased with an increase in microwave electricity and heating power. When the microwave electricity and the heating power were 130 mA and 410 W, respectively, the maximum yield of ethyl cinnamate reached 94 %. However the product's colour was dark black, it proved that ethyl cinnamate was deeply oxidized. Therefore the best reaction condition was the microwave electricity (110 mA) and the microwave heating power (340 W). It was supposed that the microwave heating power, the amount of the catalyst, the molar ratio of cinnamic acid to ethanol were 340 W, 3.75 g and 1.0:7.0, respectively. Table 2 presented effect of the different microwave heating time on the yields of ethyl cinnamate. The yields of ethyl cinnamate increased with an increase of the microwave heating time. The optimal condition was the microwave heating time (30 minutes).

Table 1. The relationship between the microwave heating power and the yield of ethyl cinnamate

Microwave electricity (mA)	50	70	90	110	130
Microwave heating power(W)	150	200	285	340	410
Yield of ethyl cinnamate (%)	39	88	90	93	94

Table 2. The effect of the different microwave heating time on the yield of ethyl cinnamate.

Microwave heating time(min)	10	15	20	25	30
Yield of ethyl cinnamate (%)	77	89	93	94	95

SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 as a catalyst to generate n-butyl cinnamate

Chen Shufen[5] described the synthetic method of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and discussed about the effect of the reaction conditions such as the amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and the molar ratio of cinnamic acid to n-butyl alcohol on the yields of n-butyl cinnamate. It was observed that the molar ratio of cinnamic acid to n-butyl alcohol and the reaction time were 1.0:5.0 and 2 hours, respectively. Table 3 showed the relationship between the amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and the yields of n-butyl cinnamate. The yields of n-butyl cinnamate increased with an increase in amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5. It was observed that the amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and the reaction time were 1.8% of total reactant weight (g) and 2 hours, respectively. Table 4 presented effect of the molar ratio of cinnamic acid to n-butyl alcohol on the yields of n-butyl cinnamate. The yields of n-butyl cinnamate firstly increased and then decreased with an increase in the molar ratio of cinnamic acid to n-butyl alcohol. Based on the experimental results, the best amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and the molar ratio of cinnamic acid to n-butyl alcohol were 1.8% of total reactant weight (g) and 1.0:5.0, respectively. The maximum yield of n-butyl cinnamate was 92.7%.

Table 3. the relationship between the amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and the yields of n-butyl cinnamate

Catalyst, %	1.0	1.2	1.5	1.8	2.0
Yield of product (%)	85.7	88.4	90.3	92.7	92.8

Table 4. The effect of the molar ratio of cinnamic acid to n-butyl alcohol on the yields of n-butyl cinnamate

Molar ratio	1.0:2.0	1.0:3.0	1.0:4.0	1.0:5.0	1.0:6.0
Yield (%)	65.3	78.4	86.3	92.7	90.2

SO_4^{2-}/TiO_2 as a catalyst to produce n-butyl cinnamate

Xia Jinhong [6] introduced the preparation of SO_4^{2-}/TiO_2 and also studied the effect of the reaction time on the yield of butyl cinnamate. It is observed that the optimal molar of cinnamic acid to n-butyl alcohol and the amount of SO_4^{2-}/TiO_2 were 0.015 mol, 0.045 mol and 17.09% of total reactant weight (g), respectively. Table 5 showed the relationship between the reaction time and the yields of n-butyl cinnamate. The yields of n-butyl cinnamate firstly increased and then decreased with an increase in the reaction time. The best reaction time was 3.0 hours. The maximum yield of n-butyl cinnamate was 92.8%.

Table 5. The relationship between the reaction time and the yields of n-butyl cinnamate

Reaction time, h	1.5	2.0	2.5	3.0	4.0
Yield (%)	85.0	91.8	92.5	92.8	88.2

CONCLUSION

Based on the above discussion and review, using cinnamic acid and ethanol or n-butyl alcohol as feedstocks and heteropoly acids ($H_8SiW_{12}O_{42} \cdot XH_2O$) and solid super acids (SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and SO_4^{2-}/TiO_2) as catalysts, effect of the microwave heating power, the microwave heating times, the amount of the catalyst, the molar ratio of cinnamic acid to n-butyl alcohol and the reaction time on the yields of alkyl cinnamates have been discussed. The experimental results obtained are as follows:

(1) When the microwave electricity, the microwave heating power and the microwave heating times were 130 mA, 410 W and 30 minutes, respectively, the maximum yield of ethyl cinnamate reached 94 % by the addition of $H_8SiW_{12}O_{42} \cdot XH_2O$.

(2) The best amount of SO_4^{2-}/La_2O_3-ZrO_2-HZSM-5 and the molar ratio of cinnamic acid to n-butyl alcohol were 1.8% of total reactant weight (g) and 1.0:5.0, respectively.

(3) The maximum yield of n-butyl cinnamate was 92.8% when the reaction time was 3.0 hours by the addition of SO_4^{2-}/TiO_2.

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CHROMATOGRAPHY OF WINE

Tibor Cserháti and Maria Szőgyi

The objectives of the review are the collection, concise description and evaluation of the various chromatographic technologies applied for the separation and quantitative determination of macro- and microcomponents present in wines.

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Introduction

Chromatographic methods have been developed and successfully employed for the separation and quantitative determination of a wide variety of organic and inorganic compounds even present in complicated accompanying matrices at the trace level. The rapid development of new chromatographic instrumentation, the increase of the precisity and sensitivity of the newest apparates facilitated the solution of separation problems which were impossible some years ago.

Wines are produced and consumed all over the world. Because of their considerable commercial importance the adequate quality control of wines is of paramount importance. Both traditional and up-to date techniques found application in the analysis of wines. Because of the their advantageous separation characteristics chromatographic methods are frequently employed in the evaluation of wine quality.

The objectives of the review are the collection of the newest results obtained in the chromatographic analysis of wines, the critical evaluation of the results and the elucidation of the similarities and dissimilarities among the results obtained by various analytical procedures.

The application of sample preparation and chromatographic technologies in the analysis of selective biogenic amines, methylxanthines and water-soluble vitamins has been earlier reviewed.[1]

Gas chromatography (GC)

Gas chromatographic (GC) technologies are generally employed for the separation and quantitative determination of volatile compounds. Non-volatile compounds can be derivatized to increase volatility, however, the method is time consuming and decreases the reliability of the procedure. As the majority of the constituents of are volatile or semi volatile GC methods have been frequently application in the analysis of wine. Wine contains not only natural constituents but also synthetic products to inrease yield and to improve the quality of the end product. Both kind of analytes can be easily investigated by GC methods.

Gas chromatography of natural analytes

The volatile composition of alcoholic beverage was investigated by headspace solid-phase microextraction method (HS-SPME) followed by gas chromatography mass spectrometry (GC-MS). It was established that volatile compounds such as alcohols, esters and terpenes influence the flavor of fortified wines. It was further found that m-thymol, o-thymol, eugenol and their esters play a considerable role in the formation of aroma profile.[2] The impact of different over-lees ageing was also investigated in detail (ageing on fine white lees, on fine red lees, fine second passage lees, rough red lees). The measurements indicated that the type of lees exerts a considerable effect on the composition of Cabernet wine modifying polysaccharide and volatile composition of the end product.[3] Both sensorial and chromatographic methods were simultaneously employed for the investigation of the aroma profile of *Garnacha Tintorera*-based sweet wines. It was established that the main volatile components were sotolon (73 µg L^{-1}) and acetoin (122 µg L^{-1}).[4] Solid phase microextraction (SPME) combined with gas chromatography-olfactometry (GC-O) and cryogenic trapping was employed for the study of the aroma-active compounds of Shiraz wine. The preconcentration procedure was carried out by using various fibre coatings such as polyacrylate (PA), and triple-phase polydimethylsiloxane/divinylbenzene/carboxene (PDC).

It was concluded from the data that the method is suitable for the investigation of the aroma active compounds of Shiraz wine.[5] The impact of soil management on the composition of aroma compounds of Negroamaro wine was elucidated by using a GC-MS method. The influence of two agronomic practices (cover cropping, and soil tillage) on the free and bound aroma compounds was measured. The investigations revealed the presence of alcohols (55.7 mg L^{-1}), fatty acids (7.0 mg L^{-1}), and esters (6.6 mg L^{-1}). It was established that soil tillage enhances the amount of aroma compounds such as esters, carboxylic acids, alcohols, acetamides and phenols. It was further established that the concentration of sulfur compounds decreased while the soil

management did not influenced the amount of glycoside volatile compounds.[6] The application of various SPME methods has been earlier discussed in various accompanying matrices and their use was in discussed in detail.[7] The impact of deficit irrigation and kaolin particle film on the grape composition and volatile compounds in Merlo grape (*Vitis vinifera*) was investigated in detail. The analytes were separated and quantitatively determined by stir-bar sorptive extraction-gas chromatography-mass-spectrometry (SBSE-GC-MS). It was established that the treatments modify considerably the following volatile compounds: hexanal, trans-2-hexenal, 1-hexanol, nerol, geraniol, β-damascenone, 3-hydroxy-β-damascenone, 1,1,6-trimethyl-1-2-dihydro-naphtalene, 3-oxo-α-ionol, 13-norisoprenoids and anthocyanins.[8] The addition of oak chips on the composition of volatiles in *Moravia Agria* wines has been investigated by GC-MS. Chips were added during alcoholic fermentation, during malolactic fermentation and in young red *Moravia Agria* wines. The results indicated that the addition of oak chips to the fermentation broth influences the amount of ethyl esters of straight-chain fatty acids, ethyl, hexyl, isoamyl acetates and superior alcohols. It was further found that the presence of oak chips increases the concentration of benzene compounds, oak lactones and furanic compounds.[9]

Gas chromatography of synthetic analytes

Besides of natural components wines contain various organic and inorganic pollutants added to the wine intentionally (i.e. pesticides) or unintentionally. These additional compounds may increase the yield, and may modify the aroma profile of the wine. An easy and reliable GC method using electron capture detector (ECD) was developed for the detection of chlorpyrophos (CP) and its metabolite chlorpyrophos-oxon (CPO) in wine. The quick, easy and cheap preparation method employs extraction with acetonitrile, followed by dispers phase extraction using primary secondary amine. It was found that the acuracy of the method was (92.3 ± 18.2) % for chloropyrophos and (96.6 ± 16.1) % for chlorpyrophos-oxon. LOD and LOQ values were 0.04 and 0.15 ng mL^{-1} for CP and 0.49 and 1.62 ng mL^{-1} for CPO.[10]

Both high performance liquid chromatography-photodiode-array detection electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS) and solid-phase micro extraction-gas chromatography-mass spectrometry (SPME-GC-MS) were employed for the identification and isolation of spoilage yeasts. It was established that the combined method is suitable for the identification of this class of yeasts[11]. A new extraction procedure was developed and tested for the determination of volatile compounds in white and red wines. The investigations indicated that the recovery values of the method are good and depend considerably on the character of the volatile compound under investigation. It was further established that the employment of matrix-matched calibration curve is necessary to obtained reliable quantitative results.[12] A new SPE-GC method was developed for the analysis of alpha- and beta tujone in alcoholic beverages. It was stated that the yield of extraction is high (over 98%), the LOD is 0.033 mg L^{-1}, RSD is below 1.8 % and the relationship between the amount of analytes and the detector response is linear.[13] Membrane ethanol biosensor was prepared using *Methylobacterium*

organophilium-immobilized egg: shell membrane and an oxygen electrode.

It was established that the relationship between the amount of ethanol in the sample and the biosensor response was linear between 0.050 – 0.75 mmol L^{-1}, the detection limit was 0.025 mmol L^{-1}, RSD was 2.1 %. The results were compared with those obtained by GC, it was stated that the biosensor is suitable for the determination of the alcohol content in alcoholic beverages.[14] An automatic headspace in-tube extraction (ITEX) technique was developed for the determination of volatile components in wine and beer. It was stated that the method is suitable for the analysis of very volatile compounds (acetaldehyde, ethyl acetate, diacetyl) in different matrices.[15] A new GC-MS method was developed for the determination of aldehydes in grape pomace distillates (Orujo). Sampled were diluted and derivatised with O-(2,3,4,5,6-pentafluorobenzyl) hydroxyl amine, then microextracted by SPME before GC-MS analysis. The LOD value was 0.7 ng L^{-1}, the repeatability was excellent.

It was found that the application of demetilant column considerably modified the quality profile of the distillate.[16]

High performance liquid chromatography

Because of high separation capacity and variability high performance liquid chromatographic technologies (HPLC) have been frequently employed in the up-to date analytical chemistry even in the separation and quantitative determination of the macro- and microcomponents of wines and in the determination of environmental pollutants. The application of cyclic voltametry and HPLC have been simultaneously employed for the determination of coffein in aqueous, acid, and alcoholic media. The investigations were motivated by the pharmacological properties of coffein (stimulation of the central nervous system, peripherial vasoconstriction, relaxation of the smooth muscle and myocardial stimulation).[17] Gas- diffusion microextraction (GDME) was applied for the extraction of some aliphatic amines such as methylamine, dimethylamine, and ethyamine in fermented beverages (white, red and rose wines, dark and pilsner beers). Analytes were derivatised by phenyl isothiocyanate and analysed by HPLC. It was found that LOD varied between 12 – 46 µg L^{-1}, while LOQ ranged 39 – 153 µg L^{-1}. Because of its simplicity the method was proposed for the analysis of these compounds.[18] Biogenic amines (histamine, tyramine, spermine, spermidine, putrescine, cadaverine and phenylethylamine were also investigated by reversed phase (RP-)HPLC. Analytes were pre-column derivatized using phase-centered central composite design. The optimal conditions for the derivatization with dansyl chloride were 60 ^{0}C and 60 min. Biogenic amines were separated on an octadecylsilica (ODS) column at a column temprature of 40 ^{0}C using gradient elution of acetonitrile-water. Analytes were detected at 254 nm. Calibration in matrix fitted resulted in $R^2 > 0.997$. Repeatability ($n = 6$) and intermediate precision ($n = 3$) in matrix showed RSD values >2.34 % and 3.16 %, respectively. It was established that the concentration of biogenic amines in Chilean young variatel wines varied between 18.12 – 39.84 mg L^{-1}.[19] The phenolic profile of *Sercial* and *Tinta Negra Vitis vinifera L.* grape skins was investigated by HPLC-DAD-ESI-MS (high performance

liquid chromatography-diode array detection-electrospray ionisation tandem mass spectrometry). The presence of 3-hydroxybenzoic acids, 8-hydroxycinnamic acids, 4-flavanols, 5-flavanones, 8-flavonols, 4-silbenes and 8-anthocyanins was established. It was stated that 10 new compounds were identified in the grape skins of *Sercial* and *Tinta Negra Vitis vinifera L.*: protocatechic acid glucoside, p-hydroxybenzoyl glucoside, caffaric and vanilloyl pentoside, p-coumaric acid-erythroside, naringenin hexose derivate, eriodictyol-glucoside, taxifolin-pentoside, quercetin-gluronide-glucoside, malylated kaempherol-glucoside and resveratrol dimer. Grape skins were proposed as sources of bioactive compounds.[20] A new generic sample treatment method was developed for the simultaneous analysis of multiclass pesticides and mycotoxins. Analytes were extracted by SPE and separated by HPLC TOF method. It was established that the retention capacity of cartridges showed considerable differences. The analytes were identified according to the retention time and accurate mass measurements of the protonated analyte molecules. LOD were lower then 1 µg L^{-1} for the 87% of the molecules included in the investigation.[21] The application of molecularly imprinted polysiloxane microspheres (MIPS) for the improved selective extraction of 3,3'-dichlorobenzidine has been recently reported. The interest in the analysis of dichlorobenzidine and degradation products was motivated by the carcinogen character of this class of molecules. It was established that MIPS show marked selectivity toward 3,3'-dichlorobenzidine the binding capacity being seven time larger than for diphenylamine. The new microspheres were proposed for the selective extraction of 3,3'-dichlorobenzene from different accompanying matrices.[22] A combined method including derivatisation, solid-liquid extraction, solid-phase purification and HPLC-DAD was employed for the determination of dithiocarbamate (DTC) fungicide in various matrices such as apples, wine grapes, lettuces, peppers, tomatoes and strawberries. Fungicide residues exceeding maximum residue limit was found only in 6% of the samples.[23] A HPLC method combined with tandem mass spectrometry detection was employed for the study of the migration of phthalates into 12% ethanol simulating alcoholic beverages. Measurements were carried out on a RP-HPLC column (50 x 3.0 mm; 2.5 µm). Analytes were separated by gradient elution using water-methanol mixtures as mobile phase containing 0.1% acetic acid, the flow rate was 300 µL min^{-1}. The target compounds dibutylphtalate, di-isononylphtalate, di-isodecylphtalate and butyl-benzyl phtalate were applied for the validation of the procedure. It was found that the detection limit of the method allows its application in legislation procedures.[24] The retention of bioactive anthocyanins by wine polymers was investigated by RP-HPLC. The investigations were motivated by the fact that red wine is important source in the dietary intake of phenolic derivatives showing marked antioxidant activity. Moreover, anthocyanins play a considerable role in the prevention of cardiovascular diseases and cancer. The measurements indicated that anthocyanins bind to wine polymers and the strength of the binding depends on the chemical character of the interacting compounds. It was further established that the results may facilitate the rational dosage of wine anthocyanins.[25] The reaction between malvidin-3-glucoside-(O)-catechin, catechin and malvidin-3-glucoside was studied by LC-DAD/ESI-MS. Measurements were performed both in the positive and

negative ion mode. The results of HPLC measurements indicated the formation of methylmethine-linked cat-mv3glc-(O)-cat ([M-H](-) m/z 1097) and mv3glc-mv3glc-(O)-cat(+)[m/z 1301] adducts.[26] As mannoproteins improve considerably the quality of wine many analytical methods have been developed for their determination in various wine varieties. A new chromatographic method was developed for the quantitative determination of mannoproteins in wines. Target analytes were isolated by size exclusion chromatography followed by acid hydrolysis, elimination of acids by weak anionic exchange solid phase extraction. The method was proposed for the analysis on monosaccharides by ion exclusion HPLC. It was stated that the method uses low volume samples, makes possible the parallel measurement of multiple samples and the absence of precipitation steps.[27] A versatile targeted metabolomics method was developed for the rapid quantification of phenolics in fruits and beverages. The investigations were motivated by the well known health benefits of these classes of molecules. It was established that the procedure suitable for the analysis of 135 phenolics such as benzoates, phenylpropanoids, coumarins, stilbenes, dihydrochalcones, and flavonoids. It was stated that the method is suitable for the evaluation of food quality.[28] The profile of low molecular weight phenolic composition of various chips (Portuguese chestnut, French, American and Portugueses oak chips) were investigated using HPLC-DAD and LC-DAD/ESI-MS. Target compounds (phenolic acids, phenolic aldehydes, and furanic derivatives) were separated by pressurized liquid extraction and analysed by HPLC technologies. The results indicated that the botanical species exerts higher influence on the composition of phenolic compounds then the geographic origin.[29] The importance of cork taint as a major organoleptic defect in wine, the analytical methods applied for the separation and quantitative determination of the compounds responsible for the cork taint, the enumeration and short discussion of the analytical procedures (solid-phase microextraction, stir-bar sorptive extraction, microextraction in packed syringe) has been recently published.[30]

Liquid chromatography combined with tandem mass spectrometry was employed for the analysis of pesticides in drinking water and beverages. Pesticide samples were extracted by shaking a mixture of 10 mg of sample and 20 mL of extracting agent (acetonitrile). After shaking vigorously the mixture 1 g of sodium chloride and 4 g of magnesium sulfate were added to the mixture. The organic phase was treated with graphic carbon black/PSA column. The samples were concentrated and reconstituted with 25 % methanol containing aqueous solution than analysed with LC-MS/MS. The measurements indicated that the recovery from alcoholic beverages was lower than of nonalcoholic ones. It was tentatively explained by the impact of ethanol on the separation process. It was stated that the method can be applied to monitor pesticide residues in drinking water and various beverages.[31] The metabolic profile of resveratrol has been investigated in healthy men using SPE coupled to LC-ESI-MS/MS. It was established that the food matrix influences considerably the bioavailability of resveratrol. The method identified 17 resveratrol and piceid derivatives. The results indicated that that supplement intake may be used to bring resveratrol to humans.[32] The impact of non-volatile compounds on the different in-mouth attributes such as astringency has been studied in detail. The target

compounds were separated with various HPLC technologies. It was found that aconitic acids, polymeric proanthocyanidins, caftaric, caffeic, coutaric acids and quercetins can considerably influence the astringency of Spanish red wines. Marked antagonisms was observed between the pairs cis/trans-aconitic acids, between aconitic acid and caffeic acids, and caffeic acids and quercetin-3-O-galactoside and quercetin-3-O-glucoside. It was concluded from the data that the interaction between the target molecules is a very complicated one and needs further investigations.[33] A RP-HPLC technology was developed and successfully applied for the measurement of Total Antioxidant Potential (TAP) It was stated that the method can be employed both for the analysis of pure compounds and complex mixtures. Hydroxyl radicals can be analysed indirectly as products of their reaction with p-hydroxybenzoic acid (pHBA) or terephthalic (TPA) acids. The 3,4-dihydroxybenzoic or hydroxyterephthalic acids can be separated with RP-HPLC method. Target compounds can be detected with electrochemical or fluorescence methods. It was stated that the method can be used for the determination of TAP values of some alcoholic beverages.[34] A similar HPLC method combined with fluorometric detection was applied for the determination of TAP values of low molecular mass molecules such as biogenic polyamines (spermidine, spermine, 1,7-diaminoheptane, putrescine and cadaverine). It was found that the HPLC procedure is suitable for the determination of the TAP values of complicated mixtures such as foods, herbal extracts, blood, alhoholic,, and non alcoholic beverages, etc.[35] In situ C-13 labelling coupled with LC-DAD-MS/MS was employed for the study of the phenolic biosynthesis in *Vitis vinifera*. The measurements indicated that the method can be used for the investigation of the biosynthesis of phenolics.[36] A reversed-phase HPLC column was applied for the separation and quantification of 9 organic acids (acetic, formic, citric, tartaric, lactic, malic, succinic, oxalic, and fumaric). The column dimensions were: 300 x 3 mm; mobile phase: consisted of Li_2SO_4, the flow rate was 0.5 mL min^{-1}. The standard deviation of the method was below 5 %.[37] A novel, simple and rapid method was developed for the separation and quantitative determination of pesticide residues in red wine. Pesticide residues were extracted into acetonitrile by QuEChERS (quick, easy, cheap, effective, rugged and safe). Samples were further purified with dSPE (dispersive solid phase extraction) followed by the separation of organic acids, sugars and polyphenolic pigments. Recoveries were between 81.6 % and 112.2 %. Final separation step was carried out by LC-MS-MS. The LOD and LOQ values varied 0.01-0.40 and 0.05-1.33 ng mL^{-1}, respectively.[38] Chromatographic profiles of wines were measured at different UV-Vis absorption wavelengths (280, 310, 370, and 520 nm) and by fluorescence at 260 nm excitation and at 360 emission. Principal component analysis (PCA) established that thirteen phenolic compound makes possible the differentiation between the Spanish varieties (*Penede, Rioja* and *Ribera del Duero*). The selected compounds were employed to build partial least squares discriminant analysis (PLS1-DA and PLS2-DA). Characteristic compounds were tentatively identified by LC-MS. It was found that the gallic acid characteristic for *Penedes*; trans-coumaroyltartaric acid and trans-caffeoyltartaric acids for *Rioja* and myricetin for Ribera del Duero wines.[39] The simultaneous determination of 17 free amino acids and 8 biogenic amines was carried out on an RP-HPLC column. Target compounds were

derivatized with a precolumn method using o-phthaldialdehyde (OPA) and detected with fluorescence detector. It was concluded from the results that the most abundant free amino acids are Glu, Arg, Ala, Asp and Lys. Histamine (HIM), cadaverine CAD), methylamine (MEA) and Tyramine (TYM) have not been found in the samples. The tryptamine (TRM) content war high in aged wines but the concentration of ethanolamine (ETA) was lower. These results were employed for the preliminary classification of the samples using cluster analysis.[40] The vascular effects of an enzymatic extract of grape pomace (GP-EE) was investigated and the concentration of polyphenols in the extract was determined by HPLC. The presence of kaempferol, catechin, quercetin and procyanidines B1 and B2, resveratrol, gallocatechin and anthocyanidines was established. It has been found that GP-EE possess antioxidant and protective vascular properties and can be applied as a functional food.[41] Capillary HPLC combined with laser-induced fluorescence detection (LIF) was employed for the determination of ochratoxin A in wines. Dispersive liquid-liquid microextraction (DLLME) was applied for the preconcentration of the target analyte. The LOD was 5.5 ng L^{-1}, recoveries ranged from 91.7 to 98.1%.[42] Both GC and HPLC were employed for the study of the effect of mixed culture fermentation (*Candida, Hanseniaspora, Saccharomyces*) on yeast populations and aroma profile. The measurements revealed considerable differences between the behaviour of mixed cultures.[43] HPLC-DAD was employed for the investigation of the distribution and concentration of anthocyanins and flavonols berries from *Vitis vinifera L.* cv. The measurement revealed the presence of the derivatives of five anthocyanins (malvidin, peonidin, petunidin, delphinidin and cyanidin) and six flavonols (quercetin, myricetin, kaempferol, laricitrin, isohamnetin and syringetin. It was stated that the berries from *Vitis vinifera L. cv. Brancellao* are suitable for the production of high quality red wine[44]. The phenolic profile of various grape skins was measured by HPLC-DAD-electrospray ionisation tandem mass spectrometry (HPLC-DAD-ESI-MS). The method identified 40 phenolic compounds including 3 hydroxybenzoic acids, 8 hydroxycinnamic acids, 4 flavanols, 5 flavanones, 3 flavonols, 4 stilbenes and 8 anthocyanins. The investigation found new compounds in grape skins such as protocatechuic acid-glucoside, p-hydroxybenzoyl glucoside, caffaric acid vanilloyl pentoside, β-coumaric acid-erythroside, naringenin hexose derivative, eriodictiol-glucoside, taxifolin-pentoside, quercetin-glucuronide, malylated kaempferol-glucoside, and resveratrol dimer. The application of grape skins as sources of bioactive compounds was proposed.[45] The phenolic profile of some spine grape varieties was determined using wet methods and HPLC. It was established that variety *Junzi*1* has the highest phenol content (total phenolic, flavonoids, flavanols, and anthocyanins) and the highest antioxidant capacity (DPPH radical-scavenging capacity, cupric-reducing capacity, and hydroxyl radical-scavenging activity). HPLC analysis of the samples established that (+)-catechin is the most abundant phenol derivatives and hydroxycinnamic acids are the most important phenolic acids. It was concluded from the results that *Junzi*1* has the best health promoting properties.[46] The conditions influencing the release of anthocyanins from the berry skins was investigated in detail. Samples were analysed by HPLC and the date were compared. The measurements indicated that the anthocyanin profile of berry skins depended

considerably on the environmental conditions such as ripeness, extraction kinetics. It was concluded from the results of the investigation that the data can be applied for the improvement of wine quality and for purposeful creation of different styles of wines.[47] The application possibility of LC-MS technologies for the analysis of foods and food products has been earlier discussed and it was established that LC-MS can be successfully employed the for the solution of problems of authentication and adulteration. Moreover the review deals with an outlook on future tendencies.[48] Ochratoxin A (OTA) is a possible human carcinogenic (IARC). The impact of alcoholic fermentation on the concentration of OTA has been investigated by HPLC-fluorescence detection (HPLC-FL). The measurements proved the detoxifying effect of alcoholic fermentation. It was further established that 5-12 of OTA is adsorbed on grape skins and both commercial strain were suitable for the reduction of the concentration of OTA during the alcoholic fermentation process.[49] A HPLC method was developed for the separation and quantitative determination of biogenic amines such as histamine, tyramine, spermine, spermidine, putrescine, cadaverine and phenylethylamine. The investigations indicated that wines show marked differences in the composition and concentration of biogenic amines, the amount of putrescine being the highest in each sample.[50] A new effective extraction and separation method was developed for the analysis of anthocyanins from black grapes. The possible application of grape anthocyanins in electronic and photonic devices is discussed in detail[51]. The aroma profile and phenolic content of some grapes grown in Italy were determined by various chromatographic technique and the result were compared by using multivariate mathematical statistical evaluation methods (cluster analysis, principal component analysis). Volatiles were extracted by using headspace solid phase microextraction (HS-SPME) carried out on PDMS fiber and were analysed by GC-MS. Ethyl hexanoate, ethyl decanoate, and ethyl octanoate were the dominating esters while phenyl ethanol, 3-methyl-1-butanol were the dominating alcohols.

HPLC-MS detected gallic acid, p-coumaric acid, trans ferulic acid, caffeic acid, trans-resveratrol, (+)-catechin and (-)-epicatechin. The total phenolic content of the wines varied between 30.4 – 61.9 mg L^{-1}.[52] HPLC and spectrophotometry were employed for the study of copigmentation and anti-copigmentation in grape extract. The investigations established that many components in wine are able for copigmentation modifying in this manner the color of the wine.[53] A HPLC procedure was optimized for the determination of biogenic amines (histamine, methylamine, ethylamine, tyramine putrescine, cadaverine, phenethylamine, isoamilamine).[54]

Abbreviations

CAD	cadaverine
CP	chlorpyrophos
CPO	chlorpyrophos-oxon
CT	cryogenic trapping
DAD	diode array detection
dSPE	dispersive solid phase extraction
DTC	dithiocarbamate
ESI-MS	electrospray ionisation tandem mass spectrometry
ETA	ethanolamine
FL	fluorescence detection
GC-O	gas chromatography-olfactometry
GC-MS	gas chromatography – mass spectrometry
HIM	histamine
HPLC	high performance liquid chromatography
HPLC-DAD-ESI-MS	high-performance liquid chromatography-photodiode-array detection-electrospray ionization mass spectrometry
HS-SPME	headspace solid-phase microextraction
LOD	limit of detection
ITEX	headspace in-tube extraction
LOQ	limit of quantitation
MEA	methylamine
MIPS	molecularly imprinted polysiloxane microspheres
ODS	octadecyl silica
OPA	o-phthaldialdehyde
OTA	ochratoxin A
PA	polyacrylate
PDC	triple-phase polydimethylsiloxane/carboxane
pHBA	p-hydroxybenzoicacid
QuEChERS	quick, easy, cheap, effective, rugged and safe
SBSE-GC-MS	stir bar sorptive extraction-gas-chromatography-mass spectrometry
SPME-GC-MS	solid phase microextraction-gas-chromatography
SPME	solid phase microextraction
TAP	total antioxidant potential
TPA	terephtalic acid
TRM	tryptamine
TYR	tyramine

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CORROSION RESISTANCE OF METALS IN SIMULATED

RINGER SOLUTION-A

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Corrosion resistance of three metals namely mild steel, 22 carat gold and 18 carat gold in Ringer Solution –A has been evaluated by polarization study. The corrosion resistance of metals in simulated Ringer Solution-A is follows; 22 carat gold > 18 carat gold > mild steel. This is due to the variation in composition of various types of gold

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INTRODUCTION

Recently, the development of biodegradable implants has attracted much attention in some biomedical applications where just require a temporary implant.[1,2] This kind of implants would be dissolved, absorbed or excreted gradually by human body after the fulfilment of their purpose at the site of implantation. Since then, biodegradable implants possess the essential potential to reduce/overcome the limitations posed by the permanent implant, such as elastic modulus mismatches between surrounding tissue and the implant, physical irritation or chromic inflammation etc.[3] Furthermore, such a biodegradable implant is expected to remove the need for a second surgical intervention to remove the implants after the successful treatment. This should certainly be deemed beneficial to patients.[1] Currently, the available biodegradable implants are primarily polymer-based. However, the insufficient mechanical strength, a drawback of their intrinsic mechanical properties, has limited their wide applications.[4,5] Thus, other biodegradable implants with sufficient mechanical properties are required urgently to be developed.

Corrosion resistance of meals and alloys in various body fluids such as artificial saliva,[6,7,8] artificial sweat,[9,10,11] blood plasma,[12,13,14] Artificial urine[15,16,17] and Ringer solution[18,19,20] has been investigated. The present work is undertaken to investigate the corrosion resistance of three metals namely, mild steel, 22 carat gold and 18 carat gold in Ringer Solution-A by polarization study.

Usually corrosion behavior metals and alloys have been studied in Ringer solution-A (RSA) whose composition[18,19,20] is given in Table 1.

Table 1. Composition of Ringer Solution-A

Substance	Amount, g L^{-1}
NaCl	6
KCl	0.075
CaCl$_2$	0.1
NaHCO$_3$	0.1

Ringer's solution [18,19,20] is the name given to a solution of several salts dissolved in water for the purpose of creating an isotonic solution relative to the bodily fluids of an animal. Ringer's solution typically contains sodium chloride, potassium chloride, calcium chloride and sodium bicarbonate, with the latter used to balance the pH. Other additions can include chemical fuel sources for cells, including ATP and dextrose, as well as antibiotics and antifungal. Ringer's solution is frequently used in *in vitro* experiments on organs or tissues, such as in vitro muscle testing. The precise mix of ions can vary depending upon the taxon, with different recipes for birds, mammals, freshwater fish, marine fish, etc. It may also be used for therapeutic purposes, such as arthroscopic lavage in the case of arthritis. Ringer's solution is named after Sydney Ringer in 1882, who found out that solution per fusing a frog's heart must contain sodium, potassium and calcium salts in a definite proportion if it has to beat for long. Ringer's solution is frequently used in human medicine in the form of Lactated Ringer's solution, a solution used in physiological experiments. It contains sodium, potassium, calcium, and magnesium chlorides; sodium bicarbonate, dextrose, and water.

MATERIALS AND METHODS

Three metal specimens namely mild steel, 22 carat gold and 18 carat gold were used in the present study. The composition of 22 carat gold,[21] 18 carat gold[22] and mild steel[23] are given in Table 2, 3 and 4 respectively.

Usually the study is carried out at 37 °C.

Table 2. Composition of 22 carat gold

Gold	91.67%
Silver	5%
Copper	2%
Zinc	1.33%

Table 3. Composition of 18 carat gold

Gold	75%
Copper	5-15 %
Silver	10-20%

Table 4. Composition of mild steel

Sulphur	0.026%
Phosphorous (Max)	0.06%
Manganese	0.4%
Carbon	0.1%
Iron	Balance

POLARIZATION STUDY

Polarization studies were carried out in a CHI-Electrochemical workstation with impendence Model 660A. A three-electrode cell assembly was used. The working electrode was mild steel. A saturated calomel electrode (SCE) was the reference electrode and platinum was the counter electrode. From the polarization study, corrosion parameters such as corrosion potential (E_{corr}), corrosion current (I_{corr}) and Tafel slopes (anodic=b_a and cathodic=b_c) were calculated.

It is observed from the Figure, 2 that for 18 carat gold,
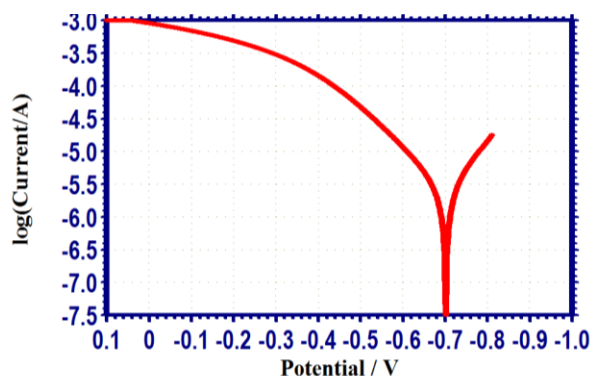
Figure 1. Polarization curve of mild steel immersed in Ringer solution A
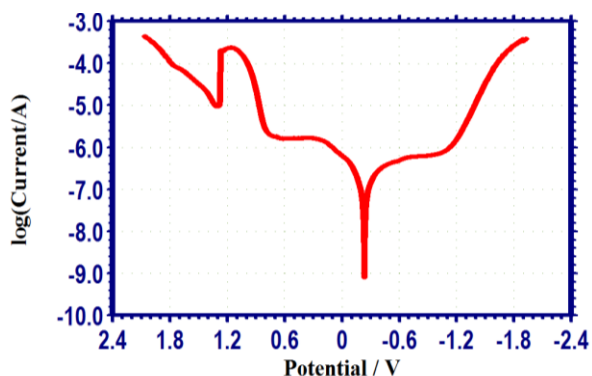
Figure 2. Polarization curve of 18 carat gold immersed in Ringer solution A
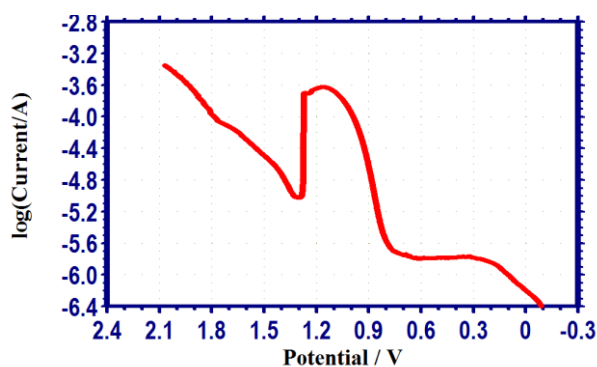
Figure2a. (Polarization curve of 18 carat gold immersed in Ringer solution A. Active passive region enlarged.
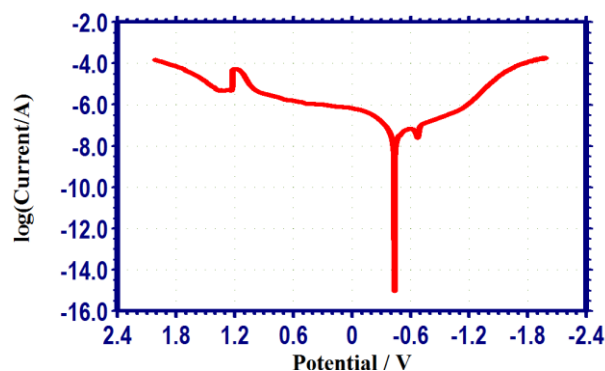
Figure 3. Polarization curve of 22 carat gold immersed in Ringer solution A
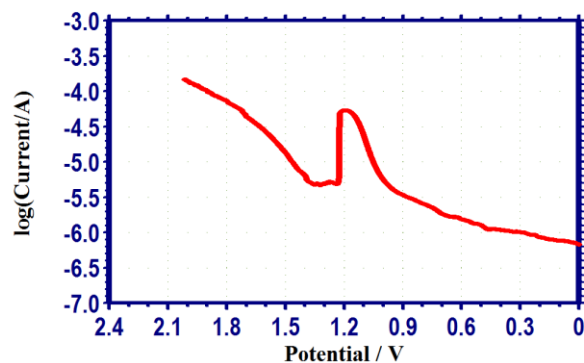
Figure 3a. Polarization curve of 22 carat gold immersed in Ringer solution A (active passive region enlarged.)

when the corrosion potential is shifted to noble side, that is from 241 mV to 760 mV, the corrosion remains constant. Afterwards as the corrosion potential is shifted to positive side, the passive film is broken and the corrosion current increases. When the corrosion potential 1253 mV is reached, the corrosion current sharply decreases. This is due to the fact a passive film is once again formed at this potential. When the potential is further increased to the positive side, the passive film is broken and the corrosion current increases. This is due to the presence of copper ions.

It is also observed that during cathodic polarization, the corrosion current remains constant in the potential range -1055 mV to -620 mV.

Table 5. Corrosion parameters of metals immersed in Simulated Ringer Solution-A obtained by potentiodynamic polarization study

System	E_{corr} mV vs SCE	b_c mV decade^{-1}	b_a mV decade^{-1}	LPR Ohm cm^2	I_{corr} A cm^{-2}
mild Steel	-701	125	143	10332	$2.803 \cdot 10^{-6}$
gold 18	-233	234	176	456194	$0.09563 \cdot 10^{-6}$
gold 22	-433	244	178	1279207	$0.03495 \cdot 10^{-6}$

During cathodic polarization, the corrosion current decrease sharply, when the potential changes from –1947 mV to –1061 mV.

It is observed from the Figure 3 that for 22 carat gold, as the corrosion potential is shifted to noble side, the corrosion current sharply decreases when, the corrosion potential is 1223 mV Vs ECE. A passive film is formed at this stage. Afterwards as the corrosion potential is shifted to noble side, the corrosion remains constant up to 1378 mV. Then the corrosion current increases.tha is, the passive film is stable in the potential range of 1223 mV to 1378 mV. This is due to the presence of copper ions.

RESULT AND DISCUSSION

Corrosion resistance of three metals namely mild steel, 22 carat gold, 18 carat gold in Ringer Solution-A has been investigated by polarization study.

Polarization study has been used to investigate the formation of protective film formed on the metal surface during corrosion process. If corrosion resistance increases, linear polarization (LPR) value increases. Corrosion current (I_{corr}) decreases.[24-33]

It is well known to everyone that mild steel should not be implanted in inside the body, because it will undergo corrosion due to the electrolytes present in body fluids. However in the present study, mild steel is used just for comparison.

The polarization curve of metals immersed in simulated Ringer solution-A are shown in Fig. 1. The corrosion parameters such as corrosion potential (E_{corr}), Tafel slopes (b_c=Cathodic; b_a=Anodic), linear polarization resistance (LPR) and the corrosion current (I_{corr}) are given in Table5.

When mild steel is immersed in SRAS, LPR value is 10332 Ω cm^2, corrosion current is $2.803 \cdot 10^{-6}$ Acm^{-2} and corrosion potential is –701 mV vs SCE.

When 18 carat gold is immersed in SRSA, LPR value (Fig 2) is 456194 Ω cm^2, corrosion current is 9.563×10^{-8} A cm^{-2} and corrosion potential is -233 mV vs SCE. It is observed LPR value increases and corrosion current value decreases. It is inferred that gold 18 is more corrosion resistant than mild steel. It is observed that the corrosion potential has shifted to the anodic side. This indicates that 18 Carat Gold is nobler than mild steel, due to the formation of passive film on the metal surface.

When 22 carat gold is immersed in SRSA, LPR value is 1279207 Ω cm^2, Corrosion current is $3.495 \cdot 10^{-8}$ A cm^{-2} and corrosion potential is -433 mV vs SCE. It is observed LPR value increases and corrosion current value decreases. It is inferred that gold 22 is more corrosion resistant than mild steel and also 18 carat gold. It is observed that the corrosion potential is shifted to the anodic side. This indicates that 22 carat gold is nobler than mild steel, due to the formation of passive film on the metal surface.

Thus, polarization study leads to the conclusion that the decreasing order of corrosion resistance of metals under investigation, in simulated Ringer Solution-A is, 22 carat gold > 18 carat gold > mild steel

The above order may be explained by the fact that, there is variation in compositions of various types of gold.

CONCLUSION

Corrosion resistance of three metals namely, mild steel, 22 carat gold and 18 carat gold in SRSA has been evaluated by polarization study. The corrosion resistance of metals in Simulated Ringer Solution-A is follows: 22 carat gold > 18 carat gold > mild steel

This is due to the presence of variation in composition of various types of gold.

ACKNOWLEDGEMENT

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SELECTIVE RECOGNITION IN POTENTIOMETRIC SENSING BASED ON TWO COMPETITIVE RECOGNITION SITES FOR STATIC AND HYDRODYNAMIC DETERMINATION OF CAMYLOFIN AS A SMOOTH MUSCLE RELAXANT

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A poly (vinyl chloride) matrix membrane sensors for selective determination of camylofin (CY) in pharmaceutical formulations were developed based on the use of dibenzo-18crown-6 (DB18C6) as a neutral carrier and ion-association complex ([CY]2[PM])of (CY) cation with phosphomolybdate anion (PM). Subsequently, these electroactive materials were dispersed in dioctyl sebacate (DOS) as solvent mediator designed and can be easily used in flow injection system. Under static mode of operation, the sensors revealed a near Nernstain response over a wide CY^+ concentration range 8.5×10^{-6} and 5×10^{-6} to 1.0×10^{-2} mol L^{-1} with a detection limit of 6.0×10^{-6} and 2.5×10^{-6} mol L^{-1}, respectively. In flow injection potentiometry, excellent reproducibility (RSD %±0.7%), fast response, high sensitity with a near-Nernstian 53.9 ± 1.1 and 40.2 ± 0.8 mV decade^{-1}, linear range 1.0×10^{-4} -1.0×10^{-2} mol L^{-1}, detection limit 16.4 ± 0.3 and 5.9 ± 0.3 µg mL^{-1}, high sampling rate (20-22 and 40-45 sample h^{-1}) and stable baseline was observed in the presence of 0.05 mol L^{-1} citrate buffer, pH 4.5 as a carrier for ([CY]2[PM] and (DB18C6) membrane based sensors, respectively. The utility of the sensors was tested for field monitoring of CY^+ in different pharmaceutical formulations collected from the local market.

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Introduction

Camylofin (CY); N-[2-(diethylamino)ethyl]-2-phenyl-glycine isopentyl ester is a smooth muscle relaxant with both anticholinergic action as well as direct smooth muscle action. Anticholinergic action is produced by inhibiting the binding of acetylcholine to muscarinic receptors, but the action is less pronounced. Direct smooth muscle relaxation is achieved by inhibiting phosphodiesterase type IV, which leads to increased cyclic AMP and eventually reduced cytosolic calcium. Thus camylofin has a comprehensive action to relieve smooth muscle spasm.

Only a few methods for the determination of camylofin (CY) in pharmaceutical analysis were reported, including high performance liquid chromatography (HPLC),[1-3] gas chromatography (GC)[4] and atomic-absorption spectrometry (AAS).[5] Most of these methods, however, require expensive instrumentation, suffer from lack of selectivity, involve careful control of the reaction conditions or derivatization reactions, and require time-consuming pretreatment steps which affect their usefulness for routine analysis. On the other hand, application of potentiometric sensors in the field of pharmaceutical and biomedical analysis has been advocated.[6,7] The approach provides simple, fast, and selective technique for determination of various drugs.[8-13] However, as far as the available literature is concerned, no potentiometric methods have yet been reported for the determination of camylofin.

The present work describes preparation, characterization and application of two potentiometric tubular membrane sensors for continuous determination of camylofin in pharmaceutical preparations. These sensors based on the incorporation of the camylofin phosphomolybdate (CY-PMA) ion pair complex and dibenzo-18-crown-6 in plasticized PVC matrix membranes (Fig.1). Performance characteristics of both sensors reveal low detection limit, high sensitivity, fast response, long life span and application for accurate determination of camylofin in pharmaceutical preparations under static and hydrodynamic (FIA) modes of operation.
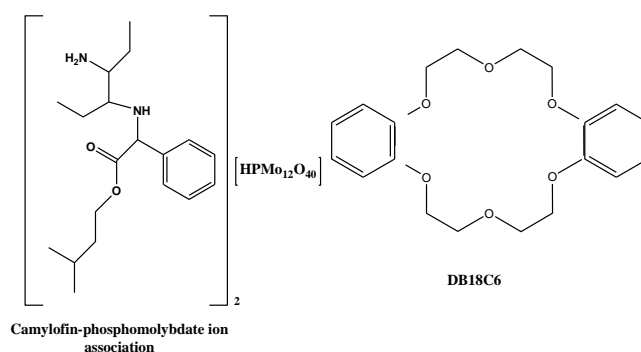
Figure 1. Structure of the CY-PMA ion association and DB18C6 ionophore.

Experimental

Equipments

All potentiometric measurements were carried out at 25±1 ºC using an EDT instruments DR 359 TX ion concentration pH meter. The sensors were used in conjunction with a double-junction Ag/AgCl reference electrode (Model 90-02) containing potassium nitrate (10% w/w) in the outer

compartment. A combination Ross pH electrode (Orion 81-02) was used for all pH measurements. Flow injection analysis (FIA) manifold consisted of a two-channel Ismatech Ms-REGLO model peristaltic pump, polyethylene tubing (0.71 mmi.d.) and an Omnifit injection valve (Omnifit, Cambridge, UK) with a sample loop of 100 μL volume. The potential signals were recorded using an Orion Model 720 SA (Cambridge, MA, USA) pH/mV meter connected to a PC through the interface ADC 16 (Pico Tech, UK) and Pico Log for windows (version 5.07) software.

Materials

All chemicals used were of analytical reagent (AR) grade and were used without further purification unless otherwise stated. Twice distilled water was used throughout all experiments. Pure grade camylofin was supplied by Drug Control Authority, Al-Haram, Giza, Egypt. Dioctyl phthalate (DOP), dioctyl sebacate (DOS), potassium *p*-chloro tetraphenyl borate (*p*Cl-TPB) and poly (vinyl chloride) (PVC) were obtained from Sigma (St. Louis, MO). Dibenzo-18-crown-6 was purchased from Aldrich Chemical Campany Inc. A 0.01 mol L^{-1} stock camylofin solution was prepared by dissolving 0.393g in 100 mL citrate buffer (0.05 mol L^{-1}) of pH 4.5.

Potentiometeric determination of camylofin

### Camylofin-phosphomolybdate ion associate complex

Camylofin phosphomolybdate ion associate complex (CY-PMA) was prepared by mixing 50 mL of 10^{-2} mol L^{-1} aqueous camylofin solution with 25 mL of 10^{-2} mol L^{-1} phosphomolybdic acid (PMA). The mixture was shaken well; the precipitate was filtered off through G4 sintered glass crucible, washed with de-ionized bi-distilled water, dried at room temperature and grounded to a fine powder. Elemental analysis of the complex gave C 38.4, H 5.4, N 4.7% for the camylofin- PMA ion-pair complex [(C$_{19}$H$_{32}$N$_2$O$_2$]$_2$[HPMo$_{12}$O$_{40}$], C 38.65, H 5.51, N 4.75%).

Camylofin-PVC membrane sensors

The plastic membranes were prepared by mixing 10 mg portion of ion pair (CY-PMA) with 190 mg of PVC, 350 mg of DOP and 7 mL THF in a glass Petri dish (5 cm diameter) covered with a filter paper and left to stand overnight to allow evaporation of the solvent at room temperature. Semi-transparent master PVC membranes of approximately 0.1 mm thick were obtained. Similarly, an another membrane sensor is made by mixing 10 mg dibenzo-18-crown-6 ionophore, 190 mg PVC, 5 mg *p*,Cl-TPB as anionic additive, 350 mg of DOP plasticizer and 7 mL THF. A disc (8 mm diameter) was cut using a cork borer and glued to polyethylene tube (3 cm x 8 mm i.d.) which was clipped onto the end of the electrode glass body. The electrode was filled with the internal reference solution consisted of an equal volume of 1×10^{-2} mol L^{-1} of camylofin and potassium chloride solutions. An Ag/AgCl internal reference wire electrode (1 mm diameter) was immersed in the internal solutions. Camylofin sensor was finally preconditioned by soaking in 1.0×10^{-2} mol L^{-1} camylofin for one day before use and was stored in the same solution when not in use.

The emf of the sensors were measured in buffer solutions obtained by transferring 0.5 mL aliquots of 1.0×10^{-2} – 1.0x10^{-6} mol L^{-1} CY$^+$ aqueous solutions to 50 mL beaker containing 10.0 mL of 0.05 mol L^{-1} citrate buffer of pH 4.5. Potential readings were recorded after stabilization to ± 0.2 mV and emf was plotted as a function of logarithm CY$^+$ concentration. Calibration graphs were used for subsequent determination of unknown CY$^+$ concentrations. General working characteristics of the CY selective electrodes were evaluated after calibration procedures carried out.

For continuous measurements (FIA), the flow cell used for detection of camylofin was designed with a constant geometry and minimum 'dead' space to accommodate small sensor size to avoid large dispersion of the sample in the cell and to give high response with short recovery time. With short tubing (10 cm) between the injector and detector, and using an injection volume of 100 μL, the dispersion in the system was kept to minimum (Fig. 2). A flow stream of 0.05 mol L^{-1} citrate buffer of pH 4.5 carrier solution were allowed to pass through the flow-cell at a flow rate 3.0 mL min^{-1}. Successive 100 μL aliquots of the standard camylofin and unknown test sample solutions were injected into the flowing stream. The corresponding potential change was measured and recorded versus time. A typical calibration plot was made and used to determine the concentration of camylofin in the unknown samples.
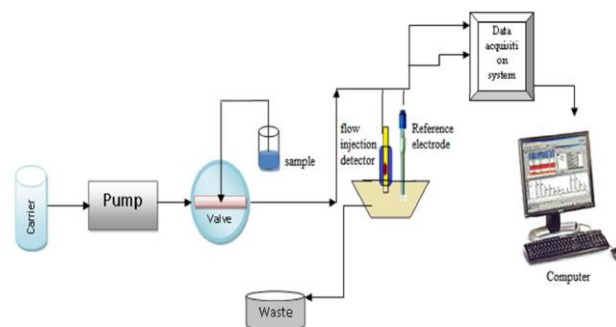
Figure 2. FIA manifold for the evaluation of camylofin. A 0.05 mol L^{-1} carrier citrate buffer solution pH 4.5; loop sample 100 μL; and flow rate 3 mL min^{-1}

Camylofin assessment

Potentiometric analysis was conducted on oral dosage forms of pharmaceutical preparations, commercially designated as Spasmopyralgin-M tablets (Kahira Pharm., Cairo, Egypt). Five tablets were reduced to a homogeneous fine powder in an agate mortar, accurately weighed, transferred to a 100 mL calibrated flask and completed to the mark with water. The contents of the flask were sonicated for 10 min to ensure complete dissolution. A 10.0 mL aliquot of the clear supernatant was diluted with 0.05 mol L^{-1} citrate solution of pH 4.5 in 50 mL measuring flask. For drug measurements under static mode of operation, a 10 mL aliquot of the drug solution was potentiometrically measured.

Potentiometric assessment of CY in urine samples

For the assessment of CY in human urine, a 1.0 mL aliquot of the human urine sample was diluted with 5x10^{-2} mol L^{-1} citrate buffer of pH 4.5 in a 50 mL calibrated flask

Table 1. Response characteristics of camylofin membrane sensors in 0.05 mol L^{-1} citrate buffer of pH 4.5.

Parameter	CY-PMA plasticized with				
	DOP (sensor I)	DOS (sensor II)	DOP (without additive) (sensor III)	DOP (with additive) (sensor IV)	DOS (without additive) (sensor V)
Slope, mV decade^{-1}	69.2	66.6	53.2	51.4±0.7	57.5±0.8
Coefficient, r (n=3)	0.9998	0.9996	0.9992	0.998	0.998
Detection limit, mol L^{-1}	2.5×10^{-6}	2.0×10^{-6}	2.5×10^{-5}	1.6×10^{-5}	6.0×10^{-6}
Linear range, mol L^{-1}	5.0×10^{-6}- 1.0×10^{-2}	5.0×10^{-6}-1.0×10^{-2}	4.5×10^{-5}-1.0×10^{-2}	4.5×10^{-5}-1.0×10^{-2}	8.5×10^{-6}-1.0×10^{-2}
Response time, s	10 - 20	10 - 20	10 - 20	10 - 20	10 - 20
Working range, pH	3.0 – 6.5	3.0 – 6.5	3.6 – 6.4	3.6 – 6.4	3.6 – 6.4
Standard deviation (%)	1.2	1.8	1.5	1.3	1.1
Accuracy (%)	99.3	99.1	98.7	98.8	99.1
Precision (%), Cv_w(%)	0.9	0.8	1.1	1.2	1.1
Between-day variability, Cv_b (%)	1.1	1.2	0.9	0.8	0.9

Table 2. Selectivity coeffients ($K^{pot}_{CY, J}$) of camylofine PVC membrane based sensors

Interferent, B	ISE I	ISE II	ISE III	ISE IV	ISE V
Camylofin	0	0	0	0	0
Quinine	-1.20	-1.25	-1.40	-1.42	-1.32
Ephedrine	-1.30	-1.15	-0.01	-0.21	-1.41
Codeine	-1.61	-1.72	-1.04	-1.05	-1.68
Caffein	-1.82	-1.85	-2.12	-2.22	-1.62
Histidin	-2.01	-2.11	-1.32	-1.36	-1.82
Glycin	-2.10	-2.15	-2.01	-1.92	-2.11
K$^+$	-3.21	-3.22	-2.21	-2.05	-2.20
Na$^+$	-4.01	-4.03	-3.21	-3.16	-3.82
Ba^{2+}	-4.02	-4.20	-1.10	-1.08	-1.91

and shaken well. A 10.0 mL portion of the diluted urine solution was transferred into a 25 mL beaker and then 0.5 mL of 10^{-2}, 10^{-3} and 10^{-4} mol L^{-1} standard drug solution were added, respectively. The working and reference electrode were immersed, and the potential readings were recorded after reaching the equilibrium response (10-20 s) and compared with the calibration plot.

Results and Discussion

Performance characteristics of the sensors

Camylofin-phosphomolybdate (CY-PMA) and Dibenzo-18-crown-6 (DB18CC6) membrane based sensors were prepared and examined for potentiometric camylofin determination with the composition 2:34:64 wt% of the ion pair complex (or ionophore), PVC and plasticizer, respectively [14]. The sensors based on (CY-PMA) exhibit a potentiometric response towards camylofin ions with near Nernstian slope of 69.2± 0.9 mV (r^2=0.999) and 66.6± 0.8 mV decade^{-1}with a detection limit 2.5×10^{-6} and 2.0×10^{-6} mol L^{-1} for the membrane plasticized with DOP [sensor I] and DOS [sensor II], respectively. For the sensors based on (DB18C6), they exhibit a near Nernstian response towards CY+ with slope of 53.2±1.1 mV (r^2=0.999) and 51.4±0.7 mV ($r2$=0.998) decade-1 with a detection limits 2.5×10^{-5} and 1.6×10^{-5} mol L^{-1} for the membrane plasticized with DOP [sensor III] and DOS [sensor IV], respectively (Fig. 3). From the previous results, it is obvious that the dielectric

constant of plasticized PVC is important when considering the material for use in ion selective electrode membrane. As shown in Fig. 3, it was found that low dielectric constant plasticizer DOS (ε=4.8) is desirable in the detection limit when measuring CY ions than DOP (ε=8). These results confirm what was reported in literature, that low dielectric constant plasticizer is desirable when monovalent ions are to be selected against divalent ions [15], and the nature of the plasticizer can influence the dielectric constant of the membrane phase, the mobility of the ionophore molecules and the state of ligands [16-18]. Addition of TPB- (30 mmol% relative to the ionophore) to membrane plasticized with DOS and incorporating (DB18C6) [sensor V] improve the response towards camylofin ions with a slope 57.5±0.8 mV (r^2=0.998) decade^{-1} and detection limit 6.0×10^{-6} mol L^{-1}. All potentiometric response characteristics of the sensors are shown in Table 1.

The validity of the proposed potentiometric method for determining camylofin was assessed by measuring the range, lower limit of detection (LOD), accuracy (recovery), precision or repeatability (CVw), between-day variability (CVb), linearity (correlation coefficient) and sensitivity (slope) [19]. Data obtained with six batches (six determinations each) of CY$^+$ solutions are shown in Table 1. From the results, it can be concluded that, the response of sensors based on (DB18C6) is based on a neutral carrier mechanism, in which the ionophore exhibits strong affinity towards CY$^+$ to create positively charged complexes in the membrane phase.
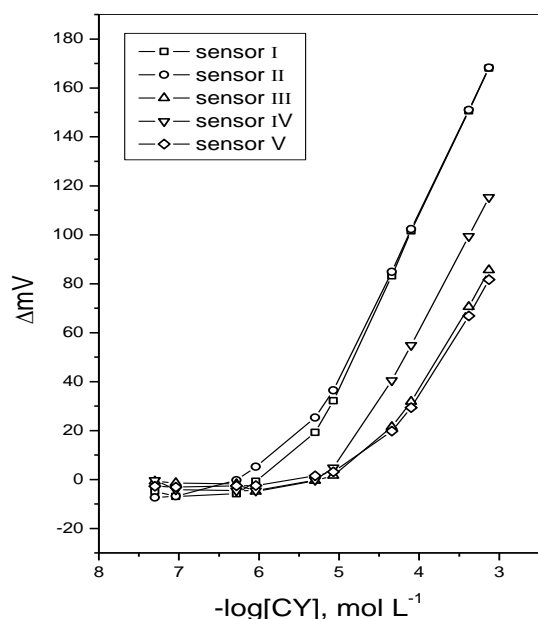
Figure 3. Potentiometric response of Camylofin membrane sensors I, II, III, IV and V using 0.05mol L^{-1} citrate buffer of pH 4.5.

To stabilize the presence of such complexes in the membrane, lipophilic anionic sites must be present [sensor V]. For sensors based on (CY-PMA), their response is mainly based on an ion exchanger mechanism, in which addition of anionic sites has no significant improvement in detection limit of the sensor.

Effect of pH and response time

The influence of pH on the potentiometric response of the proposed sensors were examined with standard 10^{-4} and 10^{-3} mol L^{-1} camylofin solutions over a pH range of 2–9. The pH of the solution was adjusted with either hydrochloric acid and/or sodium hydroxide solutions. From the pH/mV profile (Fig. 4), it is apparent that camylofin membrane based sensors reveal good stability with pH range 3.0 - 6.5 and 3.6-6.4 for sensor I and III, respectively. The potentials of both sensors considerably declined with negative drift at higher pH values due to progressive precipitation of the free camylofin base. The time required to achieve a steady potential response (±3mV) using the proposed sensors in 8.5×10^{-6} mol L^{-1} camylofin solutions with a rapid 10-fold increase in concentration were < 15 s for sensors based on [CY]2[PM] and < 30 s for sensors based on DB18C6, respectively. After several calibrations for each sensor, low potential drift, long-term stability and negligible change in sensors response were observed. When not in use, the sensors were stored and conditioned in 10^{-3} mol L^{-1}. For all sensors examined, the detection limits, response times, linear range and calibration slopes were reproducible within ±3% of their original values over a period of at least 8 weeks.

Sensor selectivity

The effect of interferents on the potentiometric determination of camylofin was investigated by the fixed interference ion method (FIM) [20]. In this method, the selectivity coefficients of CY sensors were evaluated with a fixed concentration of interferent (10^{-3} mol L^{-1}) adjusted to pH 4.5 with 0.05 mol L^{-1} citrate buffer solution. The selectivity coefficients were calculated using the following equation:

$$K_{A,B}^{pot} = \frac{a_A}{a_B^{\left[\frac{z_A}{z_B}\right]}} \qquad (1)$$

where a_A is the varying activity of the primary ion (camylofin) and a_B is the constant activity of the interfering ion.

For electrodes with membranes based on a less polar plasticizer (DOS), better selectivity toward CY$^+$ ions over interfering cations, is obtained compared to membranes with higher polar plasticizers (DOP). The selectivity order for these plasticizers were CY$^+$ > Quinine > Ephedrine > Codeine > Caffeine > Histidine > Glycine > K$^+$ > Na$^+$ > Ba^{2+} (sensor I), CY$^+$ > Ephedrine > Quinine > Codeine > Caffeine > Histidine > Glycine > K$^+$ > Na$^+$ > Ba^{2+} (sensor II), CY$^+$= Ephedrine > codeine> Ba^{2+} > Histidine > Quinine > Glycine > Caffeine > K$^+$> Na$^+$ (sensor III), and CY$^+$ ~ Ephedrine > codeine > Ba^{2+} > Histidine > Quinine > Glycine > K$^+$> Caffeine > Na$^+$ (sensor IV).
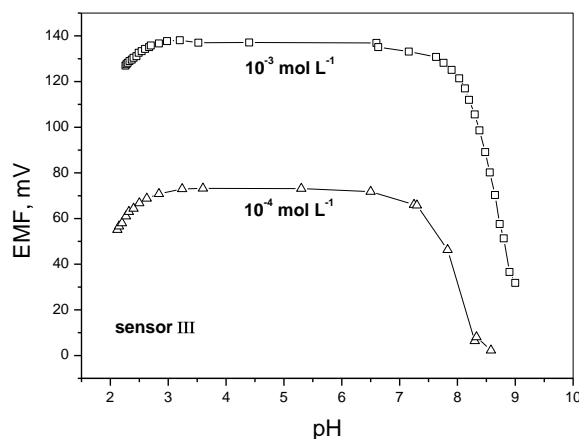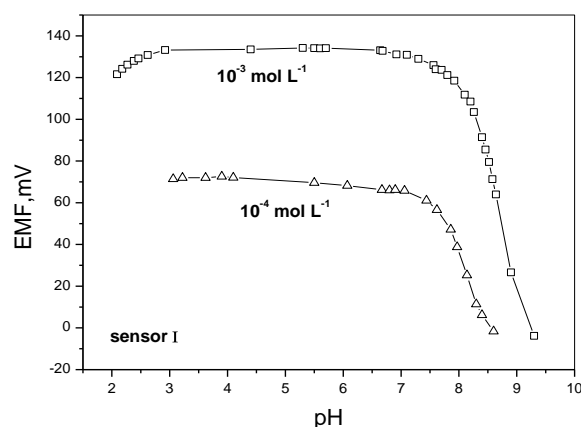
Figure 4. Effect of pH for Camylofin membrane sensor using sensors I and V.

Table 3. Response characteristics of camylofin sensors using FIA operation.

Parameter	Sensor I	Sensor IV
Slope (mV decade $^{-1}$)	53.9±1.1	40±0.8
Correlation coefficient, r	0.9997	0.9991
Lower limit of detection , µg mL^{-1}	16.4±0.3	5.9±0.1
Linear range, mol L^{-1}	1×10^{-4} -1.0×10^{-2}	1×10^{-4} -1.0×10^{-2}
Flow rate (mL min^{-1})	3.0	3.0
Sample loop volume , µL	100	100
Carrier solution	0.05 mol L^{-1} citrate buffer	0.05 mol L^{-1} citrate buffer
pH of the carrier solution	4.5	4.5
Sample output, h^{-1}	40–45	20–22

Table 4. Potentiomeric determination of camylofin in pharmaceutical preparation (Spasmograprin-M, Kahira Pharm., Egypt.) using membrane sensors I and V.

Labeled, mg/tablet	Found (mg tablet $^{-1}$)*			
	Batch		FIA	
	Sensor I	Sensor V	Sensor I	Sensor V
25	25.3±0.6	22.3±1.1	24.7±0.9	23.4±1.2

*Average of 6 measurements

The influence of the lipophilic anionic sites on the selectivity of the membrane (sensor IV) was shown in Table 2. The presence of lipophilic anionic sites helps reduce membrane resistance and limit the interference from anions at high sample activities. In addition to influencing the concentration of free carrier available for the complexing cations, they can also improve the selectivity of the ISEs [21]. The optimum concentration of such lipophilic additives in the membrane phase is dependent in part on the charge of the primary ion and its complexing stoichiometry with the carrier as compared to that of the interfering ion [22]. The selectivity order for sensor IV was CY^{+}> Quinine> Ephedrine> Caffeine> Codeine> Histidine> Ba^{2+} > Glycine > K^{+}> Na^{+}. This reflects that the presence of anionic sites in addition to the neutral carrier can affect on the mechanism by which the membrane responds. It will responds by the so called "Mixed mode mechanism", i.e. ion exchanger response mechanism and neutral carrier mechanism.

Flow injection potentiometry

For the routine control of an analyte, FIA setup is of regular selection, in view of their versatility, simplicity and suitability for large-scale analyses. The flow assembly was double-channel, and the potentiometric sensor was accommodated in a flow cell of tubular configuration, allowing full membrane/sample contact. Sensors I and V were used in this study for showing the best analytical features for the simplest membrane composition.

A sample loop (100 µL) for camylofin solution ranging from 1.0×10^{-4} to 1.0×10^{-2} mol L^{-1} at pH 4.5 with a 0.05 mol L^{-1} citrate carrier buffer, flow rate 3.0 mL min^{-1} was

chosen to study the potentiometric response (slope in mV decade^{-1}) of the proposed sensors. Main analytical features recorded under optimum flow conditions are presented in Table 3. Sensors I and V gave slopes of 53.9±1.1 and 40.0±0.8 mV decade^{-1} with detection limits of and 16.4±0.3 and 5.9±0.1 µg L^{-1} and lower limits of linear range of 1.0 × 10^{-4} mol L^{-1} for both, as shown in Fig.5.

Analytical applications

Determination of CY in pharmaceutical preparations (Spasmopralgin-M tablets [Kahira Pharm, Egypt]) collected from local market and labeled with amount of 25 mg CY tablet^{-1}, and analyzed by direct potentiometric analysis using sensors I and V. The average concentration was 25.3 and 22.3 mg per tablet, corresponding to recoveries of 101.1 and 89.2, respectively. Under hydrodynamic mode of operation, the same samples were also analyzed. The mean recovery values obtained were 99.4±1.1 and 94.9±0.8 for sensors I and V, respectively. All results for static and hydrodynamic potentiometric analysis for CY were shown in Table 4.
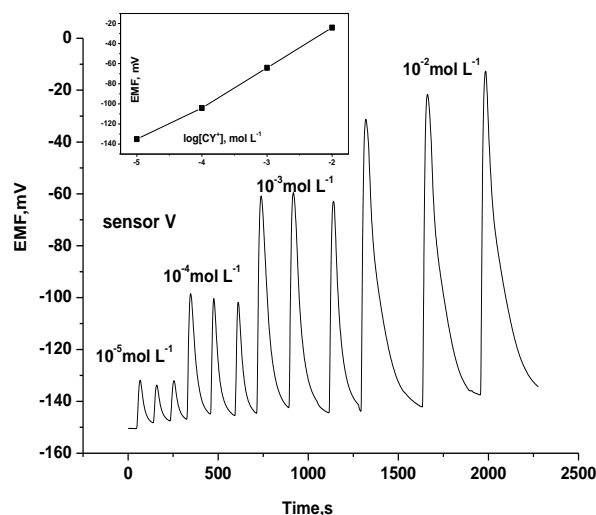
Figure 5. Typical FIA signals obtained by injecting pure camylofin standard solutions; 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} mol L^{-1} using sensors I and V.

Application of the method for determining camylofin in biological fluids was tested by spiking aliquots of human urine samples with a known concentration of standard CY^{+} in 1.0×10^{-2} mol L^{-1} Citrate buffer of pH 4.5. Internal QC sample from certified reference material (10^{-2} mol L^{-1}) were spiked into 10 mL of urine test solutions to evaluate the method procedure and recovery (φ, in %) using equation (2):

$$\varphi = 100 \frac{x_s - x}{x_{add}} \qquad (2)$$

where x_s, x and x_{add} are the results of spiked sample, mean results of un-spiked sample and of added (spiked) reference,

respectively. The results reveal average recoveries of 97.5±0.3 and 99.1± 0.7 % and mean precision of ±0.05 and (*n* = 10) for batch and FIA mode of operations, respectively.

With the standard addition method applied to urine samples, the average recoveries for sensor I were 95.5± 0.3 % and 93.0± 0.5 % for batch and FIA mode of operations, respectively. For sensor V the average recoveries were 95.8± 0.5 % and 95.7± 0.4 % for batch and FIA mode of operations, respectively. The mean recovery obtained by spiking of 20.0 μg mL^{-1} internal quality control sample to 100.0 μg mL^{-1} is 97.8±0.8 % .This confirms the applicability of the method for accurate routine analysis of camylofin in biological fluids.

Conclusions

A CY potentiometric sensors based on the use of dibenzo-18 crown-6 (DB18C6) as a neutral carrier, and ion-association complex of (CY) cation with phosphomolybdate (PMA) anion, exhibited excellent potentiometric performances such as quick response, a wide range of working pH, high sensitivity, long-term stability, good selectivity and self feasibility. The use of these sensors as detectors for the continuous monitoring of CY offered an advantage of simple design, ease of construction and possible application in the routine control of pharmaceutical drug solutions. The detectors displayed a wide range of dynamic measurement for the drug 1.0×10^{-4}–1.0×10^{-2} mol L^{-1} with detection limits 5.9±0.1 and 16.4±0.3 μg mL^{-1} under a continuous mode of operation at a flow rate of 3.0 mL min^{-1} and a sample outputs of 20–22 and 40-45 samples h^{-1} for sensors I and V, respectively.

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CONCENTRATION OF CADMIUM IN VEGETABLES GROWN ON CONTAMINATED GARDENS AND IN PURCHASED VEGETABLES

Petra Karo Bešter[a]*, Franc Lobnik[b], Ivan Eržen[c], Marko Zupan[b]

Self-supply with home-produced vegetables is very common in urban areas, where cadmium is a characteristic pollutant due to its anthropogenic origin. It has a harmful effect on human health. People are exposed to cadmium in different ways. One of the most common is through consumption of contaminated food, such as home-produced vegetables. However, different vegetables accumulate cadmium in different concentrations and as such pose different risk to human health. In this research 6 species of vegetables were sampled: endive, chicory, courgettes, tomato, onion and carrot. Vegetables (edible parts) were sampled in gardens of the Municipality of Celje, which is the third largest city in Slovenia. In some areas soils in the city and its vicinity are polluted with heavy metals (e.g. cadmium, lead, zinc) due to past industrial activities. Sampling area was stratified into 6 zones according to Slovenian legislation and the level of soil pollution with cadmium (mg/kg DW): <0.99, 1.00 - 1.99; 2.00 – 3.99; 4 – 7.99; 8 – 11.99; >12. Additionally, vegetables were purchased in local shops, markets and supermarkets in order to compare concentrations of cadmium in vegetables grown in gardens and in purchased vegetables. The main goal of this research was to determine whether purchased vegetables contain significantly lower concentration of cadmium than vegetables produced on gardens of the Municipality of Celje. Results showed that the significant difference between the average concentrations of cadmium in purchased and garden vegetables was observed in vegetables produced in gardens with soil cadmium content above 2 mg/kg DW. Therefore, the recommendation to purchase vegetable rather than to produce it at home garden is justified in areas where the contamination of soil with cadmium is above 2 mg/kg DW when producing carrot, chicory and endive or above 4 mg/kg DW when producing courgettes, tomato and onion.

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Introduction

Cadmium is a very toxic metal and has a harmful effect on human health, including promotion of osteoporosis, kidney damage, and lung cancer[13]. It occurs naturally in ores together with zinc, lead, copper and phosphorous. Cadmium compounds are used as stabilizers in PVC products, colour pigment, re-chargeable nickel–cadmium batteries and several alloys[8]. Due to its wide application in industry, cadmium is a characteristic pollutant in urban areas, where self-supply with home-produced vegetables is very common. Therefore, home gardeners are unknowingly exposed to cadmium through consumption of contaminated home-produced vegetables. However, different vegetables accumulate cadmium in different concentrations and as such pose different risk to human health. According to Pettersson[11] the order for accumulation of cadmium in vegetables was lettuce > carrot, tomato > rape, kale, radish > cucumber, peas, bean. Alexander et al.[2] reported the following order: lettuce > spinach > onion > carrot > pea >french bean. Zupan et al.[16] observed slightly different order: spinach > endive > carrot > red beet > lettuce > radish > tomato > potato > pea > Brussels sprouts > cabbage > kohlrabi.

The research area was the Municipality of Celje which is the third largest city in Slovenia. The population of the city is 37.834 people [14]. In some areas soils in the city and its vicinity are polluted with metals (e.g. cadmium, lead, zinc) due to past industrial activities (e.g. zinc smelters, brickworks, enamelware industry, heavy traffic and steel mills)[9,15]. Eržen et al.[4] concluded that increased concentrations of cadmium in soil in two areas of Municipality of Celje (Medlog and Teharje) presented a problem because surpluses of yields are sold on a market.

This research was conducted in the context of main research "Risk assessment of cadmium intake from home grown vegetables on the local population of the Municipality of Celje". The main goal was to determine whether purchased vegetables contained significantly lower concentration of cadmium than vegetables produced on gardens of the Municipality of Celje. Moreover, we wanted to determine if recommendation to buy vegetable is justified?

Materials and Methods

The sampling took place in August and September 2008. Vegetables were sampled on 59 home gardens of the Municipality of Celje and 222 samples of vegetables were collected. Purchased vegetables were sampled on 20 markets, where local population may buy vegetables and 100 vegetable samples were collected. Only edible parts were sampled.

Sampling garden area was stratified into 6 zones according to the level of soil pollution with cadmium (mg/kg DW): <0.99, 1.00 - 1.99; 2.00 – 3.99; 4 – 7.99; 8 – 11.99; >12. Slovenian legislation[3] determines three normative values of Cd concentration in soil (mg/kg DW after aqua regia dissolution): 1 - limit, 2 - warning and 12 - critical. However, the interval between warning and critical value of Cd in soil is quite wide, therefore we divided it to get more gradual transition from moderate to heavy polluted gardens in the sampling area.

The following 6 species of vegetables were included in research: endive, chicory, courgettes, tomato, onion and carrot. Samples of vegetables from gardens and markets were brought to the laboratory of the "Centre for Soil and Environmental Science", where they were cleaned with deionised water and cut with a stainless steel knife. Samples were than lyophilised and crushed in mill "Retsch ZM 100". The concentration of cadmium in vegetables was determined in "Acme Analytical Laboratories Ltd", Canada, after aqua regia dissolution by inductively coupled plasma mass spectrometry. Blanks, duplicates and standard reference materials were inserted in the sequences of analysed samples. As data of concentration of cadmium in vegetable was expressed in dry weight (DW), conversion to fresh weight (FW) was made based on literature data (Table 1).

Table 1. The water content of selected vegetables (bolded number refers to the number of reference)

Vegetables	The water content (%)					
	10	**7**	**12**	**6**	**1**	**Average**
Courgettes	94,7	91	91,3	94	91,6	92,5
Tomato	93,4	94	94,2	93,8	94,5	94,0
Onion	92,8	88	87,6	89	89,11	89,3
Carrot	89,9	90	89,7	89,2	88,29	89,4
Edive	93,7	94	94,3	93,8	93,79	93,9
Chicory	96,2	94	94,4	94,7	94,52	94,8

Allowed maximum levels of cadmium in vegetables according to European legislation[5] are set for tomato, onion and courgettes at 0.05 mg/kg FW; for carrot at 0.10 mg/kg FW; and for endive and chicory at 0.20 mg/kg FW.
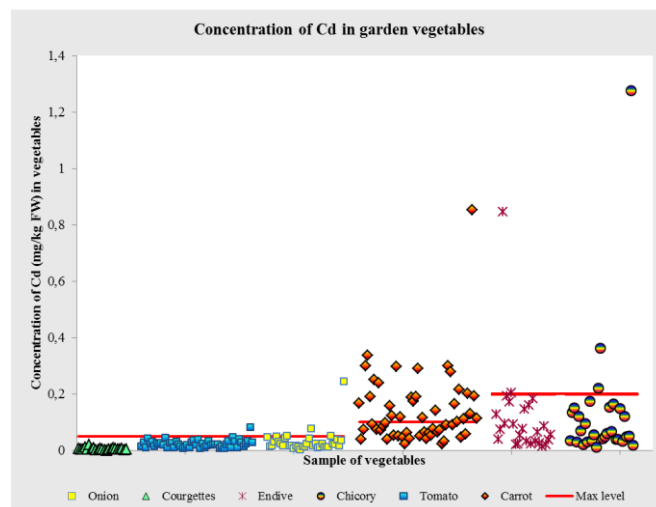
Results and Discussion

The concentration of cadmium in garden vegetables exceeded the regulatory allowed maximum level in 39 samples (Figure 1). The most frequently exceeded concentration of cadmium was in carrot (27-times), followed by onion (4-times), chicory (3-times), endive (2-times) and once in tomato. However, bear in mind that the number of sampled vegetable species was different.
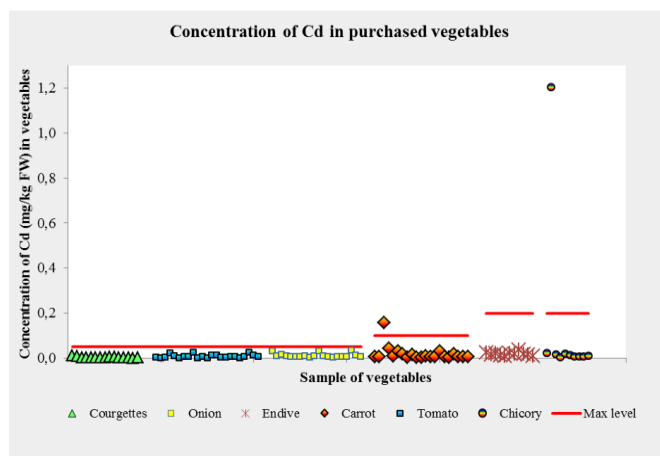
The concentration of cadmium in purchased vegetables was exceeded in two samples, in carrot (0,157 mg/kg FW) and chicory (1,203 mg/kg FW) (Figure 2). Both two samples were produced locally and bought in city market.

Figure 3 illustrates the response of garden vegetables to increase concentrations of cadmium in soil. The lowest response was noticed in courgette and the moderate response in tomato and onion. The highest responses were observed in carrot, chicory and endive, which also explain the high average concentrations of cadmium in their tissue

(Table 2). The first main increase of cadmium concentration in carrot, chicory and endive occurs at the level of garden pollution between 2.00-3.99 mg/kg DW of cadmium in soil and in courgette, tomato and onion at the level between 4.00-7.99 mg/kg DW.

Figure 1. The concentrations of cadmium in a garden vegetable samples with maximum levels (mg/kg FW)[5]

Figure 2. The concentration of cadmium in purchased vegetable sample, with maximum levels (mg/kg FW)[5]

Table 2 depicts the basic descriptive statistics for garden and purchased vegetables. The concentrations of cadmium in garden vegetables mostly varied in endive, chicory and carrot. The latter two also varied the most in purchased vegetables.

The average concentration of cadmium in vegetable was only exceeded in garden carrot (0,139 mg/kg FW). All other average concentrations of cadmium in vegetables were below the regulatory allowed maximum level. The lowest average concentration of cadmium in purchased vegetables was in courgettes (0,0034 mg/kg FW), followed by tomato (0,0074 mg/kg FW), onion (0,0104 mg/kg FW), carrot (0,0182 mg/kg FW), endive (0,0185 mg/kg FW) and chicory (0,1295 mg/kg FW). Similar order was observed in garden vegetables, with the difference in carrot being in the last place.
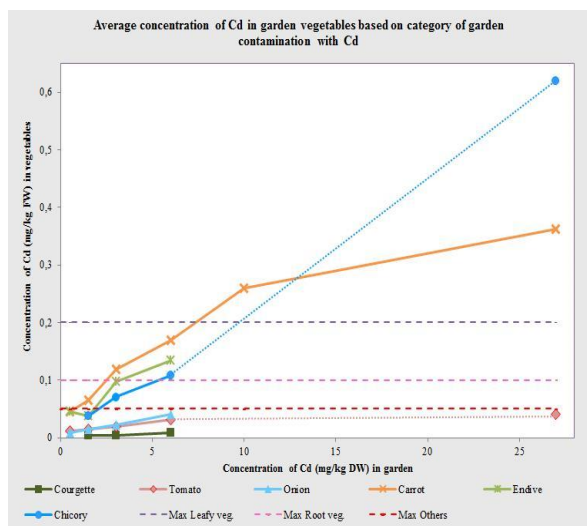
Figure 3. The average concentration of cadmium in vegetables grown on gardens with different soil cadmium content and regulatory allowed maximum levels of cadmium in vegetables. Dotted line refers to the lack of data of the average concentration of cadmium in chicory and tomato at level of soil pollution between 8.00 and 11,99 mg/kg DW of cadmium in garden.

The lowest concentration of cadmium in a sample was detected in fruit vegetables: in courgettes (0,0008 mg/kg FW) in garden vegetables and in tomato (0,0003 mg/kg FW) in purchased vegetables. The highest concentration of cadmium in a sample was detected in purchased and garden chicory (1,2028 mg/kg FW and 1,2761 mg/kg FW, respectively). Moreover, the highest difference between average value and median was noticed in chicory, but average values between purchased and garden chicory were similar (0,1295 and 0,1284 mg/kg FW, respectively).

Generally, the average concentrations of cadmium were lower in purchased vegetables than in garden vegetables. The average concentration of cadmium in courgettes was 1,7 times higher than in purchased, 3-times higher in tomato and onion, 6-times higher in endive and 7,6-times higher in carrot than in purchased ones. Likewise, the average concentration of cadmium in purchased vegetables was lower than average concentration of cadmium in garden vegetables, which were stratified in 6 garden categories, with the exception of chicory. In general, the significant difference between average concentrations of cadmium in purchased and garden carrot, chicory and endive were detected in gardens with soil cadmium content more than 2 mg/kg DW and in courgette, tomato and onion at gardens with soil cadmium content above 4 mg/kg DW.

The ratio between medians of garden and purchased courgettes, tomato and onion was similar compared to the ratio between average values of garden and purchased vegetables. However, the median of garden endive was 3,8-times higher compared to purchased, the median of chicory was 6,2-times higher and the median of carrot was 15,3-times higher compared to purchased ones.

Table 2. Descriptive statistics for vegetable samples from markets, gardens and according to category of garden contamination with Cd (mg/kg DW)

	N	Average (mg/kg FW)	±95% Conf.interval	Median	Min	Max	SD	SE	Max level (mg/kg FW)
Courgette									
MARKET	15	0,0034	0,0016	0,0023	0,0008	0,0113	0,0029	0,0007	0,05
GARDEN	23	0,0057	0,0018	0,0045	0,0008	0,0203	0,0041	0,0009	
Category of garden									
under 1	1	/	/	/	/	/	/	/	
between 1-1,9	6	0,0044	0,0010	0,0045	0,0008	0,0098	0,0032	0,0013	
between 2-3,9	9	0,0043	0,0025	0,0038	0,0015	0,0083	0,0023	0,0008	
between 4-7,9	6	0,0090	0,0027	0,0075	0,0038	0,0203	0,0060	0,0025	
between 8-11,9	0	/	/	/	/	/	/	/	
above 12	1	/	/	/	/	/	/	/	
Tomato									
MARKET	23	0,0074	0,0031	0,0060	0,0003	0,0258	0,0072	0,0015	0,05
GARDEN	52	0,0225	0,0037	0,0210	0,0060	0,0804	0,0133	0,0018	
Category of garden									
under 1	3	0,0116	0,0228	0,0066	0,0060	0,0222	0,0092	0,0053	
between 1-1,9	13	0,0145	0,0100	0,0132	0,0060	0,0288	0,0076	0,0021	
between 2-3,9	20	0,0199	0,0161	0,0210	0,0084	0,0330	0,0081	0,0018	
between 4-7,9	10	0,0317	0,0240	0,0306	0,0144	0,0456	0,0107	0,0034	
between 8-11,9	1	/	/	/	/	/	/	/	
above 12	5	0,0407	0,0124	0,0240	0,0240	0,0804	0,0227	0,0102	
Onion									
MARKET	20	0,0104	0,0044	0,0070	0,0032	0,0342	0,0094	0,0021	0,05
GARDEN	36	0,0304	0,0135	0,0193	0,0032	0,2429	0,0399	0,0066	
Category of garden									
under 1	3	0,0093	0,0031	0,0107	0,0064	0,0107	0,0025	0,0014	
between 1-1,9	10	0,0148	0,0094	0,0134	0,0032	0,0289	0,0075	0,0024	
between 2-3,9	14	0,0218	0,0151	0,0161	0,0118	0,0514	0,0115	0,0031	
between 4-7,9	6	0,0408	0,0326	0,0412	0,0310	0,0514	0,0078	0,0032	
between 8-11,9	0	/	/	/	/	/	/	/	
above 12	2	/	/	/	/	/	/	/	
Carrot									
MARKET	21	0,0182	0,0153	0,0064	0,0021	0,1569	0,0336	0,0073	0,10
GARDEN	55	**0,1386**	0,0350	0,0975	0,0223	0,8544	0,1296	0,0175	
Category of garden									
under 1	4	0,0451	0,0207	0,0498	0,0233	0,0572	0,0153	0,0076	
between 1-1,9	13	0,0650	0,0489	0,0636	0,0223	0,1177	0,0266	0,0074	
between 2-3,9	21	0,1183	0,0839	0,1018	0,0318	0,3000	0,0755	0,0165	
between 4-7,9	9	0,1696	0,1144	0,1654	0,0731	0,2979	0,0718	0,0239	
between 8-11,9	3	0,2593	0,0766	0,2502	0,1908	0,3371	0,0736	0,0425	
above 12	5	0,3623	0,0137	0,2904	0,1738	0,8544	0,2808	0,1256	
Endive									
MARKET	11	0,0185	0,0053	0,0183	0,0098	0,0378	0,0080	0,0024	0,20
GARDEN	26	0,1117	0,0654	0,0698	0,0128	0,8473	0,1618	0,0317	
Category of garden									
under 1	3	0,0460	0,0994	0,0250	0,0207	0,0921	0,0400	0,0231	
between 1-1,9	7	0,0377	0,0227	0,0329	0,0207	0,0659	0,0162	0,0061	
between 2-3,9	10	0,0980	0,0495	0,0808	0,0128	0,1928	0,0677	0,0214	
between 4-7,9	5	0,1348	0,0677	0,1269	0,0738	0,2056	0,0541	0,0242	
between 8-11,9	0	/	/	/	/	/	/	/	
above 12	1	/	/	/	/	/	/	/	
Chicory									
MARKET	10	0,1295	0,2698	0,0094	0,0042	1,2028	0,3772	0,1193	0,20
GARDEN	30	0,1284	0,0858	0,0580	0,0104	1,2761	0,2298	0,0419	
Category of garden									
under 1	0	/	/	/	/	/	/	/	
between 1-1,9	8	0,0383	0,0230	0,0372	0,0104	0,0671	0,0183	0,0065	
between 2-3,9	11	0,0708	0,0386	0,0499	0,0291	0,1659	0,0478	0,0144	
between 4-7,9	7	0,1080	0,0549	0,1217	0,0177	0,1732	0,0574	0,0217	
between 8-11,9	1	/	/	/	/	/	/	/	
above 12	3	0,6198	1,4230	0,3635	0,2200	1,2761	0,5728	0,3307	

Conclusion

Due to basic statistics parameters and the fact that the average concentration of cadmium in garden vegetables was at least two times higher than in purchased vegetables, with the exception of chicory, the purchased vegetable contribute less cadmium in food chain than garden vegetables. However, the significant difference between the average concentrations of cadmium in purchased and garden vegetables was observed in vegetables produced in gardens with soil cadmium content above 2 mg/kg DW. Therefore, the recommendation to purchase vegetable rather than to produce it at home garden in contaminated areas is justified in areas where the contamination of soil with cadmium is above 2 mg/kg DW when producing carrot, chicory and endive or above 4 mg/kg DW when producing courgettes, tomato and onion.

Acknowledgements

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