Available online at www.sciencedirect.com

ScienceDirect

Procedia Technology 22 (2016) 187 – 194

Procedia Technology

9th International Conference Interdisciplinarity in Engineering, INTER-ENG 2015, 8-9 October 2015, Tirgu-Mures, Romania

New Alternative in the Methodology of Extraction and Dyeing with Active Molecules Derived from Vegetal Sources

Diana Coman[a], Narcisa Vrinceanu[a,b,]*, Milena Dinu[a], Remus Calin Cipaian[a]

[a] *"Lucian Blaga" University of Sibiu, 10 Victoriei Blvd., Sibiu 550024, Romania*
[b] *"Al.I.Cuza" University of Iasi, 21 Carol I Street, Iasi 700506, Romania*

Abstract

The general objective of this study refers to the identification of a sustainable and physical methodology of extraction of active compounds, envisaging the preservation of the high purity active natural dye molecule from nut shell (juglone), even under the conditions of an extraction performed in a mixt solvent medium (water-ethanol). The second major objective of the study consists of the application of these above mentioned dyes onto natural and synthetic substrates, thus making a correlation between their colour attributes and the fibrous composition of the substrates they are applied on. The motivation of this research was given by the identification of an improved extraction methodology that can increase the percentage of active parts of the plant cell wall. The efficacy of the extraction protocol in a water-ethanol solvent medium from nut shells is evidenced by the complex system of investigations: HPLC chromatography, FT-Raman and IR spectroscopy. HPLC chromatography reveals an efficient ecologic extraction procedure. Moreover, the refluxing duration in the experimental protocol of juglone extraction in a water-ethanol solvent medium from nut shells is almost the same as that belonging to synthetic juglone.

Peer-review under responsibility of the "Petru Maior" University of Tirgu Mures, Faculty of Engineering

Keywords: green walnut shells; US extraction; juglone; FT-IR spectroscopy; coloristic attribute; FT-RAMAN.

* Corresponding author. Tel.: +400721428641.
E-mail address: vrinceanu.narcisai@ulbsibiu.ro

Peer-review under responsibility of the "Petru Maior" University of Tirgu Mures, Faculty of Engineering
doi:10.1016/j.protcy.2016.01.043

1. Introduction

The starting point of the research is directed toward conventional extraction techniques that have some limitations, such as: mass transfer resistances due to the involvement of more than one phase in the system [1], time consuming and long duration varying on the diffusion rates of solvents through the pores of the materials, high energy consumption [2]. Another major drawback of the conventional extraction procedure is linked up to the high temperatures and severe pressures that can destroy the active molecules and damage the quality of the extract [3-6]. A mechanical approach of the extraction should be avoided due to its poor efficacy. Chemical extraction, using a huge quantity of organic solvents, has a major and hazardous impact on both human beings and the environment [7].

Consequently, the rationale of this research was given by the identification of an improved extraction methodology, namely ultrasonication assisted extraction (UAE) that can increase the percentage of active parts from the plant cell wall. Basically, UAE is explained by the acoustic cavitations provided by ultrasonic sounds and mass transfer to the solvent.

The active molecules were extracted from green nut shells, using a green water-solvent extraction system by refluxing/UAE, for at least two hours. The dyeing methodology assumes the use of dye extract and biomordants, such as citric acid and tannic acid by comparison with a classic mordant. The retention time of the juglone extracted from walnut shells was performed by comparison with the retention duration of synthetic juglone.

The methodology we proposed in this study is an “add on” step to the existing process, having the following advantages: minimum alteration of the active molecules, application in semi-aqueous extraction where organic solvents can be partially replaced with safe solvents, shortening the extraction time. The ultrasound assisted extraction is more economic than the traditional extraction processes, being a must have for a sustainable development [8].

Another contributing response of this study has as key point the difference regarding the crystal or particle structure, during their using: a pigment maintains its structure, while the colorant loses it, by solving in a solvent system. Consequently, the research stresses the element of novelty, namely the preserving of the colorant molecule derived from walnut shells (juglone), even under its aqueous extraction conditions. Moreover, the extraction yield is relatively high.

The impact of this study consists of the application of natural extracts on polymeric supports natural/active, by investigating the mechanisms, the essence being a sustainable, innovative laboratory experiment of natural dye extraction from dried nut shells and dyeing of wool and polyamide (PA) polymeric substrates assisted by biomordants.

The juglone (5-hydroxy-1,4-naphtalen-dione), a very well-known natural colorant, is a mixture of phenols and quinones (Fig. 1) [9,10].

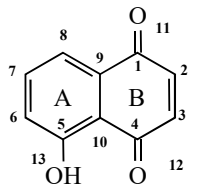

Fig. 1. The rings of phenol and quinone (A, B) depicted in atoms arrangement within juglone molecule.

Juglone can be extracted from walnut shells (*Juglans regia*). Its application in silk, polyamide (PA), etc is widely known.

2. Experimental procedure

2.1. Aqueous extraction

When dealing with compounds of low solubility, it is compulsory to extract them from a solid mixture. In this case, for colorant identification, a Soxhlet extraction can be performed.

The walnut shells are weighed. The technique is performed in a specialized piece of glassware in-between a flask and a condenser, forming the Soxhlet extractor. The basics of the technique consist of the washing of the solid by means of a solvent, extracting the desired compound into the flask. A quantity of 0.05-0.2 g was immersed into the thistle of the Soxhlet extractor, and a mixture of water/ethanol was used as solvent. The temperature of the instrument was maintained just below the boiling point of the used solvent. Several cycles of solvent were run for two hours so as to extract all the compounds from the dried walnut shells. The aqueous solution was filtered and evaporated, in order to obtain a dried residue. This residue was solved in acetronitrile and used for the analyses in HPLC (High Performance Liquid Chromatography) system.

2.2. Ultrasound (UAE) - assisted extraction

UAE was conducted by mixing dried and ground samples of green walnut shell in methanol. The sample was then placed in an Elmasonic S10 H ultrasonic chamber, for 30 min. The ultrasound chamber has a frequency of 20 Hz using different sonic powers (100-500 W) for different time intervals (15-120 min). Initially, the extraction temperature was set-up at 20- 40°C and after two hours it rose up to 60°C. The extraction was repeated two-three times and the extracts were collected. The solvent system was ethanol (EtOH) and water. 50 mL of bisolvent system were immersed in an Erlenmeyer flask of 250 mL containing 20 g of dried walnut shells. In order to avoid the evaporation of the solvent, the Erlenmeyer flask was covered with a thin film of alumina. The power level was set at a maximum (level 5) and the temperature during the 1-2 hour extraction period was maintained at 25-30°C. The temperature was controlled and maintained below 30°C through the periodical replacing of the water in the bath with cold one (15°C). Afterwards the flask was taken out and cooled down to room temperature using water. The extract was filtered using Whatman filter paper, and the solution was collected. The residue was immersed back in the ultrasonic chamber and extracted again in the same conditions. The extracts resulting from the twice-UAE extraction were mixed. It can be assumed that the UAE technique is more selective than the conventional one.

2.3. Textile supports and chemical reactives

For this study, woolen and polyamide (PA) textile fabrics with the following features: Relon 100%, raw, washed-degreased, with a weight of 67g/m2 and 11 type woolen fibers based support were used. Acetonitrile (HPLC grade) and disodic phosphate were bought from Fluka Company. The standard juglone powder was acquired from Sigma Aldrich.

The dyeing methodology assumes the use of dye extracts and biomordants like citric acid and tannic acid, as opposed to a classic mordant (copper sulphate).

The experimental dyeing protocol and the colour differences measured by reflexion spectrophotometry, can be associated with the juglone quantity, as well as the textile substrate the extracts were applied on.

2.4. Characterization systems for the juglone extracts

HPLC system consists of: a LC-112 UV Perkin Elmer detector, software for data processing, a system to record the data and a C18 column having the following dimensions: 150 × 4.6 mm, 5µm.

The experimental conditions of juglone determination, by means of its standard/reference, include an acetone percentage in mobile phase (50%), the mobile phase pH was maintained at 4, by using PBS (phosphate buffered solution), and a column temperature of 30°C. In these environmental conditions, the maximum of absorption of juglone does not interfere with other compounds extracted from walnut shells. An elution time of at least 25 min

was compulsory in order to remove the undesired compounds presented in the extract. The flow rate and the injection volume were 1.3 ml/min and 5 µl.

SERS (Surface Enhanced Raman Spectra) attributed to juglone, were measured, by means of a micro-Raman spectrometer, built-up next to an Olympus, X92 microscope. The He-Ne laser is directed toward an objective lens having a magnitude of 40×. The reference Raman signal was collected by the same objective lens.

The spectral resolution is estimated at approximately 4 cm^{-1}. IR spectra were acquired at a resolution of 1.0 cm^{-1} with a Bruker Vertex spectrophotometer 60V. The KBr pellet technique was used to prepare the powder colorant. The FT-Raman spectra of solid powder were achieved at a resolution of 4.0 cm^{-1}, with a Bruker spectrometer, by using a Nd:YAG laser, (~30 mW), running at 1064 nm.

The measurement ofcolour response of the studied samples was conducted with a stable laboratory Datacolor 110^{TM} LAV reflexion spectrophotometer.

3. Results and discussion

Figure 2 depicts the effect of refluxing time over the juglone water extraction. After two hours of refluxing irrelevant differences could be noticed, which is why a two-hour span is the most appropriate for the extraction.

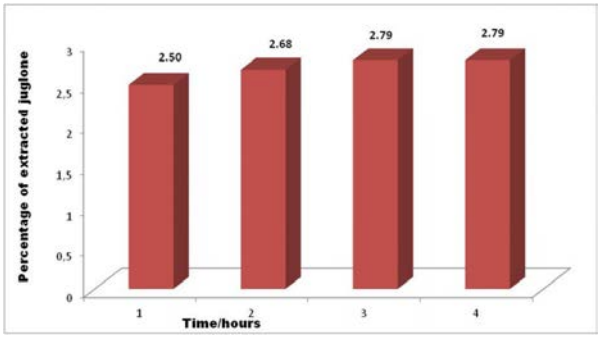

Fig. 2. The effect of reflux duration onto the percentage of juglone extracted from green walnut shells

Figure 3 presents the chromatograms assigned both to the juglone solution extracted from green walnut shells and standard juglone. In experimental conditions, the retention time for juglone is approximately 6.3 min, and its identification in the solution was made by comparison with the retention duration of standard juglone. The HPLC technique quantitatively highlights the efficiency of the ecologic procedure of juglone extraction. It can certainly be claimed that the percentage of the extracted juglone is relatively high (2.79% juglone in 0.1 g walnut shells), conducting to the remark that the 100 % juglone was extracted from a weight of 3.58 g of walnut. Technically, the yield is higher than 50%.

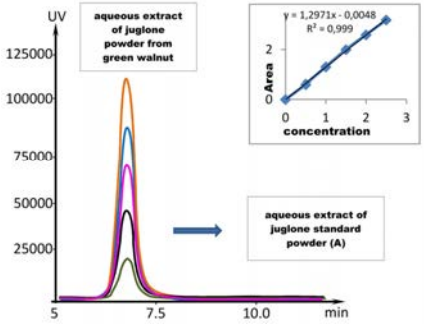

Fig. 3. The chromatogram belonging to aqueous extract of the juglone standard powder (A); the chromatogram belonging to aqueous extract of the juglone powder from green walnut shells (B)

3.1. Comments on juglone spectra

Discrete Fourier Transform calculus was performed in order to obtain IR and FT-Raman spectra of juglone powder. The solid sample of juglone has a high reflectance and thus the FT-Raman spectrum is "noisy", and the bands intensity is decreased [11-14].

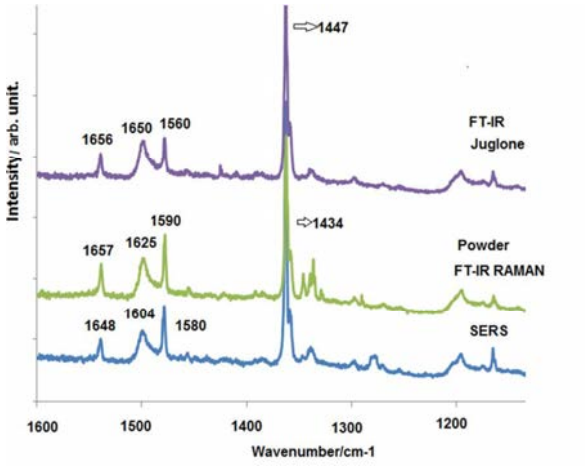

Fig.4. FT- IR, FT-Raman and SERS spectra belonging to double bond of juglone

However is noteworthy to mention that FT-IR and FT-Raman spectra are very different, with respect to bands intensity. Nevertheless, in most cases, similitude in terms of the bands frequency occurs when compare two of the spectra. The strongest bands from the region specific to double bond (1700-1400 cm−1) lead to the assumption of double bond shift, in case of the natural colorant. The strong bands are noticed, not only at double bond, but also within IR region (300 cm–1 - 1000 cm–1) belonging to SERS spectrum. Technically, the regions specific to juglone double bonds, belonging to IR, FT-Raman and SERS spectra are shown in Fig. 4 [15].

3.2. The interpretation of data provided bycolour measurement protocol

Table 1 reveals the colour coordinates, meaning luminosity andcolour differences for the woolen samples dyed with extracts A and B. The values of luminosity and colour differences are changing in terms of the mordant type used in dyeing process (copper sulphate, citric and tannic acids).

The highest colour difference is attributed to the woolen samples dyed with extract A, in the presence of copper sulphate, as mordant. The lowest differences are noticed at samples dyed with the assistance of tannic and citric acid (concentration of 3%) as biomordants, with reasonable colour changes with respect to the reference.

Table 1. The values of luminosity andcolour differences attributed to the woolen samples dyed with extract A (water: ethanol 1:1) and B (water: ethanol 3:1) consisting of green walnut shells

Woolen sample, mordant	CIE DL*		CIE Da*		CIE Db*		CIE DC*		CIE DH*		CIE DE*	
3% copper sulphate	-6.11	3.09	-1.07	-6.63	4.39	-1.31	2.95	5.19	3.42	4.33	7.60	7.43
5% copper sulphate	-6.55	-0.82	-2.01	-6.87	3.92	-6.10	2.07	9.13	3.89	1.09	7.89	9.23
3% citric acid	-4.14	0.92	3.68	2.05	12.83	6.39	12.63	6.19	4.31	2.59	13.98	6.77
5% citric acid	-11.15	2.88	5.03	2.76	12.13	7.26	12.75	7.30	3.14	2.62	17.23	8.28
3% tannic acid	-1.28	0.97	0.61	3.20	7.56	8.12	6.54	8.26	3.85	2.83	7.70	8.79
5% tannic acid	-1.56	1.40	2.95	4.07	9.11	9.34	9.09	9.76	3.03	2.94	9.70	10.28

The luminosity dyed with extract A is diminished, the most luminous being the ones dyed with the assistance of tannic acid (concentrations of 3 and 5%). The luminosity of woolen sample is higher in case of extract B, the most luminous being the ones dyed with the assistance of 3% copper sulphate and 5% acid citric, and the lowest being the specimen dyed using 5% copper sulphate, assuming that the mordant concentration influences in a major manner the brightness of the samples.

Table 2. The values of luminosity and colour differences for the polyamide (PA1 and PA2) samples dyed with extract A (water:ethanol 1:1) and B (water:ethanol 3:1), made of green walnut shells

PA1 and PA2 samples, mordant	CIE DL*		CIE Da*		CIE Db*		CIE DC*		CIE DH*		CIE DE*	
3% copper sulphate	-6.47	2.60	12.28	-9.76	2.89	-8.61	6.48	-12.93	-10.82	1.46	18.83	13.27
5% copper sulphate	-6.00	0.92	14.85	-9.91	2.51	-8.59	7.60	-13.01	-13.00	1.61	21.27	13.14
3% citric acid	-6.30	-0.09	8.45	0.89	8.30	0.11	9.79	0.69	-6.66	-0.57	13.41	0.90
5% citric acid	-6.36	1.93	8.38	3.03	9.37	3.02	10.78	4.28	-6.46	-0.15	14.08	4.69
3% tannic acid	-4.23	3.78	6.89	2.52	7.9	3.06	8.04	3.96	-4.38	0.23	9.33	5.48
5% tannic acid	-5.46	7.44	7.65	3.50	8.05	6.98	9.76	7.53	-5.11	2.06	16.43	10.78

Table 2 also reveals the colour coordinates, meaning luminosity and colour differences for the polyamide (PA1 and PA2) samples dyed with extracts A and B. The values of luminosity and colour differences are changing in terms of the mordant type used in dyeing process (copper sulphate, citric and tannic acids).

A high colour difference is noticeable at samples dyed with extract A and B, with the assistance of both mordants: copper sulphate and tannic acid. The lowest differences are shown at samples assigned to experiment paths using acid citric as biomordant (concentrations of 3% and 5%), consequently the colour changes compared to the reference are detectable. Generally speaking, the luminosity of polyamide (PA1 and PA2) samples is decreased, the most luminous being the ones dyed with the assistance of tannic acid (concentrations of 3% and 5%), for both extracts A and B.

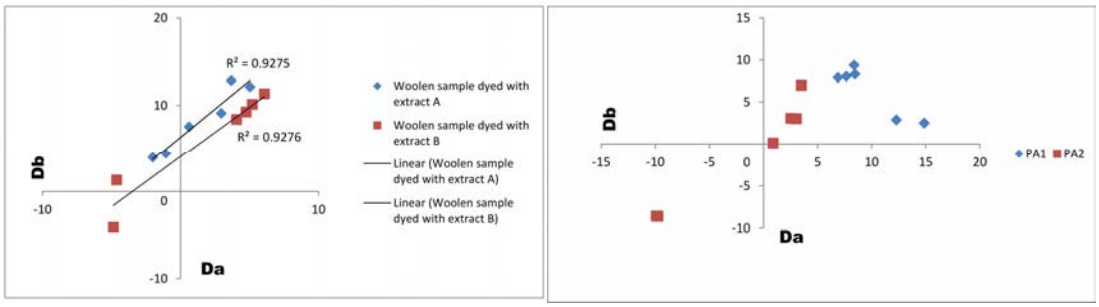

Fig.5. The graphic representations of colour attributes onto the red-green and yellow-blue axis, for the woolen and polyamide (PA1, PA2) samples dyed with extract A (water:ethanol 1:1) and B (water:ethanol 3:1)

The woolen samples dyed with extract A have red-yellow tint and slightly greenish as well, and those dyed with extract B, are partially red-yellowish tinted and partially greenish-bluish. The polyamide (PA1 and PA2) samples dyed with extract A are red-yellow tinted, while the ones colored with extract B, are different tinted: some of them are red-yellowish tinted, and some of them are both greenish-yellowish and even blue-greenish. The presence of colour intensity, meaning the dye deposition is noticeable where the colour modifications are detectable. In case of colour difference is not consistent; the biomordant occurrence would be successfully used in the dyeing technology.

The values of colour difference for polyamide (PA1 and PA2) samples dyed with both extracts (A and B), are well depicted, stressing the linear correlation assigned to the same mordant concentration 3% or 5%, the colour modification progressively diminishing from the samples dyed by assistance of copper sulphate (3%, 5%), towards the 5% concentration of tannic acid. The small values of colour difference assigned to woollen samples can be associated to the small concentrations of mordant, for both extract A and B. Thus, undoubtedly a 3% concentration of citric acid attributed to extract B, conducted to an acceptable modification of colour.

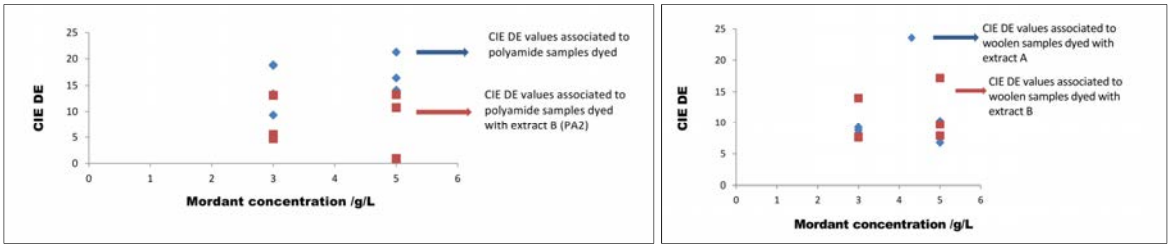

Fig.6. The graphic representations of colour difference – mordant concentration for: woolen samples dyed with extracts A and B; polyamide (PA1 and PA2) samples dyed with extracts A (water:ethanol 1:1) and B (water:ethanol 3:1)

4. Conclusions

This study revealed the efficiency of juglone extraction protocol in an aqueous medium, from green walnut shells, through a complex system of investigation: HPLC, IR-Raman, and Surface Enhanced Raman Spectroscopy (SERS), preserving the high purity active natural dye molecule from nut shell (juglone), even under the conditions of an extraction performed in a water-ethanol solvent medium. Secondly, the study envisages the application of these above mentioned dyes onto natural and synthetic substrates, making a correlation between their colour attributes and the fibrous composition of the substrates they are applied on. HPLC chromatography reveals an efficient ecologic extraction procedure. Besides, the refluxing duration in the experimental protocol of juglone extraction in water-ethanol solvent medium, from nut shells is almost the same with that belonging to synthetic juglone. It can be assumed there is a correlation between polymeric supports – dyeing stability/color intensity. The woolen samples have higher colour intensities, fact sustained by the specific surface of the fibres compared with polyamide fibre, consequently, the dye molecule is protected/preserved by the wool fibre morphology. It is realistic to presume that the histo-morphological profile of wool fibre provides the dye stability inside it. This aspect is more pregnant if biomordants are used. Regarding the FT-IR and Raman spectra, they are completely different, in terms of bands intensity. However, similitude occurs with respect to bands frequencies, in most cases, when we compare two spectra. The strongest bands, from the region specific to double bond (1700-1400 cm^{-1}) suggest the essence of double bond shift, in case of the natural dye, compared to the standard juglone. The strong bands are noticed, not only at double bond, but also within the IR 300 cm^{-1}- 1000 cm^{-1} domain of SERS. This research is a key point in the identification of potential technological alternatives applied in ecologic finishing of synthetic and natural textile supports, quantified and controlled by colorimetric response/ attributes.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0474.

References

[1] Jadhav D, Rekha BN, Gogate PR, Rathod VK. Extraction of vanillin from vanilla pods: a comparison study of conventional soxhlet and ultrasound assisted extraction. J Food Eng 2009; 93:421–426.

[2] Lianfu Z, Zelong L. Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. Ultrason Sonochem 2008; 15:731-737.
[3] Da Porto C, Decorti D. Ultrasound-assisted extraction coupled with under vacuum distillation of flavour compounds from spearmint (carvone-rich) plants: comparison with conventional hydrodistillation. Ultrason Sonochem 2009;16:795-799.
[4] Khan MK, Vian MA, Tixier ASF, Dangles O, Chemat F. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel. Food Chem 2010; 119:851-858.
[5] Kimbaris AC, Siatis NG, Daferera DJ, Tarantilis PA, Pappas CS, Polissiou MG. Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (Allium sativum). Ultrason Sonochem 2006;13:54–60.
[6] Adje F, Lozano YF, Lozano P, Adima AF, Chemat, Gaydou EM. Optimization of anthocyanin, flavonol and phenolic acid extractions from Delonix regia tree flowers using ultrasound-assisted water extraction. Ind. Crops Prod 2010;32:439-444.
[7] Pena RM, Barciel J, Herrero C, Martın SG. Comparison of ultrasound-assisted extraction and direct immersion solid-phase microextraction methods for the analysis of monoterpenoids in wine. Talanta 2005;67:129-135.
[8] Vilkhu K, Mawson R, Simons L, Bates D. Applications and opportunities for ultrasound assisted extraction in the food industry—a review. Innovative Food Sci Emerg Technol 2008;9:161-169.
[9] Scherrer NC, Stefan Z, Francoise D, Annette F, Renate J. Spectrochim Acta A 2009;73:505.
[10] Barber EJW. Prehistoric Textiles: The Development of Cloth in the Neolithic and Bronze Ages with Special Reference to the Aegean. Princeton University Press;1991.
[11] Bowie JH, Cameron DW, Williams DH. J Am Chem Soc 1965;87: 5094.
[12] Lee AS, Mahon P J, Creagh C. Vib Spectrosc 2006;41:170.
[13] Whitney AV, Van Duyne P, Casadio F. J Raman Spectrosc 2006;37:993.
[14] Pawar AB, Jadhav KD, Gonewar N R, Sarawadekar R G. J Pharm Res 2011; 4:2051.
[15] Pavia DL, Lampman, GM, George SK. Introduction to Spectroscopy. 3rd ed. New York: Brooks Cole; 2000.