Spectrophotometric determination of molybdenum with *Syzygium jambolanum* DC leaf extracts

J. R. Mudakavi* and K. Puttanna

Department of Chemical Engineering, Indian Institute of Science, Bengaluru 560 012, India

A novel spectrophotometric method for the determination of molybdenum (Mo) in industrial materials has been developed using the leaf extract of *Syzygium jambolanum* DC based on the reaction of Mo (VI) at pH 7.0 to produce an orange–yellow complex with an absorption maximum at 426 nm. The molar absorptivity of the complex is 4.27×10^4 l mol^{-1} cm^{-1} and the absorption is linear in the range 0.05–1.0 ppm. Sandell sensitivity coefficient was found to be 2.25×10^{-3} μg/cm^2. The method is ten times more sensitive than the aqueous thiocyanate system. It has been applied successfully in micronutrient fertilizer, artificial freshwater and sea-water analyses.

Keywords: Green chemistry, molybdenum, plant extract, spectrophotometry, *Syzygium jambolanum*.

CONCERN for environmental issues in analytical methods has increased to such an extent in recent years that, analysis of environmental samples and the consequent use of toxic reagents and solvents have themselves become a cause of worry. Thus we are forced to look for environmentally friendly alternatives[1]. The need for controlling laboratory wastes and collect residues to avoid contamination of water, air and soil is well recognized.

Green analytical chemistry (GAC) started as a search for practical alternatives to the off-line treatment of wastes and residues in order to replace toxic reagents and methodologies with clean ones[2–7]. Green analytical chemistry is essentially a part of the sustainable development concept. Miniaturization of analytical devices, reduction in chemical reagent volumes and shortening the analysis time between performing analyses and obtaining reliable analytical results are important aspects of green analytical chemistry.

Although atomic absorption spectrophotometry and inductively coupled atomic plasma emission spectrometry are preferred for determination of metal ions, a large number of determinations are still being made using reagent kits based on spectrophotometric methods. Spectrophotometry is ideally suited for the practice of green chemistry as naturally occurring plant extracts can be successfully employed for the determination of anions, cations and neutral molecules. This would make the analytical methods highly cost-effective and versatile. Moreover, many reagents used in spectrophotometry are detrimental to the health of the analyst and the environment. Therefore, it is desirable to replace such reagents with safer and simpler plant extracts wherever possible. This aspect of green chemistry has received little attention. Molybdenum (Mo) is an industrially important metal and a prime candidate for exploring green analytical methodology. The most commonly used reagents for molybdenum are thiocyante and dithiol[8,9]. Other reagents used include benzoinoxime, quinolin-8-ol and ortho-phenylene diamine[10].

Use of plant extracts as spectrophotometric reagents has been reviewed by Grudpan *et al.*[11]. Elm leaf extracts have been recently used as reagents for spectrophotometric determination of molybdenum[12]. *Syzygium jambolanum* (syn. *Syzygium cumini*) is a plant native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, Philippines and Indonesia. It is also known as jambhul, jambu, jambula, jamboola, Java plum, jamun, jam, kalojaam, jamblang, jambolan, black plum, damson plum, duhat plum, jambolan plum, Portuguese plum, or Malabar plum. It is an evergreen tropical tree of the family Myrtaceae[13]. The leaves and fruits of *S. jambolanum* have been used in Brazil to treat infectious diseases, diabetes and stomach ache[14]. The seeds of this plant exhibit anti-fungal and anti-bacterial activity[15,16]. The leaves of this tree are known to contain flavonoids such as myrcetin and quercetin[17]. The aqueous extract of the dried, powdered leaves gives an orange–yellow colour with molybdenum (VI) in slightly acidic solutions. The present communication describes the development of a spectrophotometric method for molybdenum based on this reaction.

A double-beam diode array spectrophotometer (350–800 nm; Biochrome Libra S6) was used in this study. All the chemicals used were of analytical grade. The following reagents were used:

(1) Stock molybdenum solution (100 ppm): For this, 0.1288 g of ammonium molybdate crystals was dissolved in deionized water by heating on a hot plate, cooling to room temperature and making up to the volume 100 ml.

(2) Standard molybdenum solution (10 ppm): For this, 10 ml of the stock molybdenum solution was diluted to 100 ml with deionized water.

(3) Ammonium acetate buffer (pH 7.0, 0.1 M): For this, 0.7708 g of *ammonium acetate* was dissolved in water and diluted to 100 ml.

(4) Reagent solution (*S. jambolanum* leaf extract): *S. jambolanum* leaves were dried at 110°C and ground to 32 mesh (500 μm) in a porcelain pestle and mortar. Next, 1 g of the leaf powder was shaken with 20 ml deionized water for 30 min and filtered through Whatman No. 42 filter paper. The filtrate was allowed to stabilize for at least one day and used as such.

The absorption spectrum of the colour produced by 10 μg of molybdenum and 2 ml of 5% aqueous *S. jambolanum* leaf extract was measured against the reagent

*For correspondence. (e-mail: jrm@chemeng.iisc.ernet.in)

blank using a spectrophotometer (Figure 1). The absorption maximum was found at a wavelength of 426 nm. The colour was stable for one day.

To study the effect of reagent concentration on the absorbance of molybdenum to 10μg of molybdenum in 10 ml volumetric flasks varying volumes (4, 20, 40, 60, 80, 100, 200, 400, 800, 1000 μl) of the *S. jambolanum* leaf extract and 2 ml ammonium acetate buffer (pH 7.0) were added. The volume was made up to 10 ml with deionized water and absorbance of the solution was measured against the respective reagent blanks at 426 nm.

Figure 2 shows the effect of reagent concentration on the absorbance of molybdenum. It can be seen from the figure that the absorbance versus concentration of the reagent curves tends to flatten above 0.6 ml of the extract and addition of 3 ml of the 5% extract will maintain sufficient excess of the reagent.

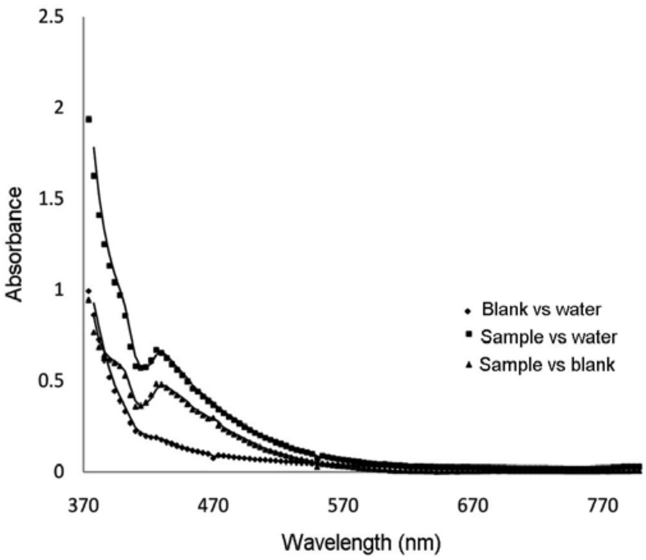

Figure 1. Absorbance spectrum of 10 μg Mo (VI) with 3 ml 5% *Syzygium jambolanum* leaf extract diluted to 10 ml final volume.

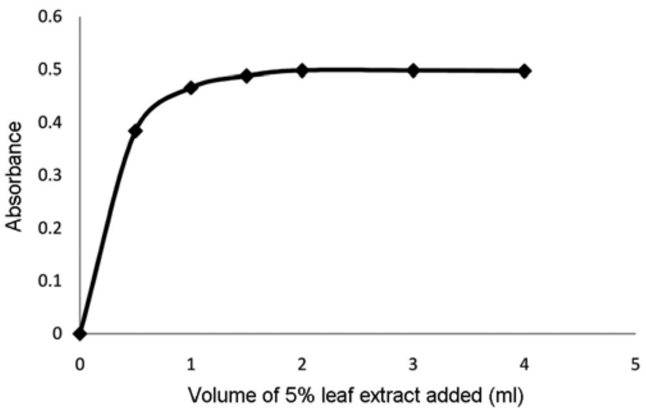

Figure 2. Effect of reagent concentration on the absorbance produced by varying volumes of 5% leaf extract 10 μg Mo (VI), 10 ml final volume.

To evaluate the effect of pH, a series of buffer solutions were prepared using sodium acetate and acetic acid. To 20 μg of molybdenum, 3 ml of the reagent solution was added. The pH of the solution was adjusted using a pH meter. The solutions were diluted to mark in 10 ml volumetric flasks and mixed. Absorbance was measured at 426 nm after 10 min.

Figure 3 shows the effect of pH on the absorbance of molybdenum complex with the reagent. It is clear from the figure that the absorbance is constant in the pH range 7–8. Therefore, it was decided to use pH 7 buffer.

The effect of reaction time was studied as follows: 2 ml 0.1 M ammonium acetate buffer solution and 3 ml reagent solution were mixed and diluted to 10 ml in a volumetric flask. This was used as a blank. Then 250 μl of 10 ppm molybdenum was treated with 1000 μl of water, 500 μl of ammonium acetate buffer and 750 μl of the re-agent in a dry, 1 cm path length, spectrophotometric cu-vette, mixed and absorbance values were measured at 30 sec intervals up to 30 min and at 1 h intervals up to 8.5 h and finally at 24 h.

Figure 4 shows the effect of reaction time on absorbance. It can be seen that the reaction is almost instantaneous as there is no change in absorbance 30 sec after

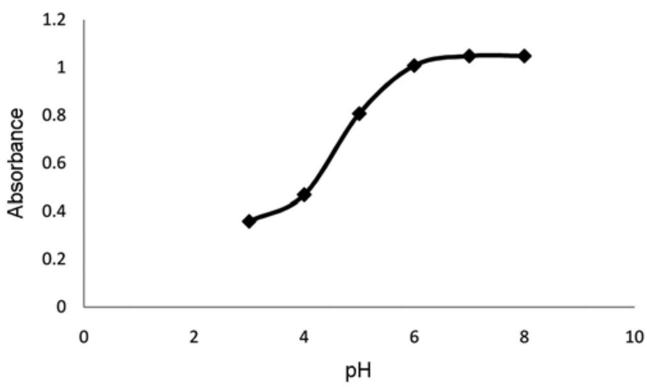

Figure 3. Effect of pH on the absorbance of 20 μg Mo (VI), 3 ml 5% leaf extract, 10 ml final volume.

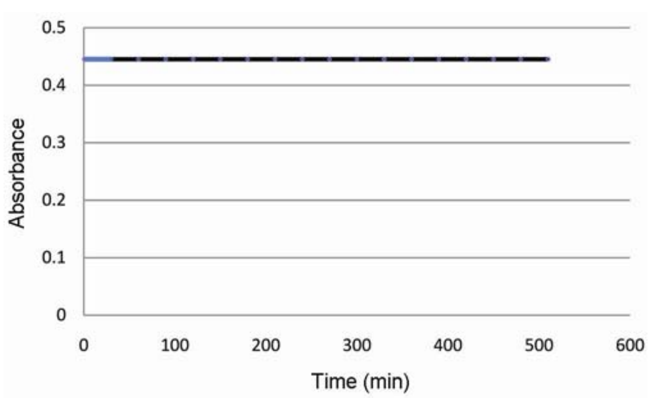

Figure 4. Effect of time after mixing on the absorbance of colour produced by 10 μg Mo (VI) with 3 ml 5% *S. jambolanum* leaf extract diluted to 10 ml final volume.

mixing the reagent. The absorbance remains constant up to 24 h. Turbidity due to fungal growth occurs sometime after 24 h. However, while analysing environmental or industrial samples, in the presence of extraneous compounds, it is advisable to measure the absorbance 10 min after mixing. Figure 4 shows data up to 8.5 h.

For preparing the calibration curve, to 0, 200, 400, 600, 800 and 1000 μl of the standard 10 ppm molybdenum solution taken in 10 ml volumetric flasks, 3 ml of the reagent solution followed by 2 ml of a buffer solution were added. The solutions were diluted to mark and mixed. Absorbance was measured at 426 nm after 10 min. The calibration curve was prepared by plotting the absorbance values against concentration.

Figure 5 presents the calibration curve. Beer–Lambert's law is obeyed between 0.5 and 8.0 ppm. The slope of the curve compares well with that of thiocyanate method[2]. The limit of detection (3.3 σ/S, where σ is the standard deviation of the slope and S is the mean slope of the calibration curve $n = 3$) was found to be 0.2 ppm Mo^{6+} and the limit of quantification (10 σ/S) was found to be 0.5 ppm Mo^{6+}.

Interference studies were conducted by adding 1000 μg of the interfering ion solution to 10 μg molybdenum in 10 ml volumetric flasks. Development of colour and absorbance measurements were carried out as above. An ion causing 10% or more error was considered to be interfering. If interference was found, attempts were made to mask the interfering ion with appropriate masking agents, such as EDTA, fluoride, etc. If the interference persisted, attempts were made to repeat with lower concentrations of interfering ions. The data will be useful when the method has to be applied to the analysis of industrial or environmental samples.

Table 1 presents the interference data. It can be seen from the table that a 100-fold excess of Mn^{2+}, Mg^{2+}, Ni^{2+} BO^{3-}, F^-, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, NH_4^+, SLS, EDTA and

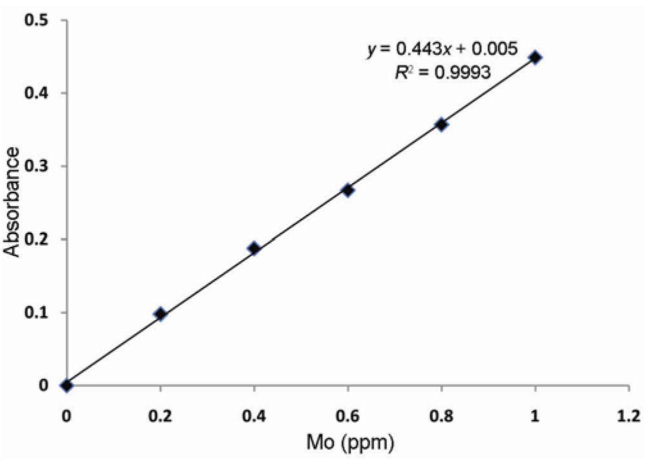

Figure 5. Calibration curve for the determination of Mo (3 ml 5% leaf extract, pH 7.0, 10 ml final volume).

ClO_4^- does not interfere in the determination of molybdenum. A 10-fold excess of Ca^{2+}, Zn^{2+}, PO_4^{3-}, citrate and tartrate and Co^{2+} concentration equal to twice that of molybdenum also do not interfere in the determination of molybdenum. In the presence of 1 ml 0.01 M EDTA, 10-fold excess of Al^{3+}, Cu^{2+}, Co^{2+} and Fe^{3+} can be tolerated. In the presence of 0.01 M fluoride, 50-fold excess of Fe^{3+} can be tolerated after filtering the solution. If carbonate is present it should be destroyed by adding dilute (0.1 N) hydrochloric acid before adding the reagents. V^{5+}, Bi^{3+}, Ag^+, Sb^{3+} and cationic surfactants interfere at all concentrations.

Recovery studies were undertaken to check the applicability of the method to real samples. Molybdenum is a known micronutrient for plants and is included in micronutrient fertilizers. Therefore, micronutrient fertilizer is a good candidate for demonstrating application of the novel method in the presence of compounds of other metals. Molybdenum also occurs in soil and natural waters. Since

Table 1. Concentration of interfering species causing less than 10% error in the determination of 1 ppm molybdenum (Mo) (VI)

Interfering species	Concentration (ppm)	Absorbance
Nil	–	0.442
Ni^{2+}	100	0.453
Mn^{2+}	100	0.440
Mg^{2+}	100	0.431
Sb^{3+}	100	0.478
NH_4^+	100	0.442
EDTA	100	0.433
SLS	100	0.432
F^-	100	0.433
Cl^-	100	0.443
Br^-	100	0.435
I^-	100	0.434
SO_4^{2-}	100	0.440
NO_3^-	100	0.430
BO_3^{3-}	100	0.409
Ag^+	10	0.463
Hg^{2+}	10	0.436
Cu^{2+}	10	0.479
Zn^{2+}	10	0.458
Ca^{2+}	10	0.447
PO_4^{3-}	10	0.424
CO_3^{2-}	10	0.479
Citrate	10	0.425
Tartarate	10	0.400
Co^{2+}	2	0.443

Species that did not interfere in the presence of 1 ml 0.01 M EDTA

Interfering species	Concentration (ppm)	Absorbance
Pb^{2+}	100	0.451
Hg^{2+}	100	0.472
Al^{3+}	50	0.434
Cu^{2+}	50	0.473
Fe^{3+}	10	0.456
Co^{2+}	10	0.453

Table 2. Recovery data

Sample	Mo spiked (ppm)	Total Mo found (ppm)	% Recovery
Zimag micronutrient fertilizer	0.5	1.05	99.12
	0.75	1.28	96.68
	1.0	1.58	102.54
			Mean: 99.44
Sea water	0.5	0.53	96.7
	0.75	0.79	99.3
	1.0	1.06	101.1
			Mean: 99.03
Artificial freshwater (medium hardness)	0.5	0.51	102.0
	0.75	0.78	103.7
	1.0	1.03	102.8
			Mean 102.8

Table 3. Aqueous extracts of leaves which gave a colour reaction with Mo(VI) qualitatively similar to *Syzygium jambolanum*

Plant	Common name	Family
Peltophorum ferrugineum	Copper pod	Caesalpiniaceae
Neolamarckia cadamba	Kadam	Rubiaceae
Terminalia catappa	Tropical almond	Combretaceae
Cymbopogon winterianus	Citronella grass	Poaceae
Vitex negundo	Nirgundi	Lamiaceae
Ricinus communis	Castor oil plant	Euphorbiaceae
Hyptis suaveolens	Horehound, mint weed	Lamiaceae
Tabebuia avellanedae	Pink tabubia	Bignoniaceae
Tabebuia roseoalba	Pale tabubia	Bignoniaceae
Cymbopogon flexuosus	Lemongrass	Poaceae
Eucalyptus pseudoglobulus	Blue gum	Myrtaceae
Callistemon citrinus	Bottle brush	Myrtaceae

approximate compositions of artificial freshwater and sea water are known, they were chosen for testing the applicability of the method. ZIMAG SPRAY, a micronutrient fertilizer manufactured by Saklaspur Agro Chem. Pvt Ltd, Bengaluru (Batch no. 001, manufacture date: June 2013) containing Zn, S, Mg, Mo, Mn, Fe, Cu and B in chelate form was procured from local market. Then 1 g of the material was treated with 5 ml concentrated HNO_3 and evaporated to dryness on a hot plate to destroy organic matter and oxidize ferrous to ferric compounds. The residue was extracted with 0.5 M Na_2CO_3 solution by warming to 80°C, cooling to room temperature and filtering into a 100 ml volumetric flask. The precipitate and filter were washed into the same flask and diluted to mark. Next, 2 ml of this extract was spiked with 0, 2.5, 5.0, 7.5 and 10 μg molybdenum and analysed using the present method.

Sea-water sample collected off Karwar coast, India was similarly spiked with molybdenum and analysed using the present method. Artificial freshwater of medium hardness[18] was prepared and spiked with molybdenum and also analysed.

Table 2 presents the recovery data. Good recoveries of molybdenum were obtained from all the samples. The micronutrient analysis also demonstrates how molybdenum can be separated from most di- and trivalent cations before analysis. While molybdenum remains in solution, di- and trivalent cations precipitate as carbonates.

To evaluate the effect of the source of leaves, *S. jambo-lanum* leaves were collected from cultivated trees with wide leaves and large fruits; wild trees with narrow leaves and small fruits; young, half-opened leaves, and old mature leaves. Apparently there was no effect of age or the source of leaves on the absorbance produced by 10 μg of Mo^{6+}. The leaf powder is stable indefinitely if kept in dry condition at room temperature.

Several other plant extracts were tested qualitatively to record colour reaction with molybdenum, similar to *jambolanum.* Chemical components of leaves are known to vary due to factors like variety, climate, soil, competition, disease, etc. Therefore, the same batch of reagents should be used for preparing the calibration curve and analysis of samples. Table 3 lists other plant extracts that gave similar colour reaction as *S. jambolanum.* Therefore, it may be concluded that compounds responsible for the colour reaction are widely distributed. The *S. jambolanum* extract gave a deep purple colour with ferric iron, lemon yellow colour with titanium and a bluish colour with vanadate ion. A compound like quercetin or tannic acid may be involved in colour development.

The age or the source of leaves did not have any effect on the absorbance produced by 10 μg of Mo^{6+}. The leaf powder was found to be stable for at least one year when kept in dry condition at room temperature. However, compositions of plant parts are known to vary due to various factors like variety, climate, soil, competition, diseases, etc. Therefore, the same batch of reagents should be used for preparing the calibration curve and analysis of samples.

Table 3 lists other plant extracts that gave similar colour reaction as *S. jambolanum*. Therefore, it may be concluded that compounds responsible for the colour reaction are widely distributed. The *S. jambolanum* extract gave a deep purple colour with ferric iron, lemon yellow colour with titanium and a bluish colour with vanadate ion. A compound like quercetin or tannic acid may be involved in colour development.

1. Koel, M. and Kaljurand, M., Application of the principles of green chemistry in analytical chemistry. *Pure Appl. Chem.*, 2006, **78**, 1993–2002.
2. Wang, J., Real-time electrochemical monitoring: toward green analytical chemistry. *Acc. Chem. Res.*, 2002, **35**, 811–816.
3. Tobiszewski, M., Mechlińska, A. and Namieśnik, J., Green analytical chemistry – theory and practice. *Chem. Soc. Rev.*, 2010, **39**, 2869–2878.
4. de la Guardia, M. and Garrigues, S. (eds), *Challenges in Green Analytical Chemistry*, The Royal Society of Chemistry, UK, 2011, p. 330.
5. de la Guardia, M. and Garrigues, S. (eds), The concept of green analytical chemistry. In *Handbook of Green Analytical Chemistry*, John Wiley, Chichester, UK, 2012; doi:10.1002/9781119940722.ch1
6. Armenta, S., Garrigues, S. and de la Guardia, M., Trends in analytical chemistry. *Green Anal. Chem.*, 2008, **27**, 497–511.
7. de la Guardia, M. and Garrigues, S., Wilson and Wilson's comprehensive analytical chemistry. In *Green Analytical Chemistry* (ed. Barcelo, D.), Elsevier, Oxford, UK, 2010, vol. 57.
8. Crouthamel, C. E. and Johnson, C. E., Spectrophotometric determination of uranium by thiocyanate method in acetone medium. *Am. Chem.*, 1952, **24**, 1780.
9. Piper, C. S. and Beckwith, R. S., *J. Soc. Chem. Ind. (London)*, 1948, **67**, 374.
10. Sandell, E. B., *Colorimetric Determination of Trace Metals*, Interscience, New York, 1950, 2nd edn, p. 334.
11. Grudpan, K., Hartwell, S. K., Lapanantnoppakhun, S. and McKelvie, I., The case for the use of unrefined natural reagents in analytical chemistry – a green chemical perspective. *Anal. Methods*, 2010, **2**, 1651–1661.
12. Monji, A. B., Zolfonun, E. and Ahmadi, S. J., Application of water extract of slippery elm tree leaves as a natural reagent for selective spectrophometric determination of trace amounts of molybdenum (VI) in environmental water samples. *J. Toxicol. Environ. Chem.*, 2009, **91**, 1229–1235.
13. Ayyanar, M. and Subash-Babu, P., *Syzygium cumini* (L.) skeels: a review of its photochemical constituents and traditional uses. *Asian Pac. J. Trop. Biomed.*, 2012, **2**, 240–246.
14. Maciel, M. C. G. *et al.*, *Syzygium Jambolanum* treatment improves survival in lethal sepsis induced in mice. *BMC Complem. Altern. Med.*, 2008, **8**, 57.
15. Kirtikar, K. R. and Basu, B. D., In *Indian Medicinal Plants, Vol. II* (eds Singh, B. and Singh, M. P.), Dehra Dun, 1991, 2nd edn, p. 1053.
16. Chandrasekaran, M. and Venkatesalu, V., Antibacterial and antifungal activity of *Syzygium jambolanum* seed. *J. Ethnopharmacol.*, 2004, **91**, 105–108.
17. Slowing, K., Sollhuber, M., Carretero, E. and Villar, A., Flavonoid glycosides from *Eugenia jambos*. *Phytochemistry*, 1994, **37**, 255–258.
18. Methods for measuring the acute toxicity of effluents and receiving waters to fresh water and marine organisms. US EPA/OW/Office of Science and Technology, Acute toxicity manual, Section 7–10, 2007, p. 33.

ACKNOWLEDGEMENTS. We thank Dr R. J. Deshpande, Department of Metallurgy, Indian Institute of Science, Bengaluru for AAS analysis and Miss. Farheen Iqbal, Project Trainee, Over Reach Programm, IISc for assistance.

Received 29 December 2014; accepted 2 June 2015

Fruit extract dyes as photosensitizers in solar cells

Mozhgan Hosseinnezhad[1,]*, Siamak Moradian[2,3] and Kamaladin Gharanjig[1,3]

[1]Department of Organic Colorants, Institute of Color Science and Technology, P.O. Box 16656118481, Tehran, Iran
[2]Faculty of Polymer and Color Engineering, Amirkabir University of Technology, P.O. Box 15875-4413, Tehran, Iran
[3]Center of Excellence for Color Science and Technology, Institute of Color Science and Technology, P.O. Box 16656118481, Tehran, Iran

Two natural dyes containing anthocyanin were extracted from sour and sweet pomegranate from Iran. Spectrophotometric evaluation of the natural dyes in solution and on a TiO_2 substrate was carried out in order to assess changes in the status of the natural dyes. The results show that the natural dyes indicate buthochromic shift on the TiO_2 substrates. Dye-sensitized solar cells (DSSCs) were fabricated in order to determine the photovoltaic behaviour of each dye and the mixture of extracts. Such evaluations demonstrate conversion efficiencies of 0.73%, 1.57% and 0.91% for sour pomegranate, sweet pomegranate and mixed extract respectively. Natural dyes are suitable alternative photosensitizers for DSSCs.

Keywords: Anthocyanin, conversion efficiencies, dye-sensitized solar cells, natural dye.

DYE-SENSITIZED solar cells (DSSCs or Grätzel cells) have become an attractive and low-cost technology for the conversion of solar light into electrical energy[1]. The

*For correspondence. (e-mail: hosseinnezhad-mo@icrc.ac.ir)