Mass transfer enhancement through optimized extraction of a natural dye from *Bougainvillea glabra* Juss. bracts

P D Patil[1]*, C R Rao[2], A I Wasif[3], S V Anekar[4] and J R Nagla[3]

[1]Department of Technology, Shivaji University, Kolhapur, Maharashtra, India
[2]Vigyan University, Vadlamudi, Guntur, Andhra Pradesh, India
[3]Textile and Engineering Institute, DKTES, Ichalkaranji, Maharashtra
[4]TKIET Warananagar, Maharashtra

Synthetic dyes are today's established dyes for applications such as textile coloration. However, there are some serious drawbacks of these new dyes. Therefore, there is increasing interest in the natural dyes. The present study was emphasized to use the optimized extraction conditions so as to get maximum amount of the dye from bracts of *Bougainvillea glabra* Juss. Taguchi design was implemented to know optimum extraction conditions: 150 micron particle size, 1:20 solid-solvent ratio, one hour extraction and enzyme addition as assistance for extraction. The dye was extracted from both optimized and control experiments. Fourier Transform Infrared Spectroscopy and UV-VIS spectroscopy techniques were used to identify chemical nature of the extracted dye. The absorbance, mass transfer rates and dye yields were compared. The optimized extraction showed mass transfer rate of about 3.3 times enhanced than that in the control extraction. The yield was 9.88 % more in case of optimized extraction.

Keywords: *Bougainvillea glabra*, Natural Dye, Taguchi optimization, Mass transfer enhancement.

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Introduction

Natural dyes are the colorants extracted from vegetative matter, animal residues or from minerals. The dyestuffs for these colorants include roots, leaves, barks, trunks, fruits and flowers of plants, animal sources like cochineal and shellfish or sometimes from soils or clay. These dyes were used in the past for various purposes such as coloration of clothing, food, leather tanning and painting[1-3]. However, in the mid 1800's, with the invention of synthetic aniline, alizarin and indigo, natural dyes lost their economic significance. Today, synthetic dyes have dominance because of their varied colors, vast and easy production and very good fastness properties. Advantages of the synthetic dyes compelled us to shift our focus from natural to synthetic[4-5]. But it is noteworthy that using the synthetic dyes has posed some serious drawbacks within a period of mere 150 years. The research has suspected many of the synthetic dyes to release harmful chemicals which are allergic, carcinogenic and detrimental to human health. About 74 azo dyes have been banned worldwide in view of their carcinogenic nature[6].

Ever increasing environment related consciousness has been making natural dyes more popular like other natural products. Most of the natural dyes are biodegradable, non-toxic and eco-friendly in nature. The other benefit of introducing natural dyes is the significant reduction in the amount of toxic effluent resulting from the synthetic dye processes[7-8]. At present, the international consumption of natural dyes is hardly 1% of the synthetic dyes. Prime limitations and technical drawbacks of the natural dyes include low color yield and inefficient dye extraction methods. It has been the major problem to reintroduce these old dyes[9]. Therefore, the greatest challenge before us is to increase the yield of these dyes. Present study has been carried out to address this particular issue. The plant part selected for study is bracts of the ornamental shrub, *Bougainvillea glabra* Juss.[10-11].

Extraction of dyes from their raw dyestuffs is basically a solid-liquid extraction operation. When a solid material is brought in contact with a solvent, the soluble components from solid material move to the solvent. The extraction of plant material results in the mass transfer of soluble colourant to the solvent. The rate of mass transfer decreases as the concentration of dye in the solvent increases, until equilibrium is reached. Thereafter, there is no longer mass transfer of dye from plant material to the solvent[12].

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*Correspondent author
E-mail: pdpchem@gmail.com

One of the steps to improve the mass transfer during extraction of dyes from their dyestuff is to investigate the optimum conditions suitable for efficient extraction. Optimized extraction conditions as suggested by the Taguchi method have been employed to get maximum dye from the source. Further, the mass transfer rates during the extraction have been calculated[13].

Materials and Methods

Bougainvillea is a genus of flowering plants native to South America from Brazil west to Peru and south to southern Argentina. It also grows in tropical and sub tropical forests in India. It is also found in both rural and urban parts of India. It is a common woody shrub that grows to the height of 3-5 m and is sometimes referred to as paper flower because the bracts are thin and papery. These bracts can be classic red, purple, orange, white, pink and cream which bloom all over the year. *Bougainvillea glabra* Juss. (Family- Nyctaginaceae) pink coloured bracts were collected from the road side shrubs, separated from their branches and dried in a tray dryer. The dry bracts mass was ground in a domestic mixer-grinder to get mixed particle sizes. The powder was sieved to get three different sizes of the dyestuff required for experimental work.

Chemicals and equipments used

Cellulase and pectinase enzymes (HIMEDIA make) were used for enzyme assisted extraction. To maintain alkaline conditions in extraction medium, the buffer tablets (Merck) with pH 9.5 were used. To weigh the raw dyestuff and the enzymes, Shimadzu AUX220 Weighing balance was used. Microwave treatment to the raw materials was one of the extraction assistance methods. For this purpose, Samsung DE68-02233G domestic microwave oven was used. Orbital shaker-incubator (Nanolab India Model NLSIC-23#25/50) was used to carry out the extraction runs at a time. After the extraction, to ensure complete separation of extracted color from the solid waste, syringe and holders assembled with 0.2 micron porosity nylon membrane filters (HIMEDIA make) were used. The absorbance values of the colored extracts were determined by using UV-VIS Spectrophotometer (Shimadzu, Germany, Model UV1800). FTIR analysis was performed using Cary 630, Agilent Technology spectrophotometer.

Taguchi extraction optimization with method of data analysis

Dr. Genichi Taguchi's standardized experimental research design was adopted for dye extraction optimization. Since its introduction in the U.S.A. in early 1980's, the approach has been popular as 'product and process improvement tool' in the hands of engineering and scientific professionals[14]. The objective in the extraction of natural dye was to increase its yield through efficient mass transfer operation. Taguchi method was mainly used to achieve efficient extraction of dye and to significantly reduce the number of experimental trials. The factors under investigation included: raw dyestuff particle size (150, 300 and 425 microns), solid-solvent ratio (1:20, 1:30 and 1:40), extraction time (60,120 and 180 minutes) and method of assistance for extraction (microwave, enzyme and microwave plus enzyme). There were three levels of each of the factors. These levels were coded as 0, 1 and 2, respectively. Absorbance of the color extract was considered as the response variable.

L9 (3^4) orthogonal arrays with 4 factors and 3 levels were used to conduct nine experimental runs[15-16]. Each of the nine runs was conducted in duplicate. Other parameters ascertained from the literature survey included extraction temperature 60°C, speed of the orbital shaker 150 rpm, microwave power 300 Watt with irradiation time 40 second and enzymes proportion 2% of cellulase and 1% pectinase both on weight of raw dyestuff basis[17-18]. Water was used as a solvent in the extraction experiments.

Using Minitab software, analysis was conducted for the response variable. The General Linear Model (GLM) approach was used to perform analysis of variance and regression of the response variable. Calculations were performed using a regression approach. Conclusions were drawn using calculated results and the graphs[19].

Dye extraction with optimized parameters and control parameters

The solid-liquid extraction was carried out in an orbital shaker incubator using optimum parameters as suggested by the Taguchi Design. The particular experiment was named as optimized extraction experiment. Also the control experiment was conducted simultaneously with predetermined control parameters. The control parameters included coarse particle size (425 micron), 1:20 solid to solvent proportion, three hours of extraction and there was no any assistance for extraction.

It was aimed to compare control extraction performance with that of the optimized extraction. UV-VIS spectra obtained from the two experiments have been shown in Fig. 1.

Analysis of data

Statistical analysis

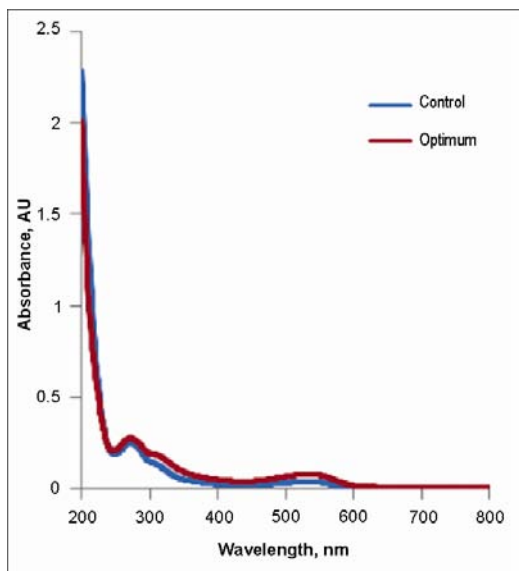The optimization analysis has been shown in Table 1. The F-value for each parameter indicated

Fig. 1- UV-VIS Spectra (λ_{max} 272nm)

which parameter has significant effect on extraction and is simply a ratio of the squared deviation to the mean of squared error. Usually, larger F-value shows greater effect on the extraction value due to the change of the process parameter. Optimal combination of process parameters was predicted using ANOVA.

Characterization of the extracted dye

To detect the UV-VIS spectrum profile of the extract from the source, the extract was scanned in the wavelength ranging from 200-800 nm by using UV-VIS spectrophotometer and the characteristic peaks were detected.

The FTIR spectrum was used to identify the functional groups of the colourant present in dye sample based on the peaks values in the region of IR radiation. The sample was passed into the system and FTIR Spectrum was recorded in the wavelength region of 4000 to 650/cm.

The peaks values of FTIR so recorded were used for functional group analysis. The results of FTIR analysis supported the presence of colorant in the dye sample (Fig. 2).

Table 1- Analysis of variance for absorbance, using adjusted SS for tests

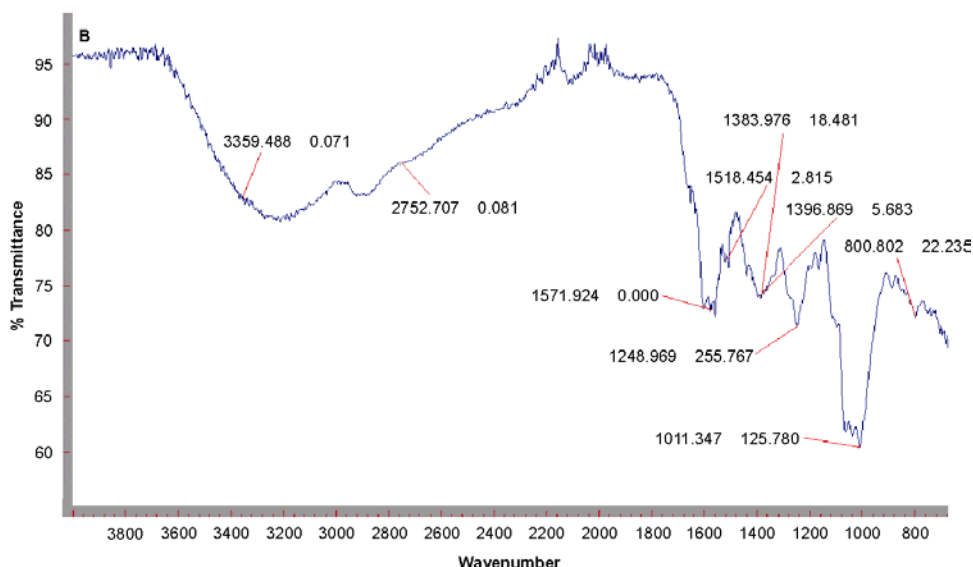| Source	DF	Seq SS	Adj SS	Adj MS	F	P
Replica	1	0.0000	0.0000	0.0000	0.0000	1.000
Particle Size	2	0.0831	0.0831	0.04155	1.70E+05	0.000
Solid-Liquid ratio	2	0.03498	0.03498	0.01749	7.00E+04	0.000
Time of extraction	2	0.0129	0.0129	0.00645	2.60E+04	0.000
Method of assistance	2	0.00914	0.00914	0.00457	1.80E+04	0.000
Error	8	2E-06	2E-06	0		
Total	17	0.14011				

Fig. 2- FTIR Spectrum of dye from *Bougainvillea glabra* bracts

Analysis of data for yield and mass transfer rate calculation

Determination of dye yield:—To find out the concentration of the extracted dye, the dye powder obtained from the spray drier was used to prepare solutions of different concentrations. These solutions were tested for their respective absorbance values using UV- VIS spectrophotometer.

The dye concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 were plotted against the respective absorbance values 0.02, 0.03, 0.05, 0.08 and 0.09. Fig. 3 showed the BEST-FIT line. The slope of this line is the product of path length and molar extinction coefficient. Using slope and absorbance, the concentration and the yields for the control extraction and optimum extraction were determined and compared.

Beer-Lambert law was used to determine concentration of an absorbing species in solution.

$$A = \log_{10} (I_o/I) = [\varepsilon \times L] \times [c] = \text{slope} \times [c] \quad \dots (1)$$

Thus, Concentration = Absorbance/Slope $\quad \dots (2)$

The equation of the trend line is

$$Y = 0.19 X - 0.003 \quad \dots (3)$$

The concentration and the respective yield calculations are as follows:

For control experiment
c = Absorbance/slope = 0.255/0.19 = 1.342 moles/L $\dots (4)$

0.5 mL of the original extract was diluted to 100ml which is 200 times the original. Therefore the original concentration was 1.342 * 200 = 268.42 moles/L.
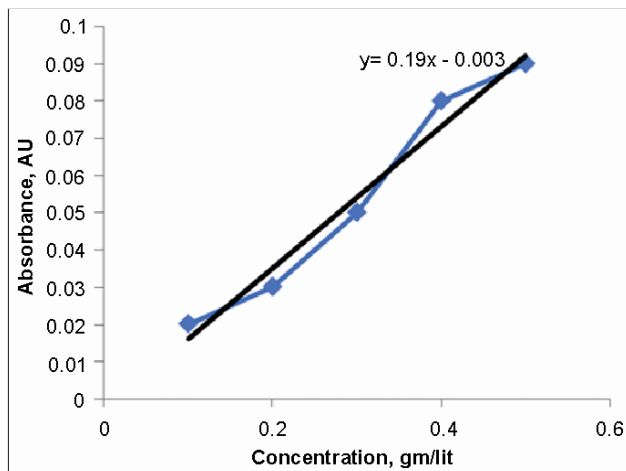

Fig. 3- Absorbance v/s concentration

The colorant with the molecular weight 551 was present in the extract. Therefore, presence of the colorant in the extract was 268.42 /551 =0.4871 g/lit which was 0.04871 g/100 mL. However, 3 g of the raw dyestuff was used for extraction.

% Yield in the control experiment
= [color extracted/quantity of raw dyestuff]*100
= [0.04871/3]*100 =1.6238 $\quad \dots (5)$

For experiment with optimized condition
c = Absorbance/slope = 0.280/0.19 = 1.473 moles/L
$\quad \dots (6)$

0.5 mL of the original extract was diluted to 100 mL which is 200 times the original. Therefore the original concentration was 1.473 * 200 =294.73 moles/L.

The colorant with the molecular weight 551 was present in the extract. Therefore, presence of the colorant in the extract was 294.73/551 =0.5349 g/L which was 0.05349 g/100 mL. However, 3 g of the raw dyestuff was used for extraction.

% Yield in the optimized experiment
= [color extracted/quantity of raw dyestuff]*100
= [0.05349/3]*100 =1.783 $\quad \dots (7)$

Mass transfer rate enhancement: —Further, using the absorbance values of the two extraction experiments, the rates of mass transfer were calculated and compared. The mass transfer rates expressed in terms of Absorbance Units (AU) per hour were calculated as below.

The formula for the rate of mass transfer is as follows:

Mass transfer Rate = absorbance/time = A/t, AU/h

For control experiment:
Rate of extraction mass transfer=
absorbance/time=0.255/3=0.085 AU/h $\quad \dots (8)$

For experiment with optimum set of parameter:
Rate of extraction mass transfer= absorbance/time
=0.280/1=0.280 AU/h $\quad \dots (9)$

When optimized extraction was compared against control, the enhancement in mass transfer rate was as follows:
Mass Transfer rate enhancement = Rate (Optimum)/ Rate (Control) $\quad \dots (10)$
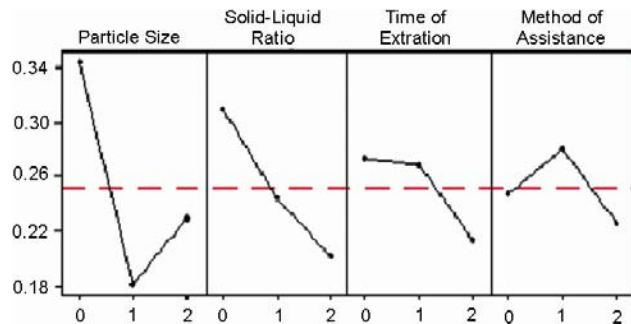
= 0.280 /0.085
=3.3 times

Fig. 4- Main Effects Plots

Results and Discussion

Optimization results

The optimal combination of the process parameters was predicted using ANOVA. The statistical analysis showed the mean values of absorbance as 0.3437, 0.1815 and 0.2302 for levels of particles 0, 1 and 2, respectively. For solid-liquid ratios with the levels 0, 1 and 2, the mean absorbance values were 0.3092, 0.2442 and 0.202, respectively. The three levels of time of extraction showed the mean absorbance values as 0.2728, 0.2685 and 0.214, respectively. Finally for the three respective levels of the method of assistance, the mean absorbance values shown were 0.2482, 0.281 and 0.2262.

Fig. 4 denoted the optimum set of conditions. The finest particle size was found as the optimum size. The optimum solid-solvent ratio found was 1:20. For one hour of extraction, the absorbance value was the highest depicting that the optimum extraction time is one hour. Extraction enhancement of the colorant was seen through the highest absorbance in the enzyme assistance method. The combined effect of these optimal parameters when used in the extraction of the dye, the absorbance obtained was 0.280 as against the absorbance 0.255 in the control experiment.

Colourant, chemical nature

The FTIR spectral analysis is important to identify the characteristic functional groups of the extracted dye. The analysis showed distinct peaks at 3359, 2757, 1571, 1011 and 800/cm, respectively. The broad and strong band at 3359/cm can be attributed to bonded -OH groups. The peak at 2757/cm is indicative of C-H symmetry in stretching mode. The wave number 1571/cm represents the carbonyl group in stretching mode associated with amide group, the peak at 1011/cm indicates ether group and finally 800/cm representing p-disubstituted aromatic group. Hence FTIR spectral analysis shows the presence of different
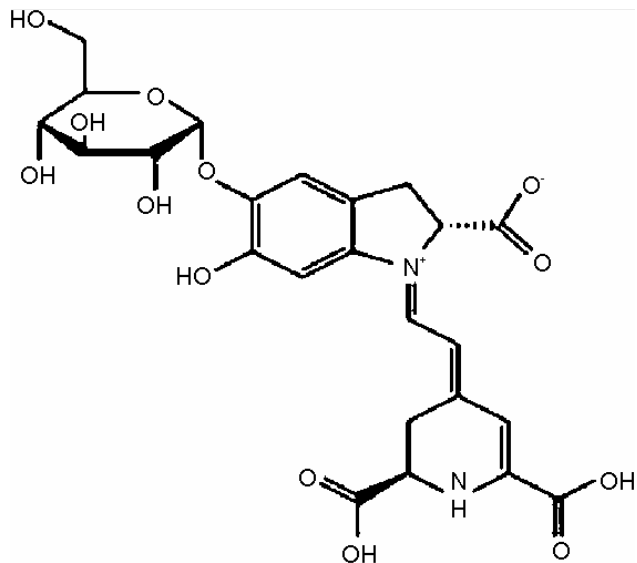

Fig. 5- Structure of betanin

chemical functional groups like OH or NH Stretch, carbonyl (O=C), R-NO$_2$ and C-O in the extracted dye.

The results of FT-IR, UV-VIS spectroscopy as well support the presence of colourant of the flavones nature. The molecular formula and molecular weight (MW) of the colourant are C$_{24}$H$_{27}$N$_2$O$_{13}$ and 551, respectively. Fig. 5 shows the structure of betanin.

Absorbance, mass transfer rates and dye yields comparison

The absorbance, mass transfer rates and dye yields showed significant improvement in case of extraction with optimized conditions when compared with the control experiment. It is observed that there was significant enhancement in the mass transfer rates. Amongst the particle sizes, the finest size was the optimum that gave rise to the increased mass transfer and the increased dye yield. It might be due to the highest contact surface area of the finest size. The optimum solid-solvent ratio was 1:20. Dynamic dye transfer equilibrium might have reached with the particular ratio. Volume greater than 20 mL was therefore, showing significantly low absorbance values. The complete transfer of dye would have been difficult to the solvent lesser than 20 mL. As regards the extraction time, one hour was found as sufficient time for complete extraction. Extraction enhancement of the desired component was seen through the highest absorbance value in the enzyme method. Enzyme treatment loosens the structural integrity of the plant material making their cells easily breakable. This might be the reason for releasing more solutes from them thereby increasing the mass transfer rate.

The enhanced mass transfer rate ultimately increases the yield of the colorant.

Thus, the rise in absorbance values, the mass transfer rates and the yields might be due to combined effect of all the parameters used at their optimal values. This has led to the finding that the optimization of extraction process is beneficial for mass transfer rate enhancement in the natural dye production.

Conclusion

Optimum extraction conditions investigated by Taguchi design were successfully used to conduct natural dye extraction operation in an efficient way. The UV-VIS and FTIR results very well supported the presence of betanin colorant in the bracts of *Bougainvillea glabra* Juss. The results showed significant increase in the absorbance of the color extract indicating the significant increase in the mass transfer rates and therefore, yield of the colorant was seen to be increased when optimal extraction conditions were used for the extraction. This has ultimate benefit in terms of reduction in time and conservation of the resource used for dye production. The study can certainly contribute to the future scale-up of this plant for dye production useful for textile, food, pharmaceutical, dye-sensitized solar cell and cosmetic applications.

Acknowledgements

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