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Synthesis of Fluorescein Dye Using Microwave Radiations and its Application on Textile Substrates

DOI: 10.5604/01.3001.0014.6090

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Abstract
Energy conservation plays a vital role in overcoming the burning issues of global warming worldwide. The synthesis of organic dyes using the conventional method consumes high levels of energy, temperature, as well as solvents, which further leads to additional costs as well as increases in energy consumption. To save energy, time and cost, the synthesis of dye using microwave based energy might be a good option. In this study, a comparison was drawn between the conventional synthesis and microwave based synthesis of fluorescein dye. The resultant dyes were characterised using the different techniques of UV-Visible and FTIR spectroscopy. Furthermore, colour values were evaluated for dyed wool and Nylon 6 fabrics. In view of this, the work in the present investigation is related to the synthesis of fluorescein with two different methods (conventional and microwave). This dye may be used for various applications in the textile industry.

Key words: *dyeing, energy saving, fluorescein, microwave, Nylon 6, wool.*

Introduction

Fluorescein dyes [1] are defined as compounds which both absorb [2] and emit [3] strongly in the visible region, and which owe their potential for application to their intense fluorescence [4] properties. Fluorescein has excellent optical properties, such as high fluorescence, a high fluorescent extinction coefficient [ε], excitation and emission wavelengths in the visible range, and innoxiousness. Since it was first synthesised one hundred years ago, fluorescein has been widely used in the chemical and biological [5] analysis fields. However, some intrinsic properties of fluorescein also limit further applications in many areas. The use of microwave radiation is a flexible and effective way to improve the fluorescence properties of dye molecules as well as the application performance by molecular designing to introduce built-in functional groups [6].

Fluorescein is a manufactured organic compound which is used as a dye. It is available as a dark orange/red colour powder slightly soluble in water and alcohol [7, 8]. Fluorescein, also called Resorcinolphthalein, has the molecular formula $C_{20}H_{12}O_5$ and has seen wide use as a synthetic colouring agent. It is prepared by heating phthalic anhydride and resorcinol over a zinc catalyst, where it crystallises as a deep red powder, with a melting point in the range of 314 °C to 316 °C. It was named for the intense green fluorescence which it imparts to alkaline solutions; a color visible even in dilutions of 1:50,000,000.

Various methods *viz*. the conventional [9], niobium pentachloride [10] and ship in a bottle [11] are available for the synthesis of fluorescein. In the conventional method, fluorescein is prepared by reacting phthalic anhydride with resorcinol using zinc chloride as a catalyst via the Friedel-Crafts reaction. In this reaction, resorcinol acts as the solvent for this reaction and zinc chloride as a catalyst. This reaction is generally carried out at 180-200 °C using pH 7-9. Niobium pentachloride and has proved to be a powerful activating agent for various organic reactions. In this process niobium pentachloride is used as Lewis acid for synthesis fluorescein. This reaction takes place at 90-100 °C and pH 4-5. In the 'ship in a bottle' method, fluorescein is entrapped inside various zeolite structures which are prepared by catalytic synthesis of the dye from its precursors (phthalic anhydride and resorcinol) adsorbed in the zeolites. The zeolites (mostly H forms) play the role of the catalyst and matrix, encapsulating the resulting dye molecules. In this method zeolites are used as a heterogeneous catalyst, and the process is carried out at 100-120 °C using pH 4-7.

This is used as dye to colour liquids in analytic tools and cosmetics [12] and as a tracer or marker [9, 12]. Halogenated derivatives of fluorescein include eosin

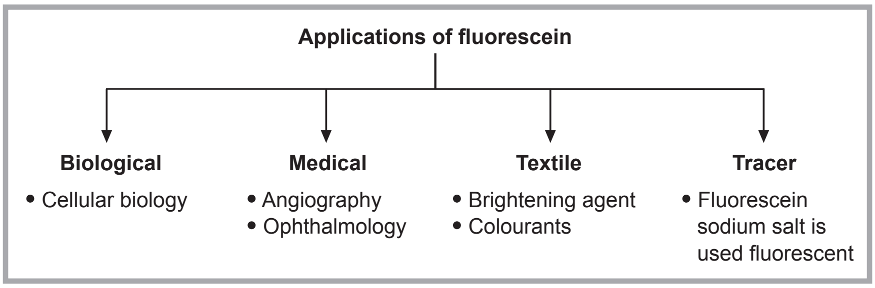

Figure 1. *Various applications of fluorescein dye.*

and erythrosine [13] which have various biological and medical applications. The fluorescent detection technique has played a significant role in the advancement of modern medicine and molecular biology and has achieved rapid development [1]. Fluorescein is one of the fluorescent probes widely used in such applications (***Figure 1***).

Based on experimental data [14], it is found that microwave-enhanced chemical reaction rates can be faster than those of conventional heating methods by as much as 1000-fold. The microwave can use higher temperature than conventional heating systems, and therefore the reactions are completed in a few minutes instead of hours, for instance, the synthesis of fluorescein, which usually takes about 10 hours by conventional heating methods, can be conducted in only 35 minutes by means of microwave heating. A few reactions which were carried out using microwave heating and their comparison with conventional heating with respect to the time and energy efficiency of the methods are assembled in ***Table 1***.

The synthesis of organic dyes using the conventional method consumes high levels of energy, temperature and solvents, which further leads to additional cost as well as increases in energy consumption. To save energy, time and cost, the synthesis of dye using microwave based energy might be a good option. In this study, a comparison was drawn between the conventional synthesis and microwave based synthesis of fluorescein dye. The resultant dyes were characterised using the different techniques of UV-Visible and FTIR spectroscopy. Furthermore, colour values were evaluated for dyed wool and Nylon 6 fabrics. In view of this, the work in the present investigation is related to the synthesis of fluorescein with two different methods (conventional and microwave). This dye may be used for various applications in the textile industry.

Material and methods

Materials

Apparatus used

All the instruments and apparatus used in the experiment are listed in ***Table 2***.

Chemicals used

Phthalic anhydride, resorcinol, and zinc chloride were obtained from the chemical store of the Chemistry Department at GNDU Amritsar, India (***Figure 2***).

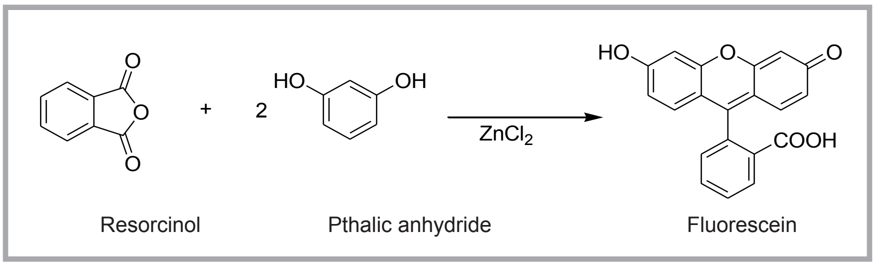

Figure 2. *Synthesis of fluorescein using $ZnCl_2$ as catalyst [9].*

Reaction setup

Set an oil bath to a temperature between 180 and 200 °C. To a large test tube or small Erlenmeyer flask, add 0.4 g of resorcinol and 0.2 g of ground powdered phthalic anhydride. To this mixture of powders, add 6 drops of 0.25 g of $ZnCl_2$. Stir the mixture briefly with a spatula. Place the test tube in the preheated oil bath. The reaction should be run at a temperature between 180 and 200 °C. (Caution: It is extremely important to monitor the temperature and keep it within this range. Overheating will cause the product to decompose.) The reaction should be run for 30 min within this temperature range. Once the reaction time is up, remove the test tube from the oil bath and allow it to cool for about 5 min.

Reaction workup

To the test tube, add 10 ml of acetone along with a stir bar. Using a ring stand and clamp, place the test tube over a magnetic stir plate and stir the solution for 5 to 10 min. The solution should turn yellow as the crude fluorescein dissolves. If the entire product did not dissolve, repeat the process with an additional 5 ml of acetone until the entire product dissolves (do not use more than 25 ml total). Combine the acetone layers in a 50 ml beaker, and then boil off the acetone, leaving a crude orange residue. Crude residue is then dissolved in 30 ml of diethyl ether and 1.5 ml of water (Caution: Even though most of the dye will end up in the organic layer, it will not dissolve unless a small amount of water is present). Place the beaker over the magnetic stir plate for several minutes until the entire solids dissolve. Move this organic solution to a separatory funnel, add 15 ml of water for washing, and discard the aqueous wash. After this, extract the ether layer once with 10 ml of a saturated NaCl solution and dry the organic layer over anhydrous sodium sulfate. Preweigh a small beaker, place the dried organic solution in it, and leave the solution to evaporate to dryness in a water bath to yield an orange solid (***Figure 3***).

Observation of fluorescence

Prepare a solution of fluorescein by dissolving 5 mg of the sample in 50 ml of 0.1 M NaOH solution. Place the solution

Table 1. *Evaluation of reaction times of microwave versus conventional heating [15].*

S. No.	Compound synthesised	Reaction time: microwave	Reaction time: conventional
1	Methyl benzoate	5 minutes	8 hours
2	4-nitrobenzyl ester	2 minutes	1.5 hours
3	Zeolite synthesis	30 seconds	60 minutes
4	Cubanite	3 minutes	3 days
5	$NaAlH_4$	2 hours	8 hours
6	$CuBi_2O_4$	5 minutes	18 hours
7	Ag_3In	2 minutes	48 hours

Table 2. *List of all instruments used.*

Instrument	Manufacturer	Function
Electronic weight balance	Citizen, India	To measure weight
Fourier Transform Infrared Spectroscopy (FTIR)	Varian, USA	Infrared spectrum
Water bath	Perfit India Ltd., India	To incubate samples in water at constant temperature
X-Ray Diffractometer (XRD)	RigakuMiniflex, Japan	X-Ray diffraction
UV-Visible Spectrophotometer	Agient Technologies, USA	Absorption spectroscopy

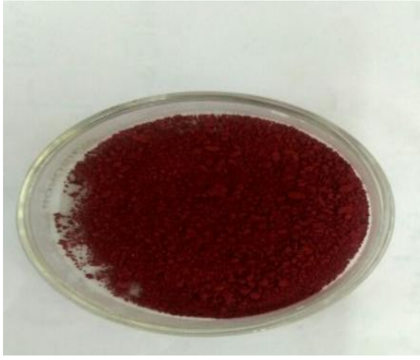

Figure 3. Dye synthesised by conventional method.

Figure 4. Test solution of synthesised dye to observe the fluorescence property.

in a vial and put it on a black non-reflective surface. Place a bright light source on the opposite side and note down the appearance of the solution. The colour observed (***Figure 4***) is primarily due to the absorbance of some wavelengths of visible light passing through the sample from the light source.

Fabrics used

100% pure wool and Nylon 6 fabrics (***Figure 5***) were selected to check the colour yield. 4 g as the initial weight of both fabrics was used for the dyeing step. The fabrics were treated in an aqueous solution for 1 h at 80 °C, then rinsed thoroughly in water, and finally air dried at room temperature.

Dyeing procedure

Wool and Nylon 6 were dyed using fluorescein dye prepared by the conventional method at optimum conditions and a liquor ratio of 50:1. The dye bath was prepared at pH 4 using acetic acid. Dyeing was initiated at 40 °C, and then the temperature was raised to 100 °C over 45 min. Subsequent to the dyeing and cooling of the dyeing baths, all dyed samples were rinsed with plain water and dried in the open air. Dye exhaustion on the wool and Nylon 6 fabrics (***Figure 6***) was further evaluated spectrophotometrically for their colour values.

Solvent free synthesis of fluorescein dye

Chemicals used

Phthalic anhydride, resorcinol and zinc chloride were obtained from the chemical store of the Department of Chemistry, Guru Nanak Dev University, Amritsar.

Synthesis of fluorescein dye using microwave irradiations

Fluorescein dye was synthesised in a microwave (***Figure 7***) using a mixture of phthalic anhydride and resorcinol at a ratio of 1:2, with zinc chloride as a catalyst. The reaction mixture was irradiated in the microwave oven at 800 W for 10 minutes at 90 °C without using any solvent (***Figure 2***). The reaction takes place in a single step. This reaction decreases the time, temperature and liquor ratio, and increases the yield of the product.

Characteristics of dye synthesised by microwave irradiations

Fluorescent dye with a yellow color was obtained, with a λ_{max} of 460 nm and melting point of > 300 °C, observed using Gallenkamp melting point apparatus. The yield obtained was 90% using ***Equation (1)***:

$$\%\text{Yield} = \text{experimental yield}/\text{theoretical yield} \times 100\%$$

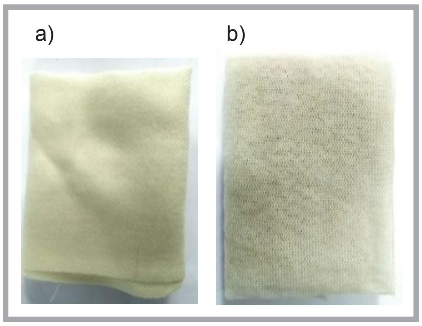

Figure 5. Undyed pure fabric samples: a) wool and b) Nylon 6.

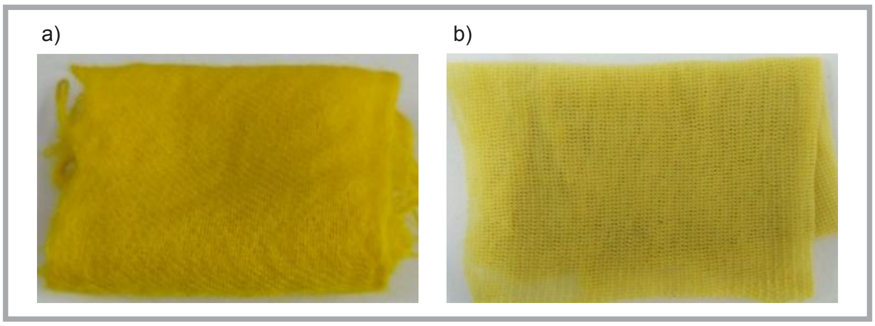

Figure 6. a) Wool sample dyed with conventionally synthesised dye and b) Nylon 6 sample dyed with conventionally synthesised dye.

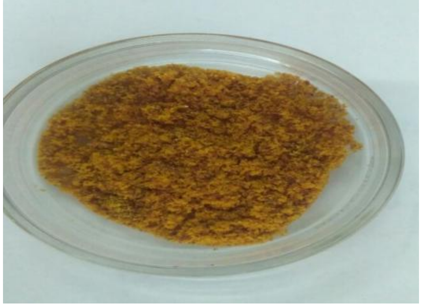

Figure 7. Synthesis of fluorescein using microwave irradiations.

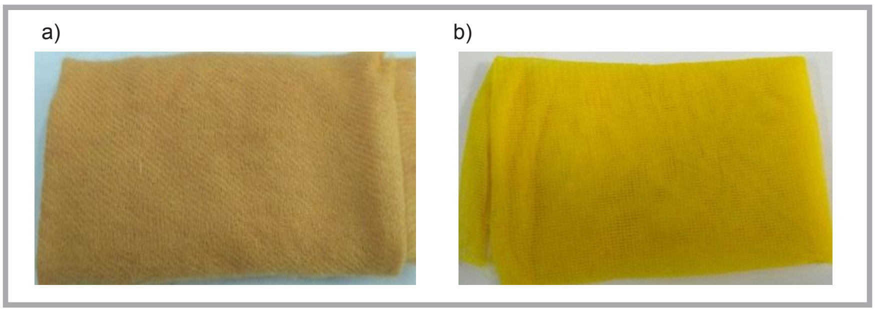

Figure 8. a) Wool sample dyed with microwave synthesised dye and b) Nylon 6 sample dyed with microwave synthesised dye.

Dyeing procedure

Wool and Nylon 6 were dyed using fluorescence dye prepared by the conventional method at optimum conditions and a liquor ratio of 50:1. The dye bath was prepared at pH 4 using acetic acid. Dyeing was initiated at 40 °C, and then the temperature was raised to 100 °C over 45 min. After dyeing, all dyed samples were rinsed with water and air dried. Dye exhaustion on the wool and Nylon 6 fabrics (***Figure 8***) were evaluated spectrophotometrically for their colour values. The wool and Nylon 6 fabrics dyed using dye prepared in a microwave were compared with the samples dyed using dye prepared by the conventional method.

Colour fastness to washing of dyed samples

The colour fastness to washing was evaluated using Standard ISO C06 C2S test. ECE reference detergent (4 gL^{-1}) and sodium perborate (1 gL^{-1}) at pH 10.5 and steel balls (25) were employed in the test. A 10 x 4 cm dyed fabric strip stitched through the short end to the SDC multifibre test fabric was washed in a Washtec (RBE) at 40 °C (for wool) and 60 °C (for Nylon 6) for 30 min. The samples were then rinsed with cold water, next air dried, and finally analysed for colour change and staining. Likewise, the colour fastness to rubbing was established using the IS-766-88 test by means of a crock meter. The light fastness was tested on a xenon arc lamp following test method ISO 105/B02, using blue wool reference samples.

Colorimetric properties [16] of the dyeings (D_{65} illumination, 10° observer) were determined using a Spectraflash 600 colorimeter (Datacolor International).

Results and discussion

UV-Spectrum

Spectrum of dye synthesised by conventional method

The absorption spectrum of fluorescein synthesised by the conventional method in ethanol was characterised by an intense 241 nm and 278 nm, which was attributed to Π-Π* transitions at 241 nm and 278 nm, and having a band at 471 nm and 498 nm, which was attributed to n-Π* transitions. The Π-Π* transitions were mainly responsible for imparting orange colour to the dye in solution form.

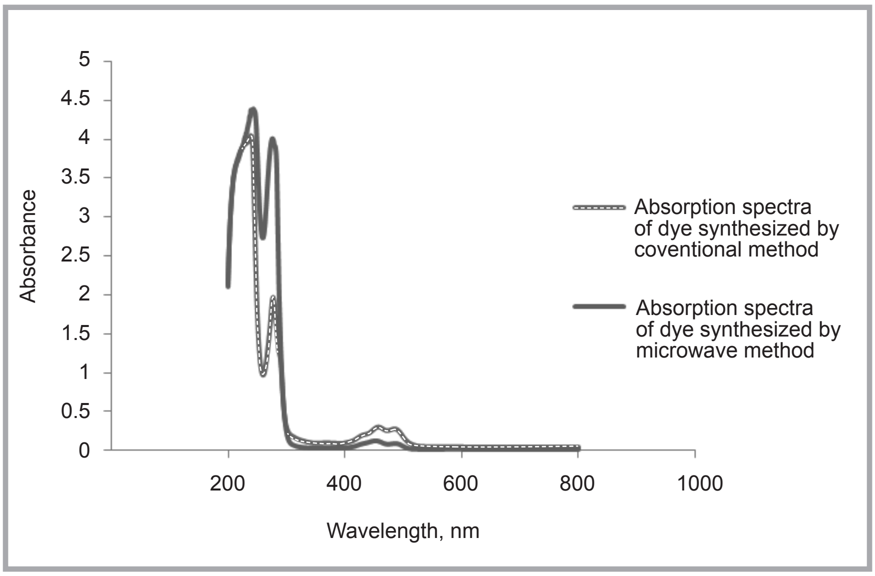

Figure 9. *Comparison of absorption spectra of dyes synthesised by conventional and microwave methods.*

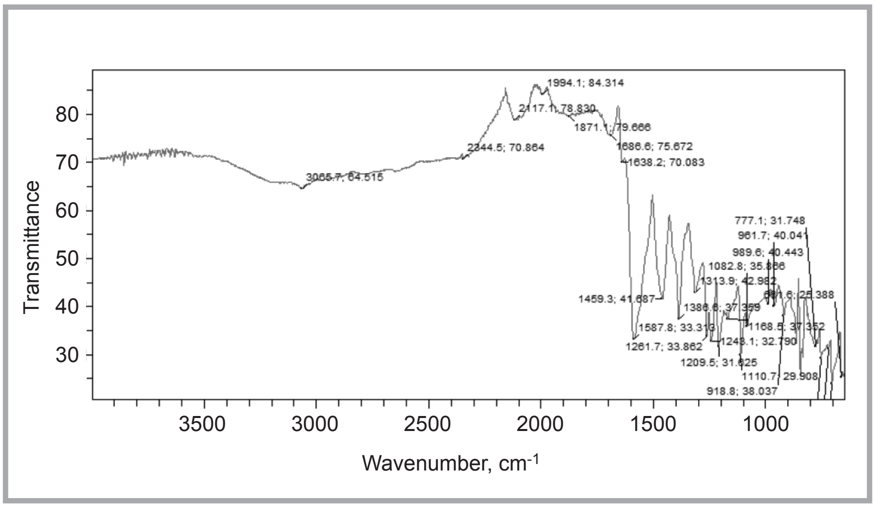

Figure 10. *FTIR spectrum of dye synthesised by conventional method.*

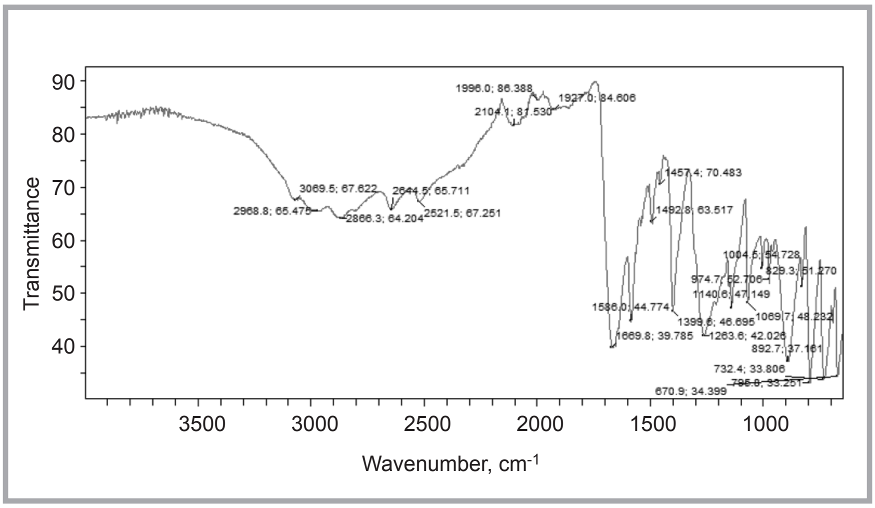

Figure 11. *FTIR spectrum of dye synthesised by microwave method.*

Table 3. *Correlation chart of functional groups and frequency.*

Functional group	Literature value, cm^{-1}	Observed value, cm^{-1}
C=O	1715	1686
C=C Aromatic	1600 and 1475	1638 and 1459
CH_2=C-O-R	1220 (strong band)	1209 (strong)
COO$^-$	1600 (asymmetric stretch) 1400 (symmetric)	1587(asymmetric stretch) 1386(symmetric)
C-H Aromatic	3150- 3050	3065

Table 4. *Correlation chart of functional groups and frequency.*

Functional group	Literature value, cm^{-1}	Observed value, cm^{-1}
C=O	1715	1669
C=C Aromatic	1600 and 1475	1586 and 1492
CH_2=C-O-R	1220 (strong band) 850 (weak band)	1263 (strong band) 829(weak band)
COO$^-$	1600(asymmetric stretch) 1400(symmetric stretch)	1586(asymmetric stretch) 1399(symmetric stretch)
C-H Aromatic	3150-3050	3069
C=CH	3000-3100	2968

Table 5. *Different shades developed using conventionally synthesised and microwave synthesised dyes.* ***Note:*** *CW – wool dyed using conventionally synthesised dye; CN: Nylon dyed 6 using conventionally synthesised dye; MW – wool dyed using microwave synthesised dye; MN – Nylon 6 dyed using conventionally synthesised dye.*

S. No.	Method used to synthesise the dye	Fabric used	Dyed samples	Sample codes	K/S
1.	Conventional	Wool		CW	21.60
		Nylon		CN	7.918
2.	Microwave	Wool		MW	8.912
		Nylon		MN	17.461

Table 6. *Washing fastness data of dyed samples (conventional and microwave). Washing conditions: $NaBO_3$ (1 g/L), ECE Ref Det. 4 g/L, Na_2CO_3 (1 g/L), pH 10.5, 60 °C/30 min. (100 ml for each sample).* ***Note:*** *Color staining rating: SCA, secondary cellulose acetate; BUC, bleached unmercerised cotton; N, Nylon 66; P, polyester; A, acrylic; WW, worsted wool.*

S. No.	Method used	Fabric used	Change in colour	Colour staining					
				SCA	BUC	N	P	A	W
1.	Conventional	Wool	4	3	4	3	3	3	3
		Nylon 6	4	4	5	4	5	5	4-5
2.	Microwave	Wool	3-4	4-5	4	4	3-4	4	4-5
		Nylon 6	3-4	5	4-5	4-5	4-5	4-5	5

Spectrum of dye synthesised by microwave method

The absorption spectrum of fluorescein by synthesized by the microwave method in ethanol was characterised by an intense 247 nm and 281 nm, which was attributed to Π-Π* transitions at 247 nm and 281 nm, and having a slight band at 471 nm and 503 nm, which was attributed to n-Π* transitions. The Π-Π* transitions were mainly responsible for imparting yellow colour to the dye in the solution.

From this comparison, it could be concluded that similar trends are noticed for both dyes. The absorption spectrum by the microwave method shows intense absorption as compared to that obtained by the conventional method. This intense absorption band is characterised as an intramolecular charge-transfer transition, while the bathochromic shift is due to the structural modification of dye synthesised by microwave radiations, which further raises electron-donating or accepting ability, as shown in ***Figure 9***.

FTIR (Fourier Transform Infrared Spectroscopy)

Spectrum of dye synthesised by conventional method

For fluorescein the FTIR Spectroscopy prepared by the conventional method provides information on the chemical structure of the material. Band assignments for the spectrum of fluorescein are summarised in ***Table 3***, which indicates that fluorescein contains a number of functional groups and structures (C=O, C=C, CH_2=C-O-R, COO$^-$, C-H, C=CH).

The positions and shape of the band at 3065 cm^{-1} are compatible with the involvement of the hydrogen in aromatic carbon, and it also contains other functional groups like carbonyl and carboxylic. The peaks in the spectra at 1587 and 1386 cm^{-1} will contain both asymmetric and symmetric stretch, respectively, which will confirm that there is a carboxylic group present as carboxylate ions and another carbonyl group present at 1686 cm^{-1}, which is at lower frequency than the normal carbonyl frequency, which will confirm that there is carbonyl present is in the conjugation with the double bond with the adjacent ring. Also, a strong peak at 1209 cm^{-1} confirms that one additional oxygen group is present as vinyl alkyl ether. The aromatic ring is further confirmed from the two peaks at 1638 and 1459 cm^{-1}.

Spectrum of dye synthesised by microwave method

Similarly, FTIR spectra of fluorescein prepared by the microwave method show similar types of peaks, like at 3069 cm^{-1}, which confirms the presence of aromatic hydrogen, as well as a carboxyl group present as carboxylate ions at 1586 and 1399 cm^{-1}. A carbonyl group in conjugation with a double bond shows a peak at 1669 cm^{-1} and vinyl alkyl ether at 1263 cm^{-1}. All these peaks in the FTIR spectra confirm that a large number of functional groups are present in the fluorescein molecule. From the FTIR study, an extra peak of alkene is observed in the case of microwave synthesised fluorescein dye.

From the results of ***Table 5***, it is seen that wool and Nylon 6 fabrics can be dyed with fluorescein dye synthesised by both the conventional and microwave methods. In comparison to the synthesised process, the use of the microwave technique to synthesise fluorescein dye offers great potential in industrial processes in terms of reduction in cost, time and energy. The colours obtained in the exhaust dyeing method were recorded and are shown in ***Table 5*** along with their K/S values. These results provide a variation in the colours and add to the colour spectra of the colour industry. Furthermore, it was observed that by using two different methods of synthesising (conventional and microwave), the wool samples were deeper in depth than the Nylon 6 (as given by the K/S Values of the dyed wool samples). The colour tone of the dyed samples also varied with the different methods of synthesising. In the case of the microwave method, the tone was shifted to a bright orange colour (for wool fabric), whereas in the case of Nylon 6, it remains fluorescent yellow.

Table 6 shows data related to the washing fastness properties of various samples dyed with the synthesised dyes by both the conventional and microwave methods. The fastness properties were relatively satisfactory in the case of the colour staining of the Nylon 6 substrate. On average, these fastness values were observed in the case of wool; on the other hand, the results related to change in colour were satisfactory.

Conclusions

The dyes synthesised by both the conventional and microwave methods can be used effectively for dyeing wool and Nylon 6 fabrics, as these dyes appear to behave as an effective dye with an optimum dye uptake, using the exhaustion method of dyeing. Fluorescein dye can be prepared in a microwave, which results in saving time (approximately 15 mins), as well as decreases in temperature (from 100 °C to 90 °C) and consumption of the solvent in comparison to the common pathways. These dyes showed very good fastness to washing in the case of Nylon 6. In the case of dyeing wool, two different colours: bright yellow and bright orange, were achieved along with average-to-good fastness properties. The results of the change in colour related to wash fastness can further be improved with a reduction in the shade percentage during the dyeing process. Energy efficiency is found to be multiple times better (> 40%) for microwave heated processes as compared to conventionally heated processes. This offers a clear benefit in reducing energy costs. It has been proved from this study that the microwave process might be adopted in the dye manufacturing industry to save energy.

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Received 30.12.2018 ***Reviewed 19.05.2020***