Pak. J. Sci. Ind. Res. 2006 **49**(6) 391-394

Effect of Base Media on the Stability of Annatto Dye in Industrial Products

A. O. Adetuyi*, L. Lajide and A. V. Popoola
Department of Industrial Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria

(received February 4, 2005; revised May 24, 2006; accepted July 6, 2006)

Abstract. Colour stability of the versatile annatto dye was examined to monitor the effects of base media on industrial products. The dye was incorporated in two media, namely, palm kernel oil used in products such as body cream and soap, and paraffin wax used in shoe polish and household candle wax. These products were exposed to various light conditions for a specific period before assessment. The melting point of the dye was determined as176-178 °C with a molar absorptivity of 13,600 l mol^{-1} cm^{-1} at 545 nm. It imparted its brilliant yellow/orange colouration in industrial products as the colour stability depends on the compounding base media of the products. Losses of β-carotene was higher and faster in non-aqueous environment as paraffin wax showed low fastness rating values, than in the palm kernel oil based aqueous medium products.

Keywords: annatto dye, base media, colour stability, industrial product, dye stability, *Bixa orellana* dye

Introduction

Apart from indigo and logwood, annatto dye is among the oldest colourants known and used by man. It is obtained from the fruit pulp from a tropical tree *Bixa orellana,* family Bixaceae. The orange-red annatto dye has been used since long by the people of the Caribbean islands and the tropical Americans to colour their bodies red during war time in order to frighten their adversaries (Kochhar, 1981). The plant occus as a large shrub to a small tree depending on the region and age of the plant. It is now grown in many places outside the tropical America, including most states in Nigeria (Noah, 1995). The fruit pod of the plant houses the seeds coated with a resinous layer of orange/red pod avils from which the commercial pigment is extracted. The annatto dye varies in hues ranging from yellow through orange to orange-red, which is due to a mixture of carotenoids and their degradation products. The structures of the major carotenoids of this dye as, *cis*- and *trans*-bixin, and *cis*- and *trans*-norbixin, have been studied and reported (Goodwin, 1980; Finar, 1975; Barber *et al*., 1961; Kuhn and L'Orsa, 1932; Karrer *et al*., 1929). Bixin, ($C_{25}H_{30}O_4$), is a monomethyl ester of a dicarboxylic acid. Under alkaline conditions the methyl group can be saponified yielding the free dicarboxylic acid termed as norbixin ($C_{24}H_{28}O_4$). With an excess of alkali, the dicarboxylic acid dissociates to form the alkali metal salt, usually potassium or sodium, in which form the pigment is water-soluble (Finar, 1975). Bixin also exhibits the typical reactivity of carotenoids. A survey of the literature has shown that annatto dye has been in use since antiquity as a colourant for food and textiles as well as cosmetics. A short review of the extraction and chemistry of the annatto dye has also been published (Preston and Richard, 1980).

The present study examines the effect of media on the stability of annatto dye in industrial products. The dye after extraction and purification was incorporated into some industrial products through various base media and their colour stability to light was assessed, or rated, on the 7th day after production using the Gray's scale, (SDC, 1992).

Materials and Methods

Source and sample preparation. The fruit pods were collected from the Staff Quarters, Federal University of Technology, Akure, Nigeria. They were sun-dried and cracked to obtain seeds. The cracking was done by packing the fruit pods into a jute bag, beaten with a heavy wood to loose the seeds from the pod. The seeds were then sieved to remove the pod pieces and stones and dried in oven at 105 °C for 3 h, cooled, and stored in an airtight desiccator.

Extraction and purification. Dried *Bixa orellana* seeds (25 g) were shaken with 100 ml chloroform in a separatory funnel for 2-3 min to extract the pigment from the seeds. The pigment was completely extracted by 2-3 repeated extractions, using 50 ml chloroform each time. All the extracted fractions were sieved through mesh (150µ) to remove seed debris from the extract. The extract was allowed to stand overnight and separated with a separatory funnel into two layers. The upper layer contained the pigment and the lower layer was a clear homogeneous deep-red solution. The pigment layer was then purified using column chromatography with silica gel as the adsorbent in the ratio of 1:25. The pigment was washed with a series of mixtures of polar and non-polar solvents at various ratios.

*Author for correspondence; E-mail: yomiadetuyi2002@yahoo.com

Determination of phyiscochemical properties. The melting point of crude and purified annatto dye was determined using the capillary tube and electrothermal methods (Furniss *et al.*, 1978). The solubility of the dye was tested in acetone, benzene, butanol, chloroform, carbon tetrachloride, commercial fragrance (benzyl propionate), dichloromethane, diethylether, ethanol, ethylacetate, groundnut oil, hexane, kerosene, methanol, petroleum ether, palm kernel oil (PKO), phenol, 0.6 M sodium hydrogen carbonate, 5% sodium hydroxide, concentrated sulphuric acid (96%), turpentine, water (distilled), and universal thinner (nitrocellulose thinner). The solubility was determined by adding 10 mg of the colourant to 5 ml each of these solvents, and heated gently in a waterbath, in case the colourant was insoluble in the cold. The presence of nitrogen, sulphur, and halogens was detected by the Lassaigne sodium fusion test (Furniss *et al.*, 1978). The phenolic hydroxy group was in the pigments detected by spraying ferric chloride-ethanol solution (1:10 w/v) on the TLC plates. The presence of blue-black colour on the plates indicated the presence of phenolic hydroxyl group in the samples. Oxidation with ethanol-$KMnO_4$ solution was used to determine unsaturation. The discolouration of the test solution to brownish colour indicated unsaturation in the dye.

Absorptiometric measurement of the colourants. Stock solution of the *Bixa* extract (1.25 x 10-4 gl^{-1}) was prepared in chloroform. Aliquots were taken for serial dilution with appropriate solvents and their absorbance was measured spectrophotometrically (Biochrom-4060-UV/vis spectrophotometer) at 545 nm. The molar absorptivity of the dye in a 1% solution and 1cm cell ($A1^{1cm}_{1\%}$) was determined using the Beer-Lambert's relationship (Giles, 1974):

$$A = \varepsilon cl \quad \text{.......} \quad (1)$$

Where:

A = absorbance

l = cell path length in cm

c = concentration in g per 100 ml (w/v)

ε = molar absorptivity or molar extinction coefficient in 1 mol^{-1} cm^{-1}

The molar extinction coefficient (ε) is represeneted as $A^{1cm}_{1\%}$,which is generaly used for the comparison of absorption intensity (Giles, 1974):

$$(A^{1cm}_{1\%}) = \frac{A}{cl} \quad \text{.......} \quad (2)$$

It is related to the molar absorptivity by the expression:

$$\varepsilon = (A^{1cm}_{1\%}) \times {}^{M}/_{10} \quad \text{.......} \quad (3)$$

The equations (2-3) shows the relationship between molar absorptivity and the molecular weight of a dye.

Colouration of industrial products and colour fastness test. A known weight (5 mg) of the purified dye was successively incorporated through an appropriate medium (paraffin wax or bleached palm kernel oil) to the following prepared products as per their formulae described in literature (Dhingra *et al*., 1991): candle sticks and shoe polish via paraffin wax; body cream and soap via bleached palm kernel oil. Each coloured product was divided into five lots (I-V) of equal weight and placed in petri dishes. These five lots were exposed to different test conditions (Table 1) for 6 h daily for 14 days. The colour stability of the dyed products was assessed after 7 and 14 days of production by visually comparing the change in colour of the products with lot # I placed in the dark as the control. A rating scale (Grays scale) used for determing colour fastness for fabric was adopted (SDC, 1992).

Results and Discussion

The physicochemical properties. The results of the physicochemical tests carried out on the colourant and the shade of the dyed articles are summarized in Table 2. The molar absorptivity ($A^{1cm}_{1\%}$) for the pigment was very high, ca 13,600 litre per mol per centimeter.The value of molar absorptivity is of economic importance as it is a measure of the strength of the dye (Griffiths,1984). This high value implied high dye strength and indicated usefulness of the dye for incorporation in commercial products, as small quantity is required for intense colouration. The solubility results (Table 3) also supported the versatile usability of the annatto dye, as it is soluble in nearly all solvents (non-polar, polar and chlorinated organic/ aromatic). This aids its easy incorporation or blending into industrial products (Kochhar, 1981). The high solubility is due to the presence of oxygen-containing functional groups in its molecule or their formation when incorporated in various media. The chemical test result does not support the presence of

Table 1. Test conditions for 6 h daily for 14 days for assessing the colour stability of annatto dyed products divided into five lots

Lot #	Experimental condition
I*	darkness
II	in the laboratory (exclusive of direct sun-light)
III	under 40 w light
IV	under 100 w light
V	open place (direct sunlight inclusive)

* = lot # I kept permanently in darkness

phenolic hydroxyl group in the extract because it gave a negative result to the ferric chloride test. It, however, supported the presence of unsaturation (conjugated compounds), which is the characteristic of bixin (Britton, 1983; Barber *et al*., 1961).

Colour stability assessment of the dyed product. The brilliant and appealing hue of yellow/orange of the annatto dye was imparted on commercial products (Table 2). The colour fastness ratings of these dyed products are given in Table 4 and Table 5. Generally, a great loss was observed in respect of such characteristics as attractiveness and colour/hue of the annatto dye in these products, ranging from paraffin-based products (candle wax and shoe polish) to bleached palm kernel- based products (body lotion and soap). The rating values also decreased with increase in the luminosity of their exposure, from the dark (experimental condition no. I) to the most intense light exposure (experimental condition no. V). This indicated that light and probably also the air (oxygen) present around the product/pigment during exposure induced fading reactions on them. This also corroborates the finding of Najar *et al.* (1988) that light was the most destructive agent for annatto dye. No change in hue of all the products in the dark was noticeable (experimental condition no. I) irrespective of the base- media. They were highly rated because the darkness forestalled the reactions that would have been induced on the products by the presence of light/air. The paraffin-based products (non-aqueous medium) had low rating values on both days of assessment as shown in Tables 4 and 5, respectively, indicating fading in colour, than was observed in the palm kernel oil-based products. Their colour was totally lost on the 14th day (Table 5) in paraffin-based products (candle and shoe polish). This fair colour stability of the PKO(palm kernel oil) based products of the annatto dye was due to the base and the chemical constituents of the products, such as fatty acids, water and hydroxides, among others, in the body cream and soap, respectively, that provided aqueous medium. The annatto pigment was highly soluble in sodium hydroxide

Table 2. The physical parameters of annato dye obtained from the annatto trees (*Bixa orellana*)

Test parameter	observation
Appearance of crude crystals	orange-red powder
Recovery level (%)	3.50
Crude melting point (°C)	185-190
Appearance of pure colourant	orange-red powder
Melting point (°C)	176-178
Lassaigne's sodium fusion test	N, S, Cl absent
Phenolic hydroxyl group test	negative
Molar absorptivity (l mol^{-1} cm^{-1}) at 545 nm	13,600
Colour shade in impacted articles*	
cotton yarn	orange
cotton fabric	orange
candle wax	golden yellow
soap	golden yellow
writing ink	nursery pink
body lotion	golden yellow
shoe polish	yellow

* = commercial emulsion paint colour chart used for coding

Table 3. Solubility test results of pure annatto dye in selected solvents

Solvents	Observation
Acetone	+
Benzene	+
Chloroform	++
Commercial fragrance	++
Diethylether	++
Distilled water	+
Ethanol	+
Groundnut oil	+
Hexane	+
Hydrochloric acid (5%)	-
Methanol	+
Palm kernel oil (PKO)	+
Paraffin oil	+
Petroleum ether	+
Phenol	++
Sodium hydrogen carbonate (5%)	-
Sodium hydroxide (5%)	++
Sulphric acid (conc.)	+
Turpentine	++
Universal thinner	++

key, + = soluble; – = insoluble; ++ = highly soluble

Table 4. Colour fastness rating of the annatto dyed products on the 7th day of exposure after production

Products	Experimental condition*				
	I	II	III	IV	V
Candle wax +	5	3	2 +	1 +	1
Shoe polish +	5	3	2	1 +	1
Body lotion ++	5	4	3 +	3	3
Soap ++	5	4	3 +	3	3

* = see Table 1 for details of the experimental conditions; + = pigment incorporated into products via paraffin wax; ++ = pigment incorporated into products via palm kernel oil (PKO); 1 = poor fastness; 2-3 = moderate fastness; 4-5 = good fastness

Table 5. Colour fastness rating of the annatto dyed products on the 14th day of exposure after production

Products	Experimental condition*				
	I	II	III	IV	V
Candle wax +	5	2	1	1	1
Shoe polish +	5	1+	1	1	1
Body lotion + +	5	3	2	1	2
Soap ++	5	3+	2+	2+	2

* = see Table 1 for details of the experimental conditions; + = pigment incorporated into products via paraffin wax; ++ = pigment incorporated into products via palm kernel oil (PKO); 1 = poor fastness; 2-3 = moderate fastness; 4-5 = good fastness

(Table 3) probably forming a salt (since the pigment is a dicarboxylic acid), which stabilized the pigment in the resulting product. This stability reduces the rate of light and oxygen (air) attacking the product in this medium as compared with paraffin based products. Ramakrishnan and Francis (1980; 1979) reported that oxygen caused losses of β-carotene were much higher in a non-aqueous solvent environment system than in aqueous solvent environment. This accounted for better colour stability in palm kernel oil-based aqueous products than in paraffin.

Conclusion

The base media of application as described in this study affect the fugitive nature of annatto dye as an added additive to commercial products, which needs to be considered in its usage. The fugitive nature of the pigment is slow in an aqueous solvent environments, which is recommended for its application.

References

Barber, M.S., Hardisson, A., Jackman, L.M., Weedon, B.C.L. 1961. Studies in nuclear magnetic resonance. Part IV. Stereochemistry of bixin. *J. Chem. Soc.* **12:** 1625-1630.

Britton, G. 1983. *The Biochemistry of Natural Pigments*, pp. 3-220, 2nd edition, Cambridge University Press, Cambridge, UK.

Dhingra, K.C., Dubey, S.C., Gupta, D.C. 1991. *20th Publication Small Scale Industries: Modern Manufacturing Formulae*, pp. 25-148, 1st edition, Small Industry Research Institute, Delhi, India.

Finar, I.L. 1975. *Stereochemistry and the Chemistry of Natural Products*, pp. 487-489, 5th edition, Longman Singapore Publishers Ltd., Singapore.

Furniss, B.S., Hannaford, A.J., Rogers, V., Smith, P.W.G., Tatchell, A.J. 1978. *Vogel's Textbook of Practical Organic Chemistry*, pp.100-222; 931-1152, 4th edition, Longman, London, UK.

Giles, C.H. 1974. *A Laboratory Course in Dyeing*, pp. 65, 126-127, 3rd edition, The Society of Dyers and Colourists, Bradford, England, UK.

Goodwin, T.M. 1980. *The Comparative Biochemistry of the Carotenoids*, vol. **1**, pp. 6-25, 2nd edition, Chapman and Hall, London, UK.

Griffiths, J. (ed). 1984. *Developments in the Light Absorption Properties of Dyes, Critical Reports of Applied Chemistry*, vol. **7**, pp. 1-28, Blackwell Scientific Publications, London, UK.

Karrer, P., Helfenstein, A., Widmer, R., Van Italie, T. B. 1929. Uber bixin. (XIII. Miteillung uber pfanzenfarbstoffe) *Helv. Chim. Acta.* **12:** 741-755.

Kochhar, S.L. 1981. *Tropical Crops: A Textbook of Economic Botany*, pp. 379-388, 1st edition, Macmillian Publishers, London, UK.

Kuhn, R., L'Orsa, F. 1932. Uber konjugiente doppelbindungen. XXV: Thermischer abbau der carotinfarbstoffe. *Ber. Deutsh. Chem. Ges.* **65:** 1873-1880.

Najar, S.V., Bobbio, F.O., Bobbio, P.A. 1988. Effects of light, air, anti-oxidants and pro-oxidants on annatto extracts (*Bixa orellana*). *Food Chemistry* **29:** 283-289.

Noah, A. 1995. *Dyes from Plants: Their Extraction and Application to Textiles*, pp. 3-104, 1st edition, Jide Publishing House, Festac Town, Lagos, Nigeria.

Preston, H.D., Rickard, M.D. 1980. Extraction and chemistry of annatto. *Food Chem.* **5:** 47-56.

Ramakrishnan, T.V., Francis, F. J. 1979. Stability of carotenoids in model aqueous system. *J. Food Anal.* **2:** 177-189.

Ramakrishnan, T.V., Francis F.J. 1980. Auto-oxidation of caratenoids and their relative polarity. *J. Food Anal.* **3:** 25-34.

SDC. 1992. *The Society of Dyers and Colourists, Standard Methods for the Determination of the Colour Fastness of Textiles and Leather, ISO (105-B02)*, pp. 1-3, 3rd edetion, SDC Publ., Bradford, UK.