

## A Quantitative Study of the Effect of pH on the Dyeing Process with Juglone

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### ABSTRACT

The use of natural dyes obtained from plants has been a well-known art for many thousands of years. This ancient practice continued unchanged until the mid 19th century when synthetic dyes were invented. Many people became aware about the hazards of chemical dyes. Recently, many cosmetic companies all over the world launched a range of hair coloring products containing natural dyes. But this practice needs scientific optimization and worth intensive research. One of these natural dyes is walnut extract which contains an effective coloring agent, juglone, a brown dye which adheres directly to the keratin fibers without a fixing agent.

The objective of this work is to study the effect of pH on the dyeing process with walnut-extract-related material (juglone).

Bleached wool felts have been dyed with juglone, the principal coloring agent of walnut. A dissolution technique that could evaluate uptake and substantivity was adopted. The mass of juglone removed from the wool was assessed from the reflectance readings, related to changes in Lab values of dyed felts which were recorded before and after extraction. A theory linking dyeing performance to electrolytic dissociation of juglone and keratin protonation was developed from the results.

**Keywords:** Walnut extract, juglone, hair dyeing, keratin.

### INTRODUCTION

Walnut (*Juglans regia* L., Juglandaceae) leaf and hull have been broadly used in traditional medicine for many years for its pharmacological effects: astringent, keratolytic, antidiarrhoeal, antifungal, hypoglycemic and sedative.<sup>(1)</sup> Herbal preparations derived from black walnut have been used as hair dyes and skin colorants.<sup>(2,3)</sup> Juglone is known to react with keratin proteins present in the skin.<sup>(4)</sup>

Different shades of hair are made by mixing walnut hull powder with henna powder; as the hands-on experience over many years has established that the pH of the medium must be acidic. This appears to be in conflict with the solubility behavior of juglone, the principal dyeing ingredient of walnut. Juglone is a weak acid and forms soluble salt in

alkaline solution, but the undissociated form has a very low solubility in water, so that in acidic suspensions of walnut, in concentrations used in hair dyes, not all the juglone is dissolved. The juglone in solution will be the active species, with the suspended excess providing a reservoir to replace dissolved juglone lost to the hair. The more juglone there is in the solution, the greater the driving force of the dyeing reaction.

The second conflict between practice and theory is that acid solutions of juglone are very faint yellow color, but as the pH values are increased it develop an intense color. Practical experience in dyeing suggests the opposite behavior; so this study attempts to rationalize these differences.

### EXPERIMENTAL

**Materials:** The material used as a model for human hair was pressed wool felt, 0.5 cm thick, supplied by Hinders-

Leslies Ltd. It was cut into pieces 2.5 cm square, each weighing 0.6g. 5-Hydroxy-1,4-naphthoquinone (juglone) was obtained from Sigma-Aldrich Chemi., GmbH.

#### Absorption spectra

Readings were obtained using Varian CARY/1E - UV-Visible spectrophotometer and 10 mm matched quartz cells.

#### Color measurement

L.a.b. values readings, were obtained using Micromatch Sphere Plus, Sheen instrument Ltd, England.

#### Dyeing procedure

Felts were washed with warm distilled water, squeezed, and dried at room temperature. A group of three felts were dyed at a time at pH values of 3, 4, 5, 6, 7, 9. Suspensions were prepared by mixing 20mg of juglone with 50 mL of distilled water. pH was adjusted to the required values with either 0.1 M NaOH or 0.1 M HCl. Felts were then placed in the suspension and pressed with glass rod, to absorb the liquid. The container was sealed with cellophane film, and placed in a shaking water bath at 40 °C for 30 min. Felts were taken out, rinsed with 50 mL distilled water for few seconds, squeezed well and dried at room temperature overnight.

#### Dissolution

Dyed felts were transferred to the rotating basket of ERWEKA dissolution rate apparatus (model DT6R), 200 mL of water was used as dissolution medium, and the basket rotated at 100 rpm. Dissolution was carried out for 60 min at 40 °C.

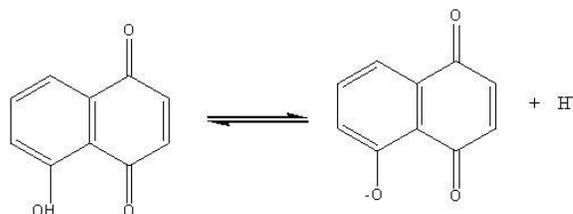
#### Influence of pH on electronic spectra

A juglone 0.005% stock solution was prepared. A series of juglone solutions at different pH values were prepared by adding 5 mL of juglone solution to 15 mL of each of the buffer solutions previously prepared.

Small quantities of each of the mixtures were transferred in turn into the glass cell of the scanning spectrophotometer, using the buffer solutions as blank. The absorbance range was selected from 200 to 350 nm, with a scan speed of 100 nm/min. Absorption spectra were overlaid using the program provided by the spectrophotometer.

#### DISCUSSION

The UV spectra of juglone solutions go through an isosbestic point of 265 nm as shown in figure (1), suggesting that the ionized and the unionized species are involved in pH-dependent equilibrium (Eq. 1).



Absorption in the visible region by juglone solutions increases with increasing pH; the tautomer in excess in alkaline solutions is therefore more deeply colored than the unionized species associated with low pH values.

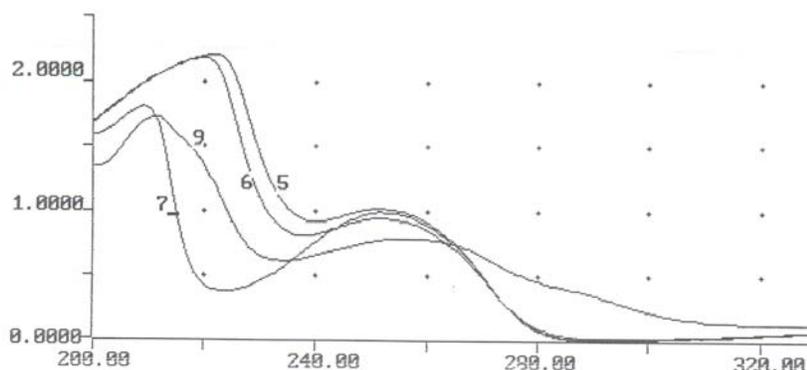


Figure 1. Electronic spectra of solutions of juglone at various pH values.

The hues of the dyed felts can be expressed in L.a.b. notation as the ratio  $a/b$ . When  $a/b = 1$ , the hue is pure orange, and when  $a/b > 1$ , it is reddish orange; the higher the  $a/b$  values the higher the red contribution. Similarly, when  $a/b < 1$ , the hue is yellowish orange, the yellow contribution increases with decreasing values of  $a/b$ . The  $a/b$  ratios of felts dyed at various pH values are shown in Table (I) and indicate that the hue was yellowish orange without significant change in acid media and changed suddenly to a slightly yellow hue in alkaline media, while the opposite was observed with juglone solutions.

This behavior can be predicted from the ionizing properties of juglone and keratin. Juglone dissociates according to Eq.1, and it is well known that the polypeptide chains of keratin contain monoacidic diamine residues, which are protonated at low pH values. If it is assumed that the dyeing process mainly involves interaction of juglone anions with the protonated groups of keratin, electrovalent bonds will result, fixing the dye firmly to the hair. Juglone has a  $pK_a$  of 3.65<sup>(5)</sup>, and keratin has an isoionic point of 6.0<sup>(6)</sup>, so that the proportions of the ionized species can be calculated. These are shown in Table (II). At pH 3, 99.5% of the groups of hair are positive and will be ready for

complexation. Only 18% of the juglone in solution is ionized, but Eq. (1) will move to the right to replace juglone anions taken up by the keratin. Similarly, juglone lost from solution will be replaced from the suspended juglone in excess of solubility.

At alkaline pH values (for example, pH 9), although the juglone is completely ionized; only 0.05% of the sites on the keratin are available for interaction. Probabilities of ionic interaction therefore correspond to the percentage of positively charged groups of the keratin, decrease with increasing pH.

Substantivity is expressed as  $\Delta a$  and  $\Delta b$ , where  $\Delta$  symbolizes the difference between results before and after extraction. These parameters are given in Table (I), and suggest that there was no significant change in color before and after dissolution, this is to say that the interaction between juglone and keratin is very strong. This can be explained by the very low solubility of juglone in water, which was found to be 0.005% w/v. Comparing these results with those of a very similar compound like lawsone, previously published,<sup>(7)</sup> which has a higher solubility (0.02% w/v) and where  $\Delta a$  and  $\Delta b$  values were much more higher, complies with this conclusion.

**Table I**  
**L.a.b. Values of Wool Felts after Dyeing with Juglone at Various pH Values**

	pH					
	3	4	5	6	7	9
<b>L</b>						
Before dissolution	48.96±0.34	48.05±0.44	47.22±0.26	47.73±0.76	52.24±0.69	54.74±0.90
After dissolution	47.89±0.63	47.93±0.96	46.76±0.96	47.06±0.54	53.26±0.29	56.76±0.55
<b>a</b>						
Before dissolution	14.29±0.36	14.17±0.49	14.44±0.36	14.16±0.51	12.74±0.33	8.69±0.11
After dissolution	14.46±0.23	14.43±0.42	14.33±0.51	13.18±0.23	13.08±0.72	8.24±0.10
<b>b</b>						
Before dissolution	31.95±0.42	31.70±0.22	31.97±1.01	31.38±0.71	27.90±0.46	23.94±0.12
After dissolution	30.94±0.43	30.90±0.62	31.10±0.12	31.53±0.43	30.77±0.40	26.37±0.46
<b>a/b</b>						
Before dissolution	0.45	0.45	0.45	0.45	0.46	0.36
After dissolution	0.47	0.47	0.46	0.42	0.42	0.31
<b>Δ a</b>	0.17	0.25	-0.11	-0.98	0.34	-0.45
<b>Δ b</b>	-1.01	-0.80	-0.87	0.14	2.87	2.42

**Table II**  
**Distribution of Charged Species in Keratin and Juglone at Various pH Values**

	pH					
	3	4	5	6	7	9
Percentage of total charged						
Species in keratin						
Positively charged	99.95	99.5	95.0	50.0	5.0	0.05
Negatively charged	0.05	0.5	5.0	50.0	95.0	99.95
Percentage of total Juglone molecules						
Anion	18.0	68.75	95.72	99.55	99.95	100.0
Undissociated	82.0	31.25	4.28	0.45	0.05	0.0
Relative probability of interaction	100	100	95	50	5	0

It has been explained above how the intensity of color of juglone solutions increase progressively as the pH increases. However, the hues of the wool felts shown in Table (I) move with dyeing pH in the opposite direction. The relative probability of interaction suggested above permits complexation of juglone anion with the positive keratin groups of the hair at acidic pH values, giving yellowish orange hue, but does not explain why the hue is more yellow when the hair is dyed at high alkaline pH values. This yellow color at high pH confirms the existence of other type

of interaction between unionized juglone and keratin.

#### ACKNOWLEDGMENT

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