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NOTE

Isolation of the natural colorant from the roots of *Arnebia nobilis* and its use as a new neutralization indicator

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Solution of natural dye from the roots of the plant *Arnebia nobilis* in methanol is an excellent acid-alkali indicator capable of working in much wider range than the usual indicators phenolphthalein and methyl orange. Volumetric titrations of strong acid-strong alkali, strong acid-weak alkali and weak acid-strong alkali are performed and very satisfactory results found in the wider range of concentrations (10^{-3} to 4 *N*) of acid and alkali. The structures of the indicator in acid and basic mediums are suggested. The p*K* of indicator is 5.21. The pH at the equivalence point in three types of titrations varies in the range of 3.75 to 11.70; λ_{max} are found to be 235 and 250 nm. Active component, arnebin-7, is isolated pure and also used as indicator to confirm the results.

Arnebia nobilis is a Boraginaceous plant and is a native of Afghanistan. The roots are the source of well known red medicinal dye. The chemical investigation of this medicinal plant was first reported in 1969[1]. When extracted with methanol, a dark maroon decoction is obtained which yields a red-black solid on the removal of methanol. Apart from the medicinal use[2–4] and the food colorant[5], the dye has different colours in acid and alkaline mediums. Certain natural colorants prove to be the better indicators[6] than the conventional indicators in use. We explored the possibility of its use as neutralization indicator for varied concentrations of acid and alkali. A comparative study with conventional indicators phenolphthalein and methyl orange has also been undertaken. Active component, arnebin-7, has been isolated and also used independently as indicator to confirm the results.

Results and discussion

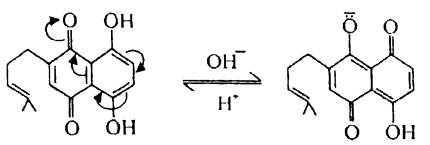
Results of the three kinds of titrations are given in Table 1.

Results of pH-metric titrations are given in Table 2 alongwith the λ_{max} values of the dye at the equivalence points of acid-base titrations.

It is evident from the results that the present natural dye obtained from *Arnebia nobilis* is a better indicator in all respects to the popularly used phenolphthalein and methyl orange indicators for different type of neutralization titrations. It is also better in range. A successful titration of 10^{-3} *N* alkali could be performed by the natural dye whereas this was not possible when phenolphthalein was used as an indicator. Moreover, while phenolphthalein fails in strong acid-weak base, and methyl orange fails in weak acid-strong base, this natural indicator is successful in strong acid-weak base as well as weak acid-strong base in most of tried ranges of titrations.

In the case of phenolphthalein in presence of dilute alkali, the lactone ring opens[7] and the triphenylcarbinol structure undergoes loss of water to produce the resonating ion, which is red. But in excess alkali the red colour disappears owing to the conversion to benzenoid structure. In the case of *Arnebia nobilis* the active coloured ingredient is arnebin-7 which is comparable to phenolphthalein. We have isolated pure arnebin-7 also (confirmed by λ_{max} 225 and 540 nm, IR (ν_{max}) 1608, 1600, 1553 and 1447 cm^{-1} and ^1H NMR single proton triplet at 3.18 τ, 4-proton multiplet at 7.40 τ, 1-proton multiplet at 4.80 τ, 2-proton singlet at 2.83 τ and singlets at –2.55 and –2.40 τ and m.p. 95°) using the method described by Shukla *et al.*[8] and used as indicator independently. The same results are obtained. This dye is red in quinonoid form but blue in other form.

It seems that a change from quinonoid form to benzenoid form in excess alkali takes place just like in phenolphthalein. In acidic medium arnebin exists in its quinonoid form (**I**). In alkaline medium the conversion to the form (**II**) takes



(I) **(II)**

Table 1. Results of three kinds of titrations

Vol. of acid taken = 5.00 ml

Strong acid-strong base titrations :

HCl *N*	NaOH *N*	Volume of NaOH required		Present dye indicator		Phenolphthalein	
		Present dye indicator	Phenolphthalein	Colour change at equiv. point	Sharpness and stability upto atleast 1 min	Colour change at equiv. point	Sharpness and stability upto atleast 1 min
0.001	0.001	5.00	–	Light Cu-pink to light purple	Sharp Stable	Could not be titrated successfully	
0.01	0.01	5.00	5.00	Cu-pink to purple	Sharp Stable	Colurless to pink	Disappears soon
0.10	0.10	5.00	5.00	Cu-pink to blue	Sharp Stable	Colourless to pink	Sharp Stable
1.00	1.00	5.00	5.00	Cu-pink to blue	Sharp Stable	Colourless to pink	Sharp Stable
4.00	4.00	4.95	4.95	Cu-pink to blue	Sharp Stable	Colurless to pink	Sharp Stable

Strong acid-weak base titrations :

HCl *N*	NH_4OH *N*	Volume of NH_4OH required		Present dye indicator		Methyl orange	
		Present dye indicator	Methyl orange	Colour change at equiv. point	Sharpness and stability upto atleast 1 min	Colour change at equiv. point	Sharpness and stability upto atleast 1 min
0.001	0.001	4.95	–	Cu-pink to purple	Sharp Stable	Could not be titrated successfully	
0.01	0.01	5.05	5.05	Cu-pink to purple	Sharp Stable	Pink to yellow	Sharp Stable
0.10	0.10	5.00	5.00	Cu-pink to purple	Sharp Stable	Pink to yellow	Sharp Stable
1.00	1.00	5.00	5.00	Cu-pink to purple	Sharp Stable	Pink to yellow	Sharp Stable
4.00	4.00	4.95	4.95	Cu-pink to purple	Sharp Stable	Pink to yellow	Sharp Stable

Weak acid-strong base titrations :

CH_3COOH *N*	NaOH *N*	Volume of NaOH required		Present dye indicator		Phenolphthalein	
		Present dye indicator	Phenolphthalein	Colour change at equiv. point	Sharpness and stability upto atleast 1 min	Colour change at equiv. point	Sharpness and stability upto atleast 1 min
0.001	0.001	4.95	–	Cu-pink to purple	Sharp Stable	Could not be titrated successfully	
0.01	0.01	5.00	5.00	Cu-pink to purple	Sharp Stable	Colourless to pink	Sharp Stable

Table-1 (contd.)

0.10	0.10	5.00	5.00	Cu-pink to blue	Sharp Stable	Colourless to pink	Sharp Stable
1.00	1.00	5.00	5.00	Cu-pink to blue	Sharp Stable	Colourless to pink	Sharp Stable
4.00	4.00	5.00	5.00	Cu-pink to blue	Sharp Stable	Colourless to pink	Sharp Stable

Table 2. pH-metric titrations and λ_{max} values

Vol. of acid or alkali taken = 15 ml, [Acid] = 1.0 *N*, [Alkali] = 1.0 *N*

Type of titration	Volume of alkali added ml	pH	Colour	Colour change	λ_{max} nm	λ_{max} nm (reverse titration)
Strong acid-strong base	14.95	3.75	Cu-pink	Sharp	235	242
	15.00	11.65	to blue	Stable		
Strong acid-weak base	15.00	3.80	Cu-pink	Sharp	235	250
	15.05	7.85	to purple	Stable		
Weak acid-strong base	14.90	6.15	Cu-pink	Sharp	229	250
	14.95	11.70	to blue	Stable		

The p*K* value of the present dye indicator is found to be 5.21.

place, responsible for blue colour.

The existence of two forms in acidic and alkaline mediums is confirmed by obtaining of two different λ_{max} values at equivalence points – first by titrating the alkali with acid in burette and second by titrating the acid with alkali in burette.

Experimental

Isolation of dye : Authentic roots of *Arnebia nobilis* were procured from Dehradun. The dye was islolated from the roots of the plant as described earlier[9]. 10 g of the crushed roots were kept in sufficient methanol in Soxhlet extractor for 24 h. Dark maroon extract with a brown tinge collected and fresh methanol added again and kept for 24 h. Procedure was repeated till colour of extract became light. All solutions were mixed and methanol was separated by vacuum distillation.

Isolation of arnebin-7 : Active component was isolated pure as described by Shukla *et al.*[8], and duly identified.

Indicator solution : The dye (1 g) was dissolved in methanol (100 ml) and its pH was determined (Systronics-324) and found to be 5.05. Arnebin-7 (0.02 g) was dissolved in 2 ml methanol. Its pH was found to be 5.26.

Volumetric titrations : All reagents used were either B.D.H., AnalaR or A.R. (C.D.H.) quality. Double-distilled water was used. Five solutions (0.001, 0.01, 0.10, 1.00 and 4.00 *N*) each of HCl, NaOH, CH_3COOH and NH_4OH were used. Phenolphthalein and methyl orange indicator solutions were prepared[7]. In each case, both the titrations (acid in burette and alkali in burette) were performed using present dye indicator and separately the phenolphthalein or methyl orange (2–3 drops). Strong acid with strong base, strong acid with weak base, and weak acid with strong base were titrated. The pH-metric titrations were also performed in each case. The reproducibility was better than ±3% .The p*K* value of the dye solution was determined using Henderson-Hasselbalch equations[7]. Similar titrations were performed using the indicator solution made of pure arnebin-7. Same results were obtained. For the determination of λ_{max}, a Systronics UV-Vis Spectrophotometer-118 was used.

References

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