European Reviews of Chemical Research

Has been issued since 2014.
ISSN 2312-7708, E-ISSN 2413-7243
2017. 4(2). Issued 2 times a year

Journal is indexed by: **CrossRef** (UK), **Electronic scientific library** (Russia), **Journal Index** (USA), **Open Academic Journals Index** (Russia), **ResearchBib** (Japan), **Scientific Indexing Services** (USA)

All manuscripts are peer reviewed by experts in the respective field. Authors of the manuscripts bear responsibility for their content, credibility and reliability.

Editorial board doesn't expect the manuscripts' authors to always agree with its opinion

Postal Address: 1367/4, Stara Vajnorska str., Bratislava – Nove Mesto, Slovakia, 831 04

Website: http://ejournal14.com/en/index.html
E-mail: evr2010@rambler.ru

Founder and Editor: Academic Publishing House Researcher s.r.o.

Release date 15.09.17.
Format 21 × 29,7/4.

Headset Georgia.

Order № 111.

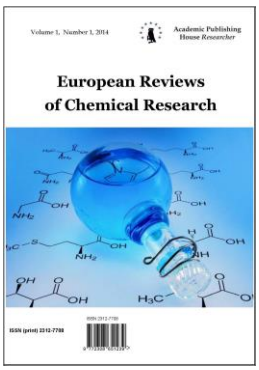
European Reviews of Chemical Research

2017

Is. 2

C O N T E N T S

Articles and Statements



Published in the Slovak Republic
European Reviews of Chemical Research
Has been issued since 2014.
ISSN: 2312-7708
E-ISSN: 2413-7243
2017, 4(2): 36-40

DOI: 10.13187/ercr.2017.2.36
www.ejournal14.com

Articles and Statements

Competence of *Hyptis Suaveolens* Leaf Extract on Treatment of Ecto-Parasites (Fleas) on Farm Animals (Goat)

M. Chindo [a, *], I.A.A. Ibrahim [b]

[a] Department of Science Laboratory Technology, School of Science and Technology, Federal Polytechnic, Nassarawa, Nigeria
[b] Department of Science Laboratory Technology, School of Science and Technology, Abubakar Tatari Ali Polytechnic Bauchi, Nigeria

Abstract

Studies were carried out to examine the effectiveness of Hyptis suaveolens leaf extract and DO force against ectoparasites (fleas) on goats. Effect of Hyptis suaveolens leaf extract and DO force on certain parameters weight, packed cell volume (PCV) and repellence were studied on goats. A total number of 18 goats were examined for the presence of fleas by physical examination and were divided into three equal groups viz: Group A (infested control group) group B (treated with Hyptis suaveolens extract) group E (treated with the DO force). On day 14 of post treatment it was discovered that the PCV level and body weight of treated groups (B and C) increased significantly and relative infestation decreased, all the goats after dipping in DO force diluted water and Hyptis suaveolens spray remained healthy, no adverse effect on goats was observed. On the other hand the PCV and body weight of control group decreased on day 14 and the number of fleas per surface area of the body increased.

Keywords: packed cell volume, ecto parastes, infestation, ethylene diamine tetraacetic acid, hyptis suaveolens, repellence.

1. Introduction

Hyptis suaveolens is a native of tropical America and west tropical African region and Found to grow in Australia since the mid-19th century. A major weed in northern queen land, Hyptis suaveolens is commonly found along side roads and water sources and overgrazed pasture. The plants are not eaten by stock. Hyptis suaveolens is a weed in many tropical areas around the world (Bieski et al., 2015).

Hyptis suaveolens which is popularly known as mint weed, bush tea, Gros baume, Vilayati tulsi, Jungi tulsi, Ganga tulasi, Gandha thulasi, Konda thuslasi, Adavi tulasi, Bhustrence, American mint, chan plant belongs to the family Lamiaceae describe as erect annual herb grow up to 3m high with a woody base. The stem is hairy and hollow. The plant is covered with glandular and non-glandular hair. Leaves are hairy ovate 2 – 10 cm long. The leaf stalk ranges between 0.5-4cm long.

* Corresponding author
E-mail addresses: eliyah2001@yahoo.com (M. Chindo), iliyasuibrahim@gmail.com (I.A.A. Ibrahim)

The fruit is a lobed capsule nutlets dark brown shield shaped 3.5-4cm long and 2.5-3mm wide. Flowers and seeds head in clusters of 1-5, 6-7mm long on stalks 0-2cm long, sepals joined to form a 5-lobed Calyx that is ribbed, green at first drying brown and bluish purple flowers and hairy lobed capsules 4-6mm long in flower late summer to winter (Srivastava et al , 2012).

Hyptis suaveolens distinguished by opposites leaves strong mint aroma when crushed, 5-bristled calyx 8-14mm long fruit petals bluish purple, joined and with the lower lip punched and bent down ward, stamens 4 bent down wards, flower and fruit not in comb-like group, fruit dividing into 2 seeds-like nutlets. Dispersal methods of fruit is spread by water and mud attached to animals (Edeoga, Gomina, 2000).

Hyptis suaveolens is popularly used in the treatment of respiratory and gastrointestinal infection, in digestion, cold, pain, fever, cramps and skin diseases (Bieski et al., 2015). The leaves are used as an anti-cancer and anti-fertility agent. While their aqueous extract has shown on antinociceptive effects and acutetoxicity, antimicrobial activity of plant extract towards drug resistant or clinically significant microbes are reported and it was observed that active constituent material seep out in organic solvent to display biological activity (Srivastava et al , 2012; Bieski et al., 2015).

Fleas belongs to the kingdom: *anlmalia,* phylum; *arthropoda,* class: *insect,* sub class: *pterygoto,* infra class: *neoptero,* super order: *endopterygota,* order: *siphonoptera*. They are wingless, with mouth part adopted, piercing skin and sucking blood. Fleas are external parasites, living by hemotophagy off the blood of mammals and birds. Some fleas species include; *spi/opsythus cunichli* (rabbit fleas), *Ctemocephalides [eiis* (cat fleas), *Csterocephalide canis* (dog fleas), *Pulex irritans* (Human fleas), *Dasypsyllus gallinulae* (moorhen flea), *Nosopsyllus fasetatus* (jnorthern rat flea), *Xenopsylla cheapis* (ureiental rat flea). Over 2,000 species have been described worlds wide (Peattie , 2013).

Fleas are wingless insects (1/10 to 1/8inch) (1.5 to 3.3mm long) that are agile usually dark coloured (for example, the reddish brown of the cat fleas) with tube like mouth parts adapted to feeding on the blood of their hosts. Their legs are long. The hind are well pair adapted for jumping. A flea can jump vertically up to 7 inches (18cm) and horizontally up to 13 inches (33cm) making the flea to be one of the best jumpers of all known animals (relatively to body size). Their bodies are laterally compressed permitting easy movement through the hair or feathers on the host's body (or in the case of human under clothing). The flea's body is hard, polished and covered with many hairs and short spire directed backward, which also assist its movement on the host (Peattie, 2013).

Fleas are holomelabolous insects, going through the four life cycle stages of egg, larva, pupa and imago (adult) (Mueller et al., 2015). Adult fleas must feed on blood before they can become capable of reproduction. The flea life cycles begins when the female lay eggs after feeding. Eggs are laid in batches of up to 20 or 50, usually on the host itself, which means that the eggs can easily rot on the ground (Van Emden, 2013). Because of these, area where the host rest and sleeps becomes a habitat of the eggs and developing fleas. The eggs take around two days to two weeks to hatch (Burgess, Cowan, 2012; Boase et al., 2014).

Expenditure on control measures, fleas related diseases account for over 50 % darmatological cases reported to the veterinary clinics (Laflamme, 2014). Regular applications of paraseticide to prevent or treat fleas infestation is a common strategies in veterinary practice which have more toxic affect to the host itself (Jacobs et al., 2015). The cost of chemicals is also high which make it inaccessible to peasant to farmers.

A lot of different parasite control plants are naturally available everywhere and easier to extract and make use of it, many of them exhibiting almost one hundred percent efficacy to treat vector parasite, many of them when used are very effective and toxic to parasites and virtually not harmful to the host so they are safer to use and at affordable price (Bradley et al., 2015).

Objectives of the research is therefore to study the efficacy of *Hyptis suaveolens* leaf extract on the repellence of fleas; to investigate the effect of *Hyptis suaveolens* on weight gain of goat; and also to examine the effect of *Hyptis suaveolens* leaf extracts on blood count or packed cell volume (PCV) of the animals.

2. Materials and methods

Experimental Site

The study was conducted at the small ruminant animal farm of the College of Agriculture Bauchi, Nigeria (10°17' N, 9° 47' E and 609m above sea level) to investigate the efficacy of *Hyptis*

suaveolens leave extract on treatment of ectoparasite (fleas) on farm animals (goat). The experiment consisted of three treatments (control, conventional drug and *Hyptis suaveolens* leave extract). The treatment were randomized and applied in a complete randomize design with six replications.

Method of Extraction

The fresh and tender leaves of *Hyptis suaveolens* were collected from the College premises which were thoroughly washed with clean water and chopped to pieces with a knife and are exposed to sunlight for five days till they drained completely. The dried leaves were mechanically grinded and sieved to fine soft powder, 500g of the powder was mixed with one liter of free chlorine water and boiled to 100°c. The solution was filtered using filter paper, the filtrate was then diluted with 500ml clean water. The solution so obtained was sprayed onto the experimented animals using sprayers.

Procedures of Data Collection.

Animal weight

The animal weight was determined by the use of weighing scales, at the beginning of the experiment the weight of assistance was taken as an initial weight and then the weight of assistance plus that of animals considered as the final weight. So the actual weight of the animal was obtained by subtracting the initial weight from the final weight. The obtained value was recorded as animal weight per unit kilogram (kg).

Total Blood count or packed cell volume (PCV)

Blood samples were collected from jugular veins of each animal and transferred to blood sample bottles that contain the material that prevent blood from clotting (EDTA), 1ml of blood were transferred into capillary tubes and placed in a haematocrit centrifuge spinning at 3000 revolutions per minute for 5 minutes, the result was read using microhaematocryt reader in percentage (%). Relative infestations were determined by physical examination of the body surface area of animals by counting the number of ecto-parasites in the selected area of individual goat.

Repellence method of application

Eighteen goats were grouped into A, Band C, each group has six infected goats. Group A was negative control (infested untreated). Group B infested treated with *Hyptis suaveolens* leaves extract solution applied by spraying and the last group C was infested treated conventional drug (acaricide D.O. force) applied by dipping method with the dilution rate of 1ml per 1 liter of distil water.

3. Results and Discussion

The effect of *Hyptis suaveolens* leaf extract and DO force on body weight of goat (Table 1) indicated that the average initial body weight of group A (control) on day 1 were 32.l kg decreased to 30.2kg on day 14 of post treatment respectively. On the other hand, the body weight was increased significantly in treated groups (Band C) from 30.8kg to 32.8kg in group B and in group C from 30.6kg to 32.8kg at days 1 and 14 respectively. This is in agreement with the findings of Sabate (1993) who reported that the means of initial body weight of group's treated with plant extract at (15 %) were 18kg at day 1 of post treatment, the body weight of calves increased significantly at group treated with plant extract.

Table 1. The effect of *Hyptis suaveolens* leaf extract and DO force on body weight (kg) of goat

Group	Treatment	Day 1	Day 14	Change
A	Control	26.3	24.3	-2.3
B	H. suaverolens	25.5	27.3	2.3
C	D.O force	26.2	29.3	3.1

The effect of *Hyptis suaveolens* leaf extract and DO force on PCV of goats (Table 2) indicated that the average PCV value of group A (control) decreased from 26.3 % on day 1 to 24.0 % on day 14 of post treatment. On the other hand the PCV value increased in all treated groups (B and C) from 25.5 %, 26.2 % to 27.8 %, and 29.3 % respectively. This is in agreement with the findings of Eguale (2007), who reported that the active ingredient does not penetrate the skin but dissolve into

the skin oil giving long time protection and increase in PCV observed due to the repellence of blood sucking parasites.

Table 2. The effect of *Hyptis suaveolens* leaf extract and DO force on PCV (%) of goat

Group	Treatment	Day 1	Day 14	Change
A	Control	55.6	62.2	0
B	H. Suaverolens	55.0	6.2	89
C	D.O force	55.5	5.5	91

The effect of *Hyptis suaveolens* leaf extract and DO force on repellence of fleas on goat (Table 3) indicated that the relative infestation increased in group A (control) from 55.6 % on day 1 to 62.2 % on day 14, on the other hand relative infestation decreased in treated groups (B and C) from 55.0 %, 55.5 % on day 1 to 6.2 %, 5.5 % on day 14 of post treatment, showing 89 % and 91 % efficacy against fleas. This is in agreement with the finding of Eguale et al., (2007) who reported that leaf extract or by controlling mint as an anti feedant with some repellence effect within days, it prevent feeding and repel midge from further feeding and egg lying. The parasite infection is dramatically reduced mainly due to the antifeedant effect of the plant extract.

Table 3. The effect of *Hyptis suaveolens* leaf extract and DO force on repellence of fleas (%)

Group	Treatment	Day 1	Day 14	Change
A	Control	32.1	30.2	-1.9
B	H. Suaverolens	30.8	32.8	2.0
C	D O force	30.6	32.1	1.5

3. Data Analysis

One way Analysis Variance (ANOVA). The P value is 0.0010, considered very significant variation among column means is significantly greater than expected by chance.

Turkey-Kermer Multiple Comparisons Test if the value of q is greater than 3.674 the P value is less than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sample from population with identical SOs. This assumption is tested using the method of Barlett. Barlett statistic (corrected) 0.09896. The P value is 0.6097. Barlett's suggests that the difference among the SOs is not significant. Assumption test; Are the data sampled from Ga ussian distributions. ANOVA assumes that the data are sampled from population that follows Gaussian distributions. This assumption is tested using the method; Kolmogorov and Smirov if the P value greater than 0.10 passed normality test. The data were collected on day 1 and day 14 that is pre-treatment and post treatment.

4. Conclusion

From the foregoing it can be deduced that hyptis suaveolens leave extract has a notable efficacy against flea infestation on goats.

Hyptis suaveolens could therefore be used as an alternative to any other conventional drug against ectoparasites, it is naturally available and cheaper. However, prolong use of conventional drug in high dose might accompanied by toxic effect on the host life.

References

Anupam et al., 2012 – *Anupam, S., Patel, S. P., Mishra, R. K., Vashistha, R. K., Ajay, S., Puskar, A. K.* (2012). Ethnomedicinal importance of the plants of Amarkantak region, Madhya Pradesh, India. *International Journal of Medicinal and Aromatic Plants*, 2(1), 53-59.

Boase et al., 2014 – *Boase, C., Kocisova, A., Rettich, F.* (2014). 6 Fleas and Flea Management. *Urban Insect Pests: Sustainable Management Strategies*, 86.

Bieski et al., 2015 – *Bieski, I. G. C., Leonti, M., Arnason, J. T., Ferrier, J., Rapinski, M., Violante, I. M. P., da Silva, D. R.* (2015). Ethnobotanical study of medicinal plants by population of

valley of Juruena region, legal Amazon, Mato Grosso, Brazil. *Journal of ethnopharmacology*, 173, 383-423.

Bradley et al., 2015 – *Bradley, F. M., Ellis, B. W., Martin, D. L.* (2015). The organic gardener's handbook of natural pest and disease control: a complete guide to maintaining a healthy garden and yard the Earth-friendly way. Rodale.

Burgess, Cowan, 2012 – *Burgess, N., Cowan, G. O.* (2012). A colour atlas of medical entomology. Springer Science & Business Media.

Edeoga et al., 2002 – *Edeoga, H. O., Ikem, C. I., Jäger, A. K.* (2002). Tannins, saponins and calcium oxalate crystals from Nigerian species of Boerhavia L. (Nyctaginaceae). *South African journal of botany*, 68(3), 386-388.

Eguale et al., 2007 – *Eguale, T., Tilahun, G., Debella, A., Feleke, A., & Makonnen, E.* (2007). In vitro and in vivo anthelmintic activity of crude extracts of Coriandrum sativum against Haemonchus contortus. *Journal of Ethnopharmacology*, 110(3), 428-433.

German et al., 2007 – *German, A. J., Holden, S. L., Bissot, T., Hackett, R. M., Biourge, V.* (2007). Dietary energy restriction and successful weight loss in obese client- owned dogs. *Journal of Veterinary Internal Medicine*, 21(6), 1174-1180.

Jacobs et al., 2015 – *Jacobs, D., Fox, M., Gibbons, L., Hermosilla, C.* (2015). Principles of veterinary parasitology. John Wiley & Sons.

Müller et al., 2015 – *Müller, W. A., Hassel, M., Grealy, M.* (2015). Development and reproduction in humans and animal model species. Springer.

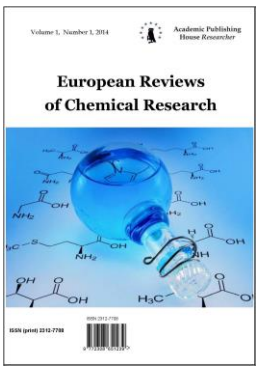
Peattie, 2013 – *Peattie, D. C.* (2013). Green laurels: the lives and achievements of the great naturalists. Trinity University Press.

Sabate et al., 1993 – *Sabate, J., Fraser, G. E., Burke, K., Knutsen, S. F., Bennett, H., & Lindsted, K. D.* (1993). Effects of walnuts on serum lipid levels and blood pressure in normal men. *New England Journal of Medicine*, 328(9), 603-607.

van Emden, 2013 – van Emden, H. F. (2013). Handbook of agricultural entomology. John Wiley & Sons.

Published in the Slovak Republic
European Reviews of Chemical Research
Has been issued since 2014.
ISSN: 2312-7708
E-ISSN: 2413-7243
2017, 4(2): 41-45

DOI: 10.13187/ercr.2017.2.41
www.ejournal14.com



Evaluation of the Effects of *Psidiumguajava* Leave Extracts on Biochemical Indices of two Liver Enzymes and Some Haematological Parameters in Rabbits

I.A.A. Ibrahim [a, *], M. Chindo [b]

[a] Department of Science Laboratory Technology, School of Science and Technology, Abubakar Tatari Ali Polytechnic Bauchi, Nigeria
[b] Department of Science Laboratory Technology, School of Science and Technology, Federal Polytechnic, Nassarawa, Nigeria

Abstract

The influence of *Psidiumguajava* leaf extract on the biochemical indices of the liver function and some haematological parameters in rabbits was assessed. The plant leaves were collected from the College of Agriculture, Bauchi garden and authenticated by two agronomist; S. Adamu and A. Bununu of the department of Agricultural Technology, college of Agriculture, Bauchi. The powdered leaves of *P. guajava* was extracted with ethanol and distilled water using maceration for 2 days. Phytochemical screening of the resulting ethanol and aqueous extracts was carried out using standard procedures. Eight rabbits were randomly grouped into four (4) groups T_1, T_2, T_3 and T_4. Rabbits in T_1 (control) were administered orally with distilled water after acclimatization period. Groups T_2, T_3 and T_4 received 200mg/kg of the ethanol leaf extract of *P. guajava* for a period of 40 days.

Preliminary phytochemical screening of the ethanol extract revealed the presence of saponins, alkaloids, flavonoids, tannins, steroids, terpenes and phenols which varies in the aqueous extract. The liver function test revealed that the serum ALT and AST were found to be within the normal range of 10-45U/L and 10-120U/L respectively. However, there is a significant increase in the red cells production and the hemoglobin concentration.

The results of the study suggested that the ethanol extract of *Psidiumguajava*leaves extract exhibit the hematopoietic potential and has no negative impact on the liver and may be hepatoprotective and hence, the findings may be of clinical importance considering the various reported medicinal values of the plant.

Keywords: *Psidiumguajava*, Hepatic functions, ALT, AST and Phytochemical screening.

1. Introduction

It is generally known that the consumption of a variety of local herbs and vegetables by man contribute significantly to the improvement of human health, in terms of curing and or prevention of diseases. Plants have long served as a useful and natural sources of the therapeutic agents (Gupta et al., 2014).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants is attributed to the chemical substance that produces a definite

* Corresponding author
E-mail addresses: iliyasuibrahim@gmail.com (I.A.A. Ibrahim), eliyah2001@yahoo.com (M. Chindo)

physiological action on the human body. Many of these medicinal plants are used as spices and food plants (Abbasi et al., 2015).

Moreover, traditional medicine is greatly relied upon especially by rural dwellers for the treatment of various ailments. Traditional doctors are the dispensers of such concoctions (Belonwu et al., 2013; Ferngren, 2014). Guava (*P. guajava*) is a common shade tree or shrub indoor-yard gardens in the tropics. The tree is easily identified by its distinctive feature as thin, smooth copper-coloured bark that flakes off showing the greenish layer beneath (Hiwale, 2015).

It has been reported that physiochemical analysis of *P. guajava* leaf products revealed the presence of more than 20 isolated compounds, including alkaloids, antholyanins, and arytenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triteerpens and vitamin C (80 mg per 100kg of *P. guajava*). The main active constituent in the plant is reported to be quercetin. Spasmolytic and antidiarrheal effect are report to be associated with its quercein derived, flaronoids and glycoside, which support use of this ancient leaf remedy in treating gastrointestinal disorders. The decoction made from the leaves and or bark of *P. guajava* has been reported to be, used by many tribes for diarrhea, destiny sore throats, vomiting, stomach upset and to regulate menstrual periods throughout the tropical Amazon and India (Hiwale, 2015). Moreover, tender leaves are also reported to be chewed for bleeding guns and bad breath and it is said to prevent hangovers if chewed before drinking. According to the report, Indians throughout the Amazon gargle a leaf decoction for mouth sore, bleeding gums or use lit as douche for vaginal discharge and to tighten vaginal wall after childbirth. The medicinal importance of the plant have been attributed to their photochemical content. Thus phytochemical analysis of the plants is predicated by the need for drug alternatives of plant origin, made imperative or essential by the high cost of synthetic drugs for example, *L. owariensi* leaves have been report to contain various secondary metabolites of medicinal value including saponins, tannins, alkalolids and flavonoids. These secondary plant metabolites extractable by various solvent exhibits varied biochemical and pharmacological actions in animals when ingested. Within the recent decades a good number of medicine plant have been reported be employed in folk medicine in the treatment of anaemia (Nath, Jain, 2015).

Anaemia is one of the clinical conditions that constitute a serious health problem in many tropical countries as result of the prevalence of different forms of parasitic infections, including malaria. Anaemia condition is characterized by a decrease in the level of circulating hemoglobin less than 13g/dI in males and 12g/dI in females. In the tropics, due to the endemicity of malaria and other parasitic infection, between 10 to 20 % of the population is reported to possess less than 10g/dI of Hb in the blood. The determination of the hematological indices provides physiological information on a proper assessment. According to (Edagha et al., 2014) accurate determination of blood parameters remain the only sensitive and reliable foundation for ethical and rational, research, diagnosis, treatment and prevention of anaemia. The major concern of scientific community with regards to medicinal plants and hematological studies focuses on the measures that can maintain the normal hematological state of being and reverse any negative hematological status associated with various anaemic conditions (Edagha et al., 2014). This study therefore is to partly assess the hematological potential of *Psidiumguajava* leaves extract in rabbit model, considering the fact that different parts of the plant have been reported to be useful in the management various diseases. On the whole, this work aims at reporting the effect of *P. guajava* leaves extract on the biochemical indices of liver function and some hematological parameters in rabbits.

2. Materials and methods

Experimental Site

The research work was carried out at Chemistry/Biology Laboratories of the School of Science and Technology, AbubakarTatari Ali Polytechnic and Pathology Laboratory section of Darusssalam Health Clinic Center, Dutsen Tanshi Bauchi, Bauchi State, Nigeria.

Collection and Identification of Plant Material

Fresh leaves of P. *guajava* were collected from the schools garden. The identification and authentication of the plant was done by a Botanist, Ibrahim Shuaibu, College Agriculture, Bauchi. The leaves were sorted, shade dried, pulverized to powder and stored in a clean container for onward analysis.

Extraction

Extraction with ethanol

The Powdered leaves (200g) was extracted with 400ml ethanol using maceration method for 2days with occasional shaking. The extract was filtered using Whatmann No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40^{o}C) to obtain a gummy greenish product (28g) subsequently referred to as the crude ethanol extract (EE).

Extraction with distilled water

The Powdered leaves (100g) was extracted with 200ml distilled water using maceration method for 2days with occasional shaking. The extract was filtered using Whatmann No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40^{o}C) to obtain a gummy greenish product (28g) subsequently referred to as the aqueous extract (AE).

Preliminary Phytochemical Investigation

Portion of the fractions each was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins and steroids/triterpenes and alkaloids using standard procedures.

Test for Alkaloids

0.5g of the extract was stirred with 5 ml of 1 % aqueous hydrochloric acid on a water bath and filtered. 3ml of the filtrate was divided into two. To the first 1ml few drops of freshly prepared Dragendoff's reagent was added. To the second, 1 drop of Meyer's reagent was added and observed.

Test for Flavonoids and Phenols

Ferric chloride test: To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

Test for Anthraquinones

0.5g of the extract was shaken with 5ml carbon tetrachloride, this was filtered and 10 % dilute ammonia solution was added. The mixture was shaken and observed.

Test for Saponins

0.5g of the extract was shaken with distilled water in a test tube. It was allowed to stand for 10 minutes and observed.

Test for Steroids and Triterpenes

Liebermann-Buchard test: A small portion of the extract was dissolved in chloroform. Equal volume of acetic anhydride and concentrated H_2SO_4 were added down the test tube and observed.

Test for Tannins

Lead Sub-acetate Test: To a small portion of the extract, distilled water was added. 3-5 drops of lead acetate solution was added and observed.

Test for Carbohydrates

Fehling's Test: To a small portion of the extract, distilled water was added. 2ml Fehling's

Test for Glycosides

Legal'stest: To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed.

Preparation of Animal Sample

A total of 8 rabbitscontaining four young and four adult rabbits of either sex (2kg) obtained in Bauchi metropolis were used for the study. They were kept in the School Laboratory Garden for 40 days so as to acclimatize with the environment. The test animals were divided into four groups of two rabbits each (containing a mixture of one young and one adult rabbit each). T_1 serves as the control, T_2, T_3 and T_4 serving as the test groups.

Mode of Administration and Dosage

T_1 serving as the control group received distilled water (placebo) while the ethanol leaf extract of *M. indica*was administered orally to the test groups (T_2, T_3 and T_4). The dosage of administration sustained was 200ml/kg daily in divided doses for a month.

Blood Collection Procedure

The blood was collected from the central auricular artery (ear) with a 20g needle. Vasodilation was achieved with the aid of heating lamps, 70 % alcohol swabs and warm compression.

Liver Function Test (ALT & AST) using Randox Reagent

The ALT & AST test procedure was conducted according to the manufacturer's instruction in the following three steps;

Step 1:

Two test tubes were set as TEST and BLANK. 200 U/L of reagent 1 was added to both test tubes. To the TEST tube, 40 U/L of serum was added to it and 40 U/L of distilled water to the BLANK tube and incubated at 37°C in water bath for 35 minutes.

Step 2:

200 U/L of reagent 2 was added to both the TEST and BLANK tubes, incubated for 20 minutes at room temperature.

Step 3:

To the TEST and BLANK tubes, 2000 U/L of sodium hydroxide (NaOH) solution was added and incubated for 5 minutes at room temperature. The result was displayed at 530 mm with the aid of photoelectric colorimeter. ALT and AST were calculated below;

ALT (U/L) = Optical density of sample mixture × concentration = OD of sample mixture x 160

AST (U/L) = Optical density of sample mixture × concentration = OD of sample mixture x 350

PCV

Capillary tube (75mm) was filled to approximately with EDTA and ant coagulated blood (3 quarter of its length). The excess blood was wiped from the outside of the tube and scatted with a sealer. The tubes were placed in a microhaematocrit centrifuge with the sealed end pointing outwards. The inner lid was firmly secured and the outer lid was also closed and centrifuge for five minutes at 11,000 revolutions per minute. When the centrifuge stopped, the tubes were removed and read as the fraction of red cells column to the total length of the sample.

3. Results

The result of phytochemical screening, effects of Psidium*guajava* leaf extracts on rabbits serum enzymes and haematological indices are presented in Tables 1-3 respectively;

Table 1. Phytochemical Constituents of Ethanol and Aqueous Extracts of P. *guajava*

Constituents	Test	Observation	Inference	
			EE	AE
Saponins	Frothing	Frothing persist for 15mins	++	+
Flavonoids	$FeCl_2$	Green or violet ppt	++	+
Tannins	Lead subacetate	Cream ppt	+	+
Steroids & Terpenes	Lieberman-Buchard	Blue-green color at interphase	+	+
Anthraquinones	Borntragers	Pink or violet	-	-
Phlobatannins	Lead subacetate	Cream ppt	-	-
Reducing Sugar	Fehling's	Bluish black color	+	+
Glycosides	Fehling's	Red ppt	++	-

\+ = present
++ = present in high concentration
\- = absent

Table 2. Effect of the ethanol leaf extract of *P. guajava* on rabbit serum enzyme

Group	ALT (U/L)	AST (U/L)
T_1	28.80	38.50
T_2	32.80	45.50
T_3	30.40	45.50
C	25.60	31.50
Normal range	10-45	10-120

Key: T=Test; C=Control

Table 3. Effect of the ethanol leaf extract of *P. guajava* on rabbit haematological indices

Group	PCV (%)	Hb (g/dl)
T_1	48.0	15.40
T_2	44.0	14.70
T_3	49.8	14.00
C	36.0	124.00
Normal range	33-50	94-174

Key: T=Test; C=Control

4. Discussion

The result of preliminary phytochemical analysis of the ethanol (EE) leaf extract of *P. guajava*revealed the presence of all the constituent tested including flavonoids, saponins, steroids, tannins except Phlobatannins and anthraquinones. The aqueous extract (AE) revealed the presence of all the constituents except anthraquinones, Phlobatanninsand glycosides. These constituents have been reported to be responsible for most biological activities of plants (Shabbir, et al., 2013).

The results obtained for serum alanineaminotransferases (ALT) and aspartateaminotransferases (AST) were found to be within the normal range of 10-45U/L for ALT and 10-120U/L for AST. However, there was no significant alteration with the level of the serum enzymes in the control rabbit which is an indication that the ethanol leaf extract of *P. guajava*did not alter the stoichiometry of the liver marker enzymes and the liver. The packed cell volume (PCV) and hemoglobin concentration were the only heamatologicalparameters tested. According to the result obtained in the analysis, there was a slight variation between the PCV and Hb concentration in the test animal and the control. Hence, the extract has no adverse effect on the circulating red blood cell as well as the Hb concentration but rather brings about the slight increase in the production of red blood cell as well as the Hb concentration. This may be attributed to the presence of active constituent that promote red cell production in the plant extracts.

5. Conclusion

The results of the study suggested that the ethanol extract of *P. guajava*maymaybe hepatoprotective and hence, the findings may be of clinical importance considering the various reported medicinal values of the plant.

References

Abbasi et al., 2015 – *Abbasi, A. M., Shah, M. H., Khan, M. A.* (2015). Ethnobotany and Ethnomedicine. In *Wild Edible Vegetables of Lesser Himalayas* (pp. 19-29). Springer International Publishing.

Belonwu et al., 2013 – *Belonwu, D. C., Onyeike, E. N., Ekere, O. U.* (2013). Comparative phytochemistry of peddled Yoruba medicinal formulations. *Indian Journal of Drugs and diseases*, *2*(3), 259-269.

Edagha et al., 2014 – *Edagha, I. A., Atting, I. A., Bassey, R. B., Bassey, E. I., Ukpe, S. J.* (2014). Erythropoietic and Hepatoprotective Potential of Ethanolic Extract of Nauclea latifolia in Mice Infected with Plasmodium berghei berghei. *American Journal of Medical Sciences and Medicine*, *2*(1), 7-12

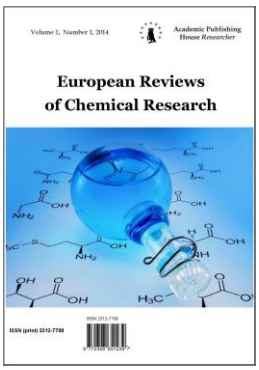
Ferngren, 2014 – *Ferngren, G. B.* (2014). Medicine and religion: A historical introduction. JHU Press.

Gupta et al., 2014 – *Gupta, P., Sharma, V. K., Sharma, S.* (2014). Healing Traditions of the Northwestern Himalayas. Springer.

Hiwale, 2015 – *Hiwale, S.* (2015). Sustainable horticulture in semiarid dry lands. Springer.

Nath, Jain, 2015 – *Nath M., Jain, S. K.* (2015). As in Today's Ethnomedicinal Perspective. Some Medicinal Plants of Indian *Purā Indian Journal of History of Science*, 50, 196-207.

Shabbir et al., 2013 – *Shabbir, M., Khan, M. R., Saeed, N.* (2013). Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. *BMC complementary and alternative medicine*, 13(1), 143.



Published in the Slovak Republic
European Reviews of Chemical Research
Has been issued since 2014.
ISSN: 2312-7708
E-ISSN: 2413-7243
2017, 4(2): 46-51

DOI: 10.13187/ercr.2017.2.46
www.ejournal14.com

Chemical Characterisation of Scrap Brass for Jewellery Making

Ruth Joel [a], Enoch G. Wuritka [a], Aje Tokan [a], Atuman S. Joel [a]

[a] Abubakar Tafawa Balewa University, Bauchi State, Nigeria

Abstract

Determination of micro element of unmodified scrap brass samples gotten from various locations in Gombe metropolis through random sampling techniques was undertaken, to help in analysing the properties needed in selecting brass scrap for jewelry making. Quantitative experimental design was adapted to know the element composition of the brass scraps. Six samples of brass scraps were collected and atomic absorption spectrometer Model AA0904M046 was used to determine the content of each element found in the scraps in part per millions. All the samples collected were found to be brass due to the percentage composition of copper and zinc present in the alloys. All the six samples have different content which makes them differ from one another. The study shows that the suitability of brass scraps for jewelry making depend on its alloying element. Sample 1 (tap heads) and 2 (car parts) are suitable for jewelry making by increasing the amount of Copper content to balance the percentage content of Iron, while samples 3 (ornaments), 4 (trumpet), 5 (fuel pipes) and 6 (dishes) can be use directly alone without modification. Finally, this study can help local jewelry producer to select suitable scrap brasses for jewelry making thereby converting waste into wealth.

Keywords: jewelry making, Scrap brass, atomic absorption spectrometer, waste, micro element.

1. Introduction

Scrap brass demonstrated high potential for use in jewelries making more especially in the case of small scale jewelry industries that mostly depend on recycled scrap metals for jewelry production (Den Besten, 2011). Small scale jewelry industries dependence on recycled metals can be linked to the difficulties encountered in mining process and the economic benefit associated in using it. Small scale jeweller gathers different types of brass not minding the elemental content in the scraps metal which may alter the quality of the jewelry produced either positively or negatively and in some cases, may result in allergic reaction to the body. Jewelries production will serve as a source of an income to the producers and revenue to the government, since jewelry is personal adornment worn to enhance the beauty of the wearer. Almost all women are using jewelries in different ways such as brasseletes, earings, bangles, neckless (Den Besten, 2011). Therefore, the use of brass scrap for jewellery production will help in bursting the economy of nations more especially the developing nations like Nigeria.

Brass is a metal compose primarily of properties including strength, machinability and wear resistance. It colour varies depending on the amount of Zinc present, the more the Zinc the lighter the colour (CDA, 2005). Brass scraps are off cut of manufacturing left-over and other materials that have reach their end of life. Brass types are classified based on its colour or its intended use. For instance, red brass contains 15 % Zinc and has reddish colour while yellow Brass consist of

35 % Zinc and has a yellowish colour (Brady et al., 1997; Terence, 2016). Alpha brasses contain a minimum of 63 % of Copper and gilding Brass contain 80–90 % copper which matched gold in colour (Helmenstine, 2009). CDA (2005) recommended that gilding Brass containing 80 % to 90 % Copper is use for jewelry purposes. Hong et al. (2014) stated that gilding Brass has rich golden colour with best combination of strong ductility and corrosion resistance.

Elemental analysis of most organic and inorganic matrices requires the partial or total dissolution of the sample prior to instrumental analysis (Oliveira, 2003). Only a few direct methods allow the introduction of the sample without any preparation and in these cases lack of reliable calibration is the major problem (Oliveira, 2003). Sample preparation allows the separation and/or pre-concentration of analytes and makes possible the use of several determination methods as reported in Oliveira, (2003). In this study, wet decomposition method was adapted because of the nature of the sample used and Spectrometry is a method that requires sample preparation (Beaty, 1988; Oliveira, 2003; Welz, Sperling, 2008)

The findings from this study will help jewellery makers improve their decision making in selecting the best brass scrap metal for jewellery production. Also, it will improve the standard of jewelleries and prevent use of inappropriate metals on the body of the wearer. Finally, money value will be improved by converting waste into wealth.

2. Materials and method

The materials used for the analysis were Taps head, Car parts such as bracket, gears and key bowls, Ornament, Trumpets, Car pipes such as fuel pipes, wire pipes and water pipes and Dishes.

Method

This study adopted qualitative research technique to interpret and explained the chemical composition of brass scrap through laboratory experiment. The procedure involves collection of samples of brass scrap metal which is then characterized using atomic absorption spectrometer (AAS) model AA0904M046 to know the alloying content of the six samples.

Study area

The area of the study was Gombe metropolis in Gombe state of Nigeria

Sample collection

Random sampling of six different brass scrap was sorted out from scrap metal scavengers and at refuse dump through on-site pickups in Gombe metropolis. The samples were then sorted into six different samples 1, 2, 3, 4, 5 and 6 shown in Figures 1, 2 and 3.

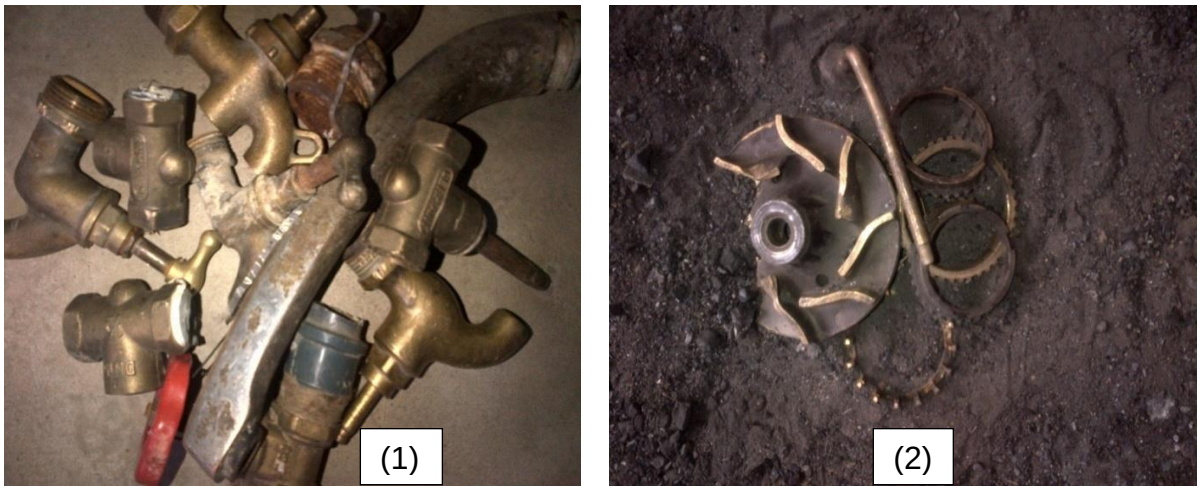


Fig. 1. Samples (1) Tap Heads (2) Car parts i.e. gears, bracket and key bowls

Fig. 2. Samples (3) Ornament (4) Trumpet

Fig. 3. Samples (5) Fuel pipes (6) Dishes

Sample preparation

The six sorted samples were separated and Atomic Absorption Spectrometer (AAS) model AA0904M046 was used to determine the content of each element in the scrap samples (i.e. in parts per million).

Wet digestion acid method was adopted for the analysis.

Preparation of reagent: The Nitric acid and Perchloric acid were both mixed together in a proportion of 3:1 (i.e. 300 mls to 100 mls) and then properly shaken.

Digestion procedure: 1 gram and less than 1 gram of the various samples were cut using Harksaw and put into a Digested flask (100 mls Cornical flask) and to each flask, 30 mls of the mixed acid was added and the flask and its contents was placed on a digested block at 25 ^{o}C for about 2 hours for digestion to take place.

After the digestion was completed, the digest was allowed 5–10 minutes to cool down. The volume of the digest was further increased to 100 mls using distilled water and properly shaken.

The micro elements were then determined from the digest using the AAS.

3. Results

Atomic absorption spectrometer was used to determine the elements in the samples as presented in Figures 4, 5 and 6.

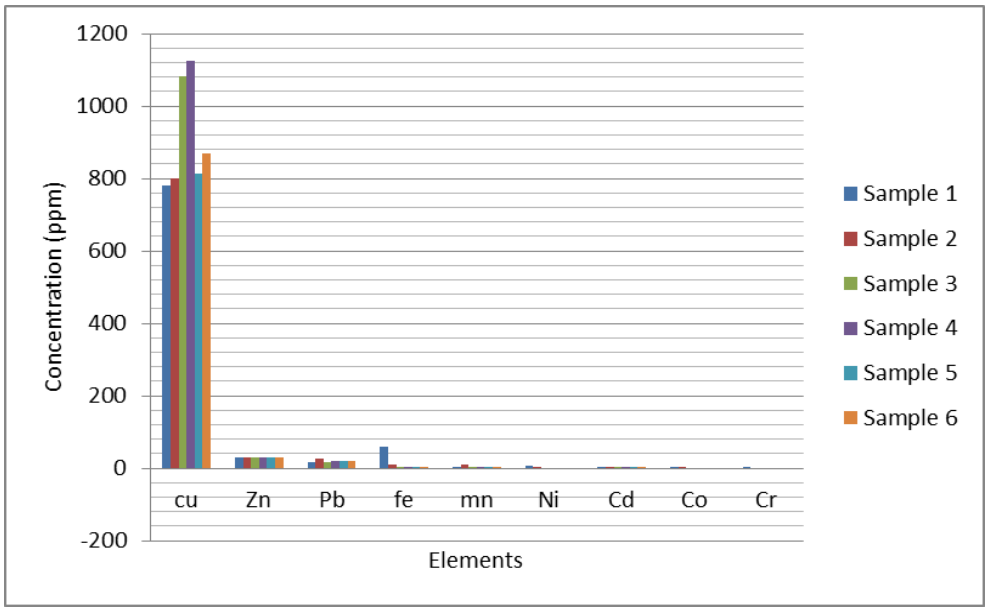


Fig. 4. Graph of sample element concentration

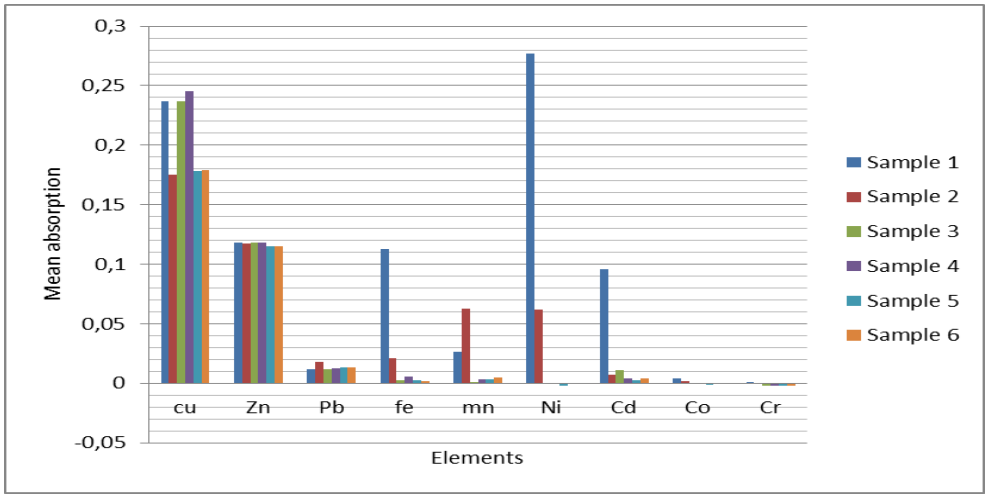


Fig. 5. Graph of sample mean absorption

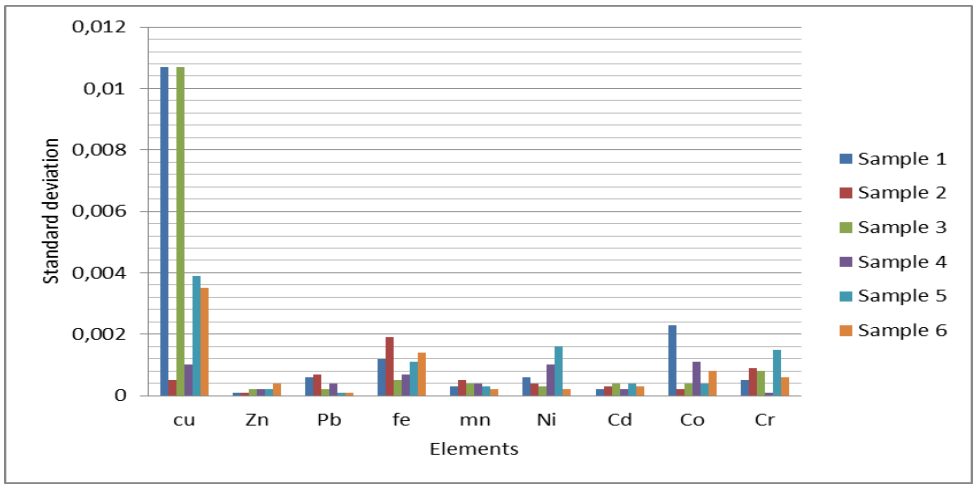


Fig. 6. Graph of sample standard deviation

3. Discussion

Brass scrap samples collected for the analysis and production contains some element with different concentration in parts per million (ppm). Analysing Sample 1, Figure 1 (Tap heads) using the AAS it was found that it contains 782.286 ppm Cu, 29.756 ppm Zn, 18.38 ppm Pb, 58.328 ppm Fe, 0.225 ppm Mn, 8.495 ppm Ni, 0.406 ppm Cd, 0.088 ppm Co, 0.055ppm Cr. In this sample, it was found that Copper is the major element found in the brass scrap, this agrees with CDA (2004), Brady et al. (1997) and Reheren (1999). The amount of iron was high which makes the metal to be too strong for cold work during production of the jewellery and the colour was doll yellowish. Also, Nickel was present in high percentage which makes its support load without breakage (CDA, 2004; Hussain, Ibrahim, 2014; Dungworth, 1997).

Cadmium and Chromium was present to improve corrosion resistance of the metal. Stromeyer (2010) and CDA (2005) works showed that Cadmium provide excellent protection from highly corrosive chemicals like acid and base. Also, Cobalt was present to improve the colour of the brass.

Lead was present which enhance the machinability, lower melting temperature of the metal, facilitate chip fracture and reduce tool wear of the metal for casting (Eco Metals Recyclers, 2014; CDA 2005; Dungworth, 1997; Hussein and Ibrahim, 2014). The lead level must be maintained at certain level because once it is high it can lead to lead poisoning more especially in children. Sample 1 was found to be the strongest and heaviest brass scrap among the samples analysed.

Sample 2, (Figure 1 Sample (2) (car parts)), the major elements are Copper 800.527 ppm and Zinc 29.504 ppm. Iron and manganese were present in almost same proportion, 10.815 Fe and 110.895 Mn these improve the strength and compactability of the metal which makes it not to break easily (CDA, 2004). Lead was present in high percentage to improve the machinability which makes the casting of the jewelleries to look smooth more than how it looks in Sample 1, but the high lead concentration may cause lead poisoning. The colour of the sample looks yellowish but darker than that of Sample 1 because the percentage of Copper is higher than that of Sample 1.

Sample 3 in Figure 2 (Ornament) consist of Copper and Zinc as the major elements present with concentration of 1082.683 ppm Cu and 29.756 ppm Zn. In the sample, the amount of Iron and Manganese is small which makes the alloy to be softer than Samples 1 and 2. This makes it easier to be cold working during production of the jewelleries. Lead was present to improve the machinability of the metal that allowed the metal to be melted and cast easily. Cadmium was present to improve corrosion resistance to the brass.

After analysing sample 4 Figure 2 (Trumpet) it was found that Copper has the highest percentage which is 1123.584 ppm Cu, which makes the metal to be softer, but the amount of iron was higher than that of sample 3, 5, and 6 that makes sample 4 to be stronger. The presence of Manganese and Iron improves the strength of the brass scrap this make's the sample to be malleable during production. Both casting and cold working can be used during production of the jewelleries. Cadmium was present to improve resistance to corrosion and cobalt to improve its colour.

Sample 5 Figure 3 (Fuel pipes) was analysed. Copper and Zinc was found to be the major alloying element and that prove that the scrap was brass scrap. Lead was present to improve the machinability in during casting. Iron and manganese was present in small amount, this makes the metal to be soft and easy for cold working. Cadmium was present to improve resistance to corrosion. There was no presence of cobalt and chromium in the sample. Due to low percentage of Zinc in Sample 5, the colour was found to be richer than sample 3 and 4.

In Sample 6 Figure 3 (Dishes) Copper and Zinc are the major elements. Lead was present to improve the machinability. Iron and manganese were present in small quantities making Sample 6 softer than all the samples selected. The colour of sample 6 is reddish and its glittering colour led to the production of fine smooth jewelleries in different design.

Copper and Zinc are the major alloying element of brass as presented in Figure 4. Iron, Manganese and Nickel present in the alloy, improves the strength. Also, Cadmium and Chromium serve as corrosion resistance and Cobalt improves the colour of the metal. That was why Cobalt was found only in Sample 1 which has high amount of Iron.

Figures 5 and 6 represent the mean absorbance and standard deviation of the elements analysed respectively. Each sample absorbance is measure five times and the mean and standard deviation is displayed and recorded.

In all the six samples collected the concentration of Lead, Chromium, Cadmium and Nickel did not exceed the Adult Jewelry Safety Standard ASTM F2999 2013 which stated that Lead

content should not exceed 600 ppm, Chromium 60 ppm, Cadmium 75 ppm and Nickel 1500.14 ppm. This proves that the samples are allergy free, unless if they corrode.

4. Conclusion

Elemental analysis of brass scrap samples was successful carried out in the study. The result of the study shows that good quality of brass scraps is directly proportional to its alloying element composition.

The study showed that not all the 6 brass scrap samples will be used for jewellery in the unmodified form. Samples 3, (ornament) 4, (trumpet) 5 (fuel pipes) and 6 (dishes) possesses all the necessary characteristics for brass jewellery making, while samples 1 (Tap heads) and 2 (car parts) are not due to their high iron and manganese content. Hence it is necessary to modified the two samples by using additives such as Copper, Chromium and Cadmium thereby reducing the percentage composition of Iron and Manganese present. Samples 3, 4, 5 and 6 can be used directly for jewellery making without additives according to ASTM 22500 standard. Sample 2 has the highest percentage composition of lead among all the samples considered. Therefore, there is a need to monitor it lead poisoning potential especially if the jewelleries will be used on children. Finally, this study will help small scale jewellers in selecting suitable scrap brasses for jewellery making.

References

Beaty, 1988 – *Beaty, R. B.* (1988). Concepts, Instrumentation, and Techniques in Atomic Absorption Spectrophotometry, Perkin-Elmer.

Brady et al., 1997 – *Brady, G. S., Clauser, H. H. and Vaccari, J. A.* (1997). "Brass" In Material Handbook, 14th ed. New York: McGraw-Hill. Available at: https://accessengineeringlibrary. com/browse/ materials-handbook-an-encyclopedia-for-managers-technical-professionals-purchasing-and-production-managers-technicians-and-supervisors-fifteenth-edition (Accessed: 04.12.16).

CDA, 2004 – Copper Development Association (CDA) (2004). Brass in focus. CDA publication No. 177. Available at: http://www.copperalliance.org.uk/docs/librariesprovider5/ resources/pub-177-brass-in-focus-pdf.pdf?Status=Master&sfvrsn=0 (Accessed: 10.12.14).

CDA, 2005 – Copper Development Association (CDA) (2005). The Brasses properties & applications. CDA publication No. 117. Available at: http://www.copperalliance.org.uk/docs/ librariesprovider5/resources/pub-117---the-brasses_whole_web-pdf.pdf?sfvrsn=0 (Accessed: 05.01.15).

Den Besten, 2011 – *Den Besten, L.* (2011). On jewellery: a compendium of international contemporary art jewellery. Stuttgart: Arnoldsche.

Dungworth, 1997 – *Dungworth, D.* (1997). Roman Copper Alloys: Analysis of Artefacts from Northern Britain. *Journal of Archaeological Science,* 24(10): 901-910.

Eco Metals Recyclers, 2014 – Eco Metal Recyclers. Brass Scrap Types. Retrieved November 16, 2014. Available at: http://www.ecometalrecyclers.com.au/brass.html

Helmenstine, 2009 – *Helmenstine, A. M.* (2009). Brass Alloys. Available at: http://chemistry.about.com/od/alloys/a/Brass-Alloys.htm (Accessed: 14.12.14)

Hong et al., 2014 – *Hong, H. L., Wang, Q. and Liaw, P. K.* (2014) Understanding the cu-zn alloy. Scientific Report Article number 7065. Available at: http://www.dx.doi.org/101155/2014 909506: (Accessed: 12.05.15)

Hussain, Ibrahim, 2014 – *Hussain, R. M and Ibrahim, O.* (2014) Influence of Al and Tin addition on micro structure and mechanical properties of leaded brass. available at: www.http/hindawa.com/journals/1jms 2014/ 909506 (Accessed: 8.05.15).

Oliveira, 2003 – *Oliveira, E.* (2003) Sample Preparation for Atomic Spectroscopy: Evolution and Future Trends, *J. Braz. Chem. Soc.,* 14(2), 174-182,

Reheren, 1999 – *Reheren, T.* (1999). Small Size, Large Scale Roman Brass Production in Germania Inferior. *Journal of Archaeological Science,* 1083-1087.

Stromeyer, 2010 – *Stromeyer, F.* (2010). The fact on cadmium. National institute of environmental health science super fund research programme. Available at: http://www.Dartmouthedu/toxmetal/toxic-metal/moremetal (Accessed: 18.10.16)

Terence, 2016 – *Terence, B.* (2016). The history of brass from coins to modern ammunition. Available at: https://wwwthebalance.com/the-history-of-brass-2340176 (Accessed: 08.08.16)

Welz, Sperling, 2008 – *Welz, B., Sperling, M.* (2008). *Atomic absorption spectrometry. John Wiley & Sons.*

Published in the Slovak Republic
European Reviews of Chemical Research
Has been issued since 2014.
ISSN: 2312-7708
E-ISSN: 2413-7243
2017, 4(2): 52-65

DOI: 10.13187/ercr.2017.2.52
www.ejournal14.com

Synthesis and Studies on New Dimethine and Tetramethine Cyanine Dyes

H.A. Shindy [a, *], A.K. Khalafalla [a], M.M. Goma [a], A.H. Eed [a]

[a] Department of Chemistry, Faculty of Science, Aswan University, Aswan 81528, Egypt

Abstract

New dimethine cyanine dyes and tetramethine cyanine dyes having the nucleus of furo[(3,2-d; ($\bar{3},\bar{2}$-d)-bis pyrazole] were prepared. The electronic visible absorption spectra of all the synthesized cyanine dyes were investigated in 95 % ethanol solution to evaluate their spectral sensitization properties. Solvatochromism for some selected dyes were examined in pure solvents having different polarities [Water (78.54), Dimethylformamide (36.70), Ethanol (24.3), Chloroform (4.806), Carbontetrachloride (2.238) and Dioxane (2.209)] to evaluate their solvatochromic properties. Halochromism (acid-base properties) of some chosen dyes were measured in aqueous universal buffer solutions having varied pH values (1.75, 2.45, 4.65, 5.80, 7.88, 8.75, 10.58 and 12.60 units) to evaluate their halochromic characterization. Structural determination were carried out through elemental analysis, visible spectra, mass spectrometer, IR and ^{1}H NMR spectral data.

Keyword: synthesis, cyanine dyes, solvatochromism, halochromism, visible spectra, methine cyanine dyes.

1. Introduction

Enhanced attention has been focused on the chemistry of cyanine dyes (Arjonat et al., 2016; Shindy et. al., 2012; Zhang et al., 2008; Park et al., 2013; Shindy et al., 2016; Deligeorgiev et al., 2007; Keisar et al., 2014; Soriano et al., 2016; Takasu et al., 2006; Park et al., 2013; Zhao et al., 2013; Shindy et al., 2016a; Shershof et al., 2013; Wang, Kim 2009; Komljenovic et al., 2016). This is because the multiplicity uses and applications of cyanine dyes in a diverse and a broad area (Liu et al., 2011; Owens et al., 2014; Matsuoka, 1990; Ansari et al., 2014; Shindy et al., 2014; Shindy, 2014; Shindy et al., 2014a; Shindy, 2015; Shindy, 2015a; Shindy et al., 2015; Shindy, 2016; Zhang et al., 2016; Chen et al., 2016). Their uses and applications includes but not limited to photographic sensitizers for silver halide emulsion in manufacturing technology of photosensitive material industry, photosensitizers for solar cells material, in modern optical technologies, photoconducting media, solvatochromic and halochromic probes, at diagnostics and treatment of cytological abnormalities, in fluorescent marker technology, in photorefractive media, information storage, optical disks as recording media, and in laser technology.

Taking in accounts and consideration the above applications and uses of cyanine dyes we prepared here new photosensitizers, solvatochromic and halochromic cyanine dyes as new synthesis contribution and spectroscopic investigation in this field and/or to may be used and/or

* Corresponding author
E-mail addresses: hashindy2@hotmail.com (H.A. Shindy)

applied in any of the metioned uses and application of cyanine dyes particularly as photographic sensitizer for silver halide emulsion in photographic industry, as probes for determining solvent polarity in physical, physical organic and/or inorganic chemistry and as pH indicators in operations of acid/base titration in analytical chemistry.

2. Results and discussion:

2.1. Synthesis

Selenium dioxide oxidation of 4, 5-dimethyl-2, 7-diphenyl-furo [(3, 2-d), ($\bar{3},\bar{2}$-d)-bis pyrazole] (1) (Shindy et al., 2017) in 2:1 molar ratios, in dioxane as organic solvent achieved the 4,5-dicarbaldehyde compound (2), Scheme (1), Table (1). Further reaction of the 4,5-dicarbaldehyde compound (2) with iodoethane quaternary salts of α-picoline, quinaldine and/or γ-picoline in 1:2 molar ratios and in ethanol containing few mls of piperidine resulted the 4,5[(2(4)]-bis dimethine cyanine dyes (3a-c), Scheme (1), Table (1).

Reaction of the 4,5-dicarbaldehyde compound (2) with acyl and/or substituted acyl such as acetaldehyde, acetone, acetophenone p-methoxyacetophenone, or p-nitroacetophenone, in 1:2 molar ratios, in ethanol containing piperidine gives the 4,5 (2)-bis (acyl ethenyl) as intermediate compounds (4a-e), Scheme (1), Table (2). Subsequent reactions of the intermediate compounds (4a-e) with 1-ethyl-2-methyl-quinolinium iodide quaternary salt in 1:2 molar ratios, in ethanol and in presence of few mls of piperidine produced the 4,5(2)-bis tetramethine cyanine dyes (5a-e), Scheme (1), Table (2).

The structures of the prepared compounds were identified through elemental analysis, visible spectra Tables (1, 2), mass spectrometer, IR (Wade, 1999) and ^{1}H NMR (Wade 1999a) spectral data, Table (3).

2.2. Spectral studies

Spectral sensitization evaluation studies for all the synthesized cyanine dyes were carried out through investigating their electronic visible absorption spectra in 95 % ethanol solution. The dyes were thought to be better spectral sensitizers when they absorb the visible light to initiate the electronic transitions at higher wavelength bands (bathochromic shifted and/or red shifted dyes). Consequently, the spectral sensitization of the dyes decreases when they absorb the visible light to initiate the electronic transitions at lower wavelength bands (hypsochromic shifted and/or blue shifted dyes). So, we may say that the spectral sensitization of one dye is lower than the other one if the wavelength of the maximum absorption spectrum of the former one is shorter than that of the latter one. In contrary, we may say that the spectral sensitization of one dye is higher than the other one if the wavelength of the maximum absorption spectrum of the former one is longer than that of the latter one. Spectral sensitization evaluation study is very important in the case of cyanine dyes because the extensive uses of these dyes in photographic industry to increase the sensitivity range of silver halide emulsion by making an increase in the range of wavelength which form an image on the film.

The electronic visible absorption spectra of the 4,5-[2(4)] bis dimethine cyanine dyes (3a-c) in 95 % ethanol solution discloses bands in the visible region (440-540 nm). The positions of these bands and their molar extinction coefficients are remarkably effected by the types of the heterocyclic quaternary salts residue (A) and their linkage positions. So, substituting A=1-ethyl-pyridinium-2-yl salt by A=1-ethyl quinolinium-2-yl salt, transferring from dye (3a) to dye (3b) causes bathochromic shifts for absorption bands by (20 nm), Table (1). This can be attributed to increasing π-delocalization conjugation in the latter dye (3b) due to the presence of the additional benzene ring system in quinoline nucleus in correspondence to pyridine nucleus system in the former dye (3a). Changing the linkage position from 2-yi salt residue to 4-yl salt residue moving from dye (3a) to dye (3c) resulted bathochromic shifts for the absorption bands by (15 nm), Table (1). This can be related to increasing the length of the π-delocalization conjugation in the latter dye (3c) due to the presence of γ-picoline nucleus in correspondence to the α-picoline nucleus in the former dye (3a).

Additionally, the electronic visible absorption spectra of the 4,5(2)-bis tetramethine cyanine dyes (5a-e) in 95 % ethanol solution reveals displacements to give bathochromic and/or

hypsochromic shifted bands depending upon the nature of the substituents in the diene side chain (R). So, substituted R = H in dye (5a) by R = CH_3 and/or R = Ph to get dyes (5b) and/or (5c) causes strong red shifts for the absorption bands by (70 nm) and/or (50 nm), respectively (Table 2). This can be related to the electron donating character of the methyl group in dye (5b) and/or increasing conjugation in the dye (5c) due to the presence of phenyl ring system. Substituting R = C_6H_5 in dye (5c) by R = C_6H_4, p.OCH_3 and/or R = C_6H_4.pNO_2 to get dyes (5d) and/or (5c), produced bathochromic shifts and/or hypsochromic shifts for the absorption bands by (30 nm) and/or (10 nm), respectively, Table (2). This can be attributed to the strong electron donating character of the methoxy group in dye (5c) and the strong electron attracting character of the nitro group in dye (5c). Electron donating groups facilitate and increase the intensity of the electronic charge transfer to the quaternary quinolinium salt residue by pushing electrons and consequently red shifts occurs. Inversely electron attracting groups makes difficult and decreases the intensity of the electronic charge transfer to the quaternary quinolinium salt residue by pulling electrons and accordingly blue shifts occurs.

General comparison between the electronic visible absorption spectra of the dimethine cyanine dyes (3a-c) and the tetramethine cyanine dyes (5a-e) reveals that the latter dyes have red shifted bands than the former dyes, Tables (1, 2). This can be illustrated in the light of the increasing of the number of methine groups in the latter dyes, Scheme (1).

From this study we could conclude that:

The electronic visible absorption spectra of the 4,5[2(4)]-bis dimethine cyanine dyes (3a-c) and the 4,5(2)-bis tetramethine cyanine dyes (5a-e) underwent displacements to give bathochromic and/or hypsochromic absorption bands accompanied with increasing and/or decreasing the number and the intensity of the absorption bands depending upon the following factors:

a-Types of the heterocyclic quaternary salt residue in the order of:
quinaldine dyes > α-picoline dyes (for the dimethine cyanine dyes).
b-Linkage positions of the hetwerocyclic quaternary salts in the order of:
γ-picoline dyes > α-picoline dyes (for the dimethine cyanine dyes).
c-Nature of the substituents (R) in the diene side chain in the order of:
(I) CH_3 dyes > Ph dyes > H-dyes (for the tetramethine cyanine dyes).
(II) C_6H_4.pOCH_3 dyes > C_6H_5-dyes > C_6H_4.pNO_2 dyes (for the tetramethine cyanine dyes).
d-Increasing the number of the methine units in the order of:
tetramethine cyanine dyes > dimethine cyanine dyes.

2.3. Solvatochromic studies

Solvatochromic evaluation studies for some selected synthesized cyanine dyes (3b) and (5a) was carried out via examining of their electronic visible absorption spectra in pure solvents having different polarities. The dyes are thought to be better solvatochromic dyes when they give a remarkable positive solvatochromism and/or negative solvatochromism in these solvents. Positive solvatochromism reveals bathochromic shifted (red shifted) absorption bands with increasing solvent polarity. Inversely, negative solvatochromism discloses hypsochromic shifted (blue shifted) bands with increasing solvent polarity. This study was carried out to select the best solvents to use of these dyes as photosensitizers when they are used and/or applied in photosensitive material industry. The other important purpose of this study is to evaluate the solvatochromic properties of these dyes to may be use and/or applied as probes for determining solvent polarity in physical, physical organic and/or inorganic chemistry.

So, the electronic visible absorption spectra of the dimethine cyanine dye (3b) and the tetramethine cyanine dye (5a) in pure solvents of different polarities (different dielectric constant) namely water (78.54), DMF (36.70), ethanol (24.3), chloroform (4.806), carbontetrachloride (2.238) and dioxane (2.209) (Shindy, et al., 2014; Shindy, et al., 2014a) are recorded. The λ_{max} (wavelength) and ε_{max} (molar extinction coefficients) values of the absorption bands due different electronic transitions within the solute molecule in these solvents are represented in Table (4).

From Table (4) it's clearly that the electronic visible absorption spectrum of the dyes (3b) and (5a) in ethanolic medium are characterized by the presence of two (dye 3b) and/or four (dye 5a) essential absorption bands. These bands can be assigned to intermolecular charge transfer transition (Shindy et al., 2014; Shindy et al., 2014a). These charge transfer is due to transfer of lone

pair of electrons from the N-phenyl pyrazole nitrogen atom to the positively charged quaternary nitrogen atom of the quinolinium salt residue, Scheme (2).

The data given in Table (4) show that the charge transfer band exhibits a hypsochromic shift in ethanol relative to DMF, dioxane, chloroform and carbontetrachloride. These effects may be attributed to the following factors:

a-The bathochromic shift in DMF relative to ethanol is a result of the increase in solvent polarity due to the increasing of dielectric constant of DMF relative to ethanol.

b-The hypsochromic shift occurs in ethanol relative to dioxane, chloroform and carbontetrachloride is a result of the solute solvent interaction through intermolecular hydrogen band formation between ethanol and the lone pair electrons of the N-phenyl pyrazole nitrogen atom, Scheme (3) (A). This decreases slightly the electron density on the N-phenyl pyrazole nitrogen atom and consequently decreases to some extent the mobility of the attached π-electrons over the conjugated system pathway to the positively charged quaternary nitrogen atom of the quinolinium salt residue, and consequently a hypsochromic shift occurs.

Also, from the data given in Table (4) it is observed that occurrence of unexpected hypsochromic shifts in water relative to ethanol and the other solvents. This can be mainly ascribed to the possible interaction of water molecules with the lone pair electrons of the N-phenyl pyrazole nitrogen atom forming intermolecular hydrogen band, Scheme (3) (B). This makes difficult the transfer of electronic charge from the N-phenyl pyrazole nitrogen atom to the quaternary nitrogen atom of the heterocyclic quinolinium salt residue, and accordingly there is observed a hypsochromic shift in water relative to ethanol and the other solvents.

From this investigation we can conclude that:

The solvatochromism of the examined cyanine dyes (3b) and (5a) in pure solvents having different polarities underwent displacements to give positive solvatochromism (occurrence of a bathochromic shift with increasing solvent polarity) and/or negative solvatochromism (occurrence of a hypsochromic shift with increasing solvent polarity) depending upon the following factors:

a. Increasing and/or decreasing the polarity (dielectric constant) of the solvents (General solvent effect).

b. Hydrogen bond and/or molecular complex formation between the solute (dyes molecules) and the solvent used (specific solvent effect).

2.4. Halochromic studies (acid-base properties)

Halochromic evaluation studies for some selected synthesized cyanine dyes (3b) and (5a) was carried out by investigating of their electronic visible absorption spectra in aqueous universal buffer solutions having varied pH values, Table (5). The dyes are though to be better halochromic dyes when they give a noticeable positive halochromism and/or negative halochromism in these buffer solutions. Positive halochromism means occurrence of a bathochromic shifted (red shifted) absorption bands with changing solution pH of the buffer solution. In contrast negative halochromism means occurrence of a hypsochromic shifted (blue shifted) absorption bands with changing the pH of the buffer solution.

The solutions of the bis dimethine cyanine dye (3b) and the bis tetramethine cyanine dye (5a) have a permanent cationic charge in basic media that then discharged on acidification. This prompted and encouraged us to study their spectral behaviour in different buffer solutions in order to select a suitable pH for use of these dyes as photosensitizers. The other important purpose of this study is to evaluate the halochromic properties of these dyes to may be used and/or applied as pH indicators in operations of acid / base titration in analytical chemistry. The acid dissociation or protonation constant of these dyes have been determined. The effect of the compounds as photosensitizers increases when they are present in the ionic form, which has a higher planarity (Shindy, et al., 2014; Shindy, et al., 2014a) and therefore more conjugation.

So, the electronic visible absorption spectra of the bis dimethine cyanine dye (3b) and the bis tetramethine cyanine dye (5a) in aqueous universal buffer solutions of varying pH values (1.75, 2.45, 4.65, 5.80, 7.88, 8.75, 10.58 and 12.60 units) showed bathochromic shifted bands at high pH media (alkaline media) and hypsochromic shifted bands at low pH media (acidic media). So, the mentioned dyes which has lone pair of electrons on the N-phenyl pyrazole nitrogen atom undergoes to protonation in low pH media (acidic media). This leads to a criterion of positive

charge on the N-phenyl pyrazole nitrogen atom and consequently the electronic charge transfer pathways to the quaternary heterocyclic quinolinium salt residue will be difficult resulting in a hypsochromic shift for the absorption bands (protonated and/or colourless-yellow structure), Scheme (4) (A).

On increasing the pH of the media, the absorption bands are bathochromically shifted due to the deprotonation of the N-phenyl pyrazole nitrogen atom, and consequently the electronic charge transfer pathways to the quaternary heterocyclic quinolinium salt residue will be easier and facilitated resulting in a bathochromic shift for the absorption bands (deprootonated and/or coloured structure), Scheme (4) (B).

Several methods have been developed for the spectrophotometric determination of the dissociation constants of weak acids. The variation of absorbance with pH can be utilized. On plotting the absorbance at fixed λ vs. pH, S-shaped curves are obtained, Table (6). An all of the S-shaped curves obtained the horizontal portion to the left corresponds to the acidic form of the indicator, while the upper portion to the right corresponds to the basic form of the indicator, since the pka is defined as the pH value for which one half of the indicator is in the basic form and the other half is in the acidic form. This point is determined by intersection of the curve with a horizontal line midway between the left and right segments (Shindy et al., 2014; Shindy et al., 2014a). The acid dissociation or protonation constants values of the dyes (3b) and (5a) are given in Table (6).

From this examination we could conclude that:

The halochromism of the bis dimethine cyanine dye (3b) and the bis tetramethine cyanine dye (5a) in aqueous universal buffer solutions having varying pH values underwent to give the following displacements changes in their absorption spectra wavelength bands:

a-Hypsochromic shifted bands in the lower pH media (acidic media) due to the protonated and/or colourless structures of the dyes in this media.

b-Bathochromic shifted bands in higher pH media (basic media) due to the deprotonated and/or coloured structures of these dyes in this media.

3. Conclusion

1. The intensity of the colours of the 4,5-[2(4)]-bis dimethine cyanine dyes (3a-c) and the 4,5(2)-bis tetramethine cyanine dyes (5a-e) can be explained in the light of the two suggested mesomeric structures (A) and (B) producing a delocalized positive charge over the conjugated system, Scheme (2).

2. These cyanine dyes can be used as:

a. Photographic sensitizers in photosensitive material industry due to their spectral and/or photosensitization properties,

b. Indicators for solvent polarity in physical, physical organic and/or inorganic chemistry due to their solvatochromic properties and

c. Indicators in operations of acid-base titrations in analytical chemistry due to their halochromic properties.

3. Because cyanine dyes have multiplicity uses and applications in various fields of science, technology, engineering, pharmacology and medicine, this research paper might be very interesting and useful for the large heterogenous community groups of chemists, biologists, physicists, biotechnologists, pharmacologists and medical scientists.

4. Experimental

4.1. General

All the melting points of the prepared compounds are measured using Electrothermal 15V, 45W 1 A9100 melting point apparatus, Chemistry department, Faculty of Science (Aswan University) and are uncorrected. Elemental analysis were carried out at the Microanalytical Center of Cairo University by an automatic analyzer (Vario EL III Germany). Infrared spectra were measured with a FT/IR (4100 Jasco Japan), Cairo University. ^{1}H NMR Spectra were accomplished using Varian Gemini-300 MHz NMR Spectrometer (Cairo University). Mass Spectroscopy was recorded on Mas 1: GC-2010 Shimadzu Spectrometer (Cairo University). Electronic visible absorption spectra were carried out on Visible Spectrophotometer, Spectro 24 RS Labomed, INC, Chemistry department, Faculty of Science (Aswan University).

4.2. Synthesis

4.2.1-Synthesis of 4, 5-diformyl-2, 7-diphenyl-furo [(3, 2-d), ($\bar{3}$,$\bar{2}$-d)-bis pyrazole] (2)

A pure crystallized sample of (1) (0.01 mol) and selenium dioxide (0.02 mol) were heated under reflux in dioxane (30 ml) for 16-18 hrs. The reaction mixtures were filtered on hot to remove selenium metal and the filtrate was cooled and precipitated by ice water mixture. The precipitated products were filtered, dried, collected, and crystallized from ethanol. To give the 4,5-dicarbaldehyde compound (2). The data are shown in Table (1).

4.2.2-Synthesis of 2, 7- diphenyl-furo [(3, 2-d), ($\bar{3}$,$\bar{2}$-d) bis Pyrazole]- 4, 5 [2(4)]-bis- dimethine cyanine dyes (3a-c)

A mixtures of unimolar ratios (0.01 mol) of compound (2) and bimolar ratios (0.02 mol) of N-iodoethane quaternary salts of α-picoline, quinaldine, γ- picoline were heated under reflux in ethanol (30 ml) containing few mls of piperidine (1-2 mls) for 4-5 hrs. The reaction mixtures which attained colours from deep brown to deep violet at the end of refluxing was filtered while hot to remove any impurities, cooled and precipitated by dilution with ice water mixture. The precipitated products was filtered, washed with water several times, dried, collected and crystallized from ethanol. The data are summarized in Table (1)

4.2.3-Synthesis of 2, 7- diphenyl-furo [(3, 2-d), ($\bar{3}$,$\bar{2}$-d)-bis pyrazole]-4, 5-bis (acyl ethenyl) as intermediate compounds (4a-e)

The 4,5-dicarbarbaldehyde compound (2) were heated under reflux with acyl and/or acyl derivatives (acetaldehyde, acetone, acetophenone, pmethoxyacetophenone, or p-nitroacetophenone) in 1:2 molar ratios in ethanol (30 ml) containing piperidine (1-2 ml) for 6 hrs. The reaction mixture, which changed from reddish colour to deep brown colour at the end of refluxing, was filtered while hot to remove any impurities, concentrated, cooled and precipitated by adding ice-water mixture to give the intermediate compounds (4a-e) which was crystallized from ethanol. The data were given in Table (2).

4.2.4-Synthesis of 2, 7- diphenyl-furo [(3, 2-d), ($\bar{3}$,$\bar{2}$-d) bis pyrazole]-4,5 (2)-bis tetramethine cyanine dyes (5a-e)

Piperidine (3-5 drops) was added to a mixture of an ethanolic solution (30 ml) of the intermediate compounds (4a- e) (0.01) and iodoethane quaternary salt quinaldine (0.02 mol). The reaction mixture was heated under reflux for 6 hrs, and attained highly violet colours at the end of refluxing. The mixture was filtered off while hot, precipitated by dilution with ice-water mixture with continues shaking. The precipitates were filtered off, washed with water several times dried and crystallized from ethanol. The data were registered in Table (2).

4.3. Visible absorption spectra

The electronic visible absorption spectra of the prepared cyanine dyes were examined in 95 % ethanol solution and recorded using 1cm Qz cell in Vis Spectrophotometer, Spectro 24RS Labomed, INC. A stock solution (1 x 10^{-3}M) of the dyes was prepared and diluted to a suitable volume in order to obtain the desired lower concentrations. The spectra were recorded immediately to eliminate as much as possible the effect of time.

4.4-Solvatochromism and halochromism

The electronic visible absorption spectra of some selected synthesized cyanine dyes were investigated in pure organic solvents of spectroscopic grade (Shindy et al., 2014; Shindy et al., 2014a) and different polarities and/or in aqueous universal buffer solutions having varying pH values and recorded using 1cm quartz cell in Vis Spectrophotometer Spectro 24 RS Labomed, INC. A stock solution (1 x 10^{-3}M) of the dyes was prepared and diluted to a suitable volume using the suitable solvent and/or the buffer solution to obtain the required lower concentrations. The spectra were recorded immediately to eliminate as much as possible the effect of time.

5. Acknowledgements

We are thankful to the Chemistry department, Faculty of Science, Aswan University, Aswan, Egypt for supporting this work.

References

Arjonat, et al., 2016 – *Arjona, A., Stolte, M., Wilerthner, F.* (2016). Angewandte Chemie, 55 (7), 2470-2473.

Shindy et. al., 2012 – *Shindy, H. A., El-Maghraby, M. A. and Eissa, F. M.* (2012). Dyes. Dyes and Pigments, 92 (3), 929-935.

Zhang, et al., 2008 – *Zhang, X. H., Wang, L. Y., Nan, Z. X., Tan, S. H., Zhang, Z. X.* (2008). Dyes and Pigments, 79 (2), 205-209.

Park, et al., 2013 – *Park, J., Kim, D., Lee, K., Kim, Y.* (2013). *Bull. korean chem.* Soc., 34 (1), 287-290.

Shindy, et al., 2016 – *Shindy, H. A., Khalafalla, A. K., Goma, M. M., Eed, A. H.* (2016). *Chemistry International*, 2 (3), 114-120.

Deligeorgiev, et al., 2007 – *Deligeorgiev, T. G., Gadjev, N. I., Vasilev, A. A., Maximova, V. A., Timcheva, I. I., Katarinopoulos, H. E.* (2007). *Dyes and Pigments*, 75 (2), 466-473.

Keisar, et al., 2014 – *Keisar, O. R., Finfer, E. K., Ferber, S., Finaro, R., Shabat, D.* (2014). *Nature Protocols*, 9 (1), 27-36.

Soriano, et al., 2016 – *Soriano, E., Holder, C., Levitz, A., Henary, M.* (2016). Molecules, 21 (1), 23-37.

Takasu, et al., 2006 – *Takasu, K., Kaiser, M., Brun, R., Ihara, M.* (2006). *Journal of Medicinal Chemistry*, 49 (15), 4795-4798.

Park, et al., 2013 – *Park, J., Kim, D., Lee, K., Kim, Y.* (2013). Bull. korean chem. Soc., 34 (1), 1-4.

Zhao, et al., 2013 – *Zhao, J., Lv, Y., Ren, H., Sun, W., Liu, Q., Fu, Y., Wang, L.* (2013). *Dyes and Pigments*, 96, 180-188.

Shindy, et al., 2016a – *Shindy, H. A., Khalafalla, A. K., Goma, M. M. and Eed, A. H.* (2016). *European Journal of Molecular Biotechnology*, 13 (3), 94-103.

Shershof, et al., 2013 – *Shershof, V. E., Spitsyn, M. A., Kuznetsova, V. E., Timofeev, E. N., Ivashkina, O. A., Abranov, I. S., Nasedkina, T. V., Zasedatelev, A. S.* (2013). *Dyes and pigments*, 97, 353-360.

Wang, Kim 2009 – *Wang, S., Kim, S.* (2009). Dyes and pigments, 314-320.

Komljenovic, et al., 2016 – *Komljenovic, D., Wiessler, M., Waldeck, W., Ehemann, V., Pipkorn, R., Shrenk, H., Debus, J. and Braun, K.* (2016). *Theranostics*, 6 (1), 131-141.

Liu, et al., 2011 – *Liu, Q., Wang, X., Wang, L. Y., Fu, Y. L., Zhang, X. H.* (2011). Dyes and Pigments, 91 (3), 370-377.

Owens, et al., 2014 – *Owens, E. A., Tawney, J .G. and Henary, M .M.* (2014). Molbank, 2014, 8, 1-8.

Matsuoka, 1990 – *Matsuoka, M.* (1990). ed., Infrared Absorbing Dyes, Plenum Press, New York.

Ansari, et al., 2014 – *Ansari, A. S., Ali, I., Haque, M. W., Khan, M. H., Narayan, B.* (2014). *Journal of Applied Chemistry*, 2 (1), 63-70.

Shindy, et al., 2014 – *Shindy, H. A., El-Maghraby, M. A., Eissa, F. M.* (2014). *European Journal of Chemistry*, 5 (3), 451-456.

Shindy, 2014 – *Shindy, H. A.* (2014). *Revue Roumaine de Chimie*, 59 (2), 117-123.

Shindy, et al., 2014a – *Shindy, H. A., El-Maghraby, M. A., Eissa, F. M.* (2014). Russ. Chem. Bull. [Izv. AN. Ser. Khim.], 707-715 (in Russian). Russ. Chem. Bull., Int. Ed., 63 (3), 707-715.

Shindy, 2015 – *Shindy, H. A.* (2015). *Revue Roumaine de Chimie*, 60 (1), 5-13.

Shindy, 2015a – *Shindy, H. A.* (2015). Organic Chemistry. *An Indian Journal*, 11 (1), 26-36.

Shindy, et al., 2015 – *Shindy, H. A., Goma, M. M., Harb, N. A.* (2015). *European Journal of Chemistry*, 6 (2), 151-156.

Shindy, 2016 – *Shindy, H. A.* (2016). *European Journal of Molecular Biotechnology*, 14 (4), 158-170.

Zhang, et al., 2016 – *Zhang, H., Niesen, B., Hack, E., Jenatasch, S., Wang, L., Veron, A. C., Makha, M., Schneider, R., Arroyo, Y., Hany, R., Nuesch, F.* (2016). *Organic Electronics*, 30, 191-199.

Chen, et al., 2016 – *Chen, H., Sun, H., Zhang, X., Sun, X., Shi, Y., Xu. S., Tang, Y.* (2016). *New J. Chem.*, 40, 1940-1943.

Shindy, et al., 2017 – Shindy, H. A., Khalafalla, A. K., Goma, M. M., Eed, A. H. (2017). *European Journal of Molecular Biotechnology*, 5 (1), 30-4.

Wade, 1999 – *Wade Jr., L. G.* (1999). Organic. Chemistry., 4th Edn., Pearson Educ. (Prentice Hall, Upper Saddle River, New Jersy 07458, USA), 500-538.

Wade, 1999a – *Wade Jr., L. G.* (1999). Organic. Chemistry., 4th Edn., Pearson Educ., (Prentice Hall, Upper Saddle River, New Jersy 07458, USA), 544-560.

Appendix

Table 1. Characterization of the prepared compounds (2) and (3a-c)

Comp. No	Nature of product			Molecular formula (M.Wt.)	Analysis %						Absorption spectra in 95 % ethanol solution	
					Calculated			Found				
	Colour	Yield (%)	M.P. (°C)		C	H	N	C	H	N	λ_{max} (nm)	ε_{max} (mole-1cm^2)
2	Red	60	130	$C_{20}H_{12}N_4O_3$ (356)	67.41	3.37	15.73	67.38	3.36	15.71	---	---
3a	Violet	45	175	$C_{36}H_{32}N_6OI_2$ (818)	52.81	3.91	10.26	52.80	3.88	10.24	440, 520	2275, 2279
3b	Deep violet	63	170	$C_{44}H_{36}N_6OI_2$ (918)	57.51	3.92	9.15	57.50	3.90	9.13	480, 540	2262, 2253
3c	Deep violet	52	180	$C_{36}H_{32}N_6OI_2$ (818)	52.81	3.91	10.26	52.80	3.88	10.24	460, 535	2242, 2251

Table 2. Characterization of the prepared compounds (4a-e) and (5a-e)

Comp. No	Nature of product			Molecular formula (M.Wt.)	Analysis %						Absorption spectra in 95 % ethanol solution	
					Calculated			Found				
	Colour	Yield (%)	M.P. (°C)		C	H	N	C	H	N	λ_{max} (nm)	ε_{max} (mole-1cm^2)
4a	Deep red	61	160	$C_{24}H_{16}N_4O_3$ (408)	70.57	3.92	13.72	70.55	3.90	13.70	---	---
4b	Deep red	50	175	$C_{26}H_{20}N_4O_3$ (436)	71.55	4.58	12.84	71.53	4.52	12.82	---	---
4c	Deep red	45	190	$C_{36}H_{24}N_4O_3$ (560)	77.14	4.28	10.00	77.12	4.25	9.98	---	---
4d	Deep red	55	195	$C_{38}H_{28}N_4O_5$ (620)	73.54	4.51	12.90	73.52	4.50	12.89	---	---
4e	Deep red	55	200	$C_{36}H_{22}N_6O_7$ (650)	66.46	3.38	12.92	66.46	3.38	12.92	---	---
5a	Deep violet	54	170	$C_{48}H_{40}N_6OI_2$ (970)	59.38	4.12	8.65	59.36	4.10	8.64	450, 520, 540, 570	2276, 2229, 2127, 1332
5b	Deep violet	51	165	$C_{50}H_{44}N_6OI_2$ (998)	60.12	4.40	8.41	60.10	4.39	8.40	600, 640	2229, 1332
5c	Deep violet	45	195	$C_{60}H_{48}N_6OI_2$ (1152)	62.50	4.16	7.29	63.36	4.35	7.64	450, 550, 570, 620	2276, 2141, 1332, 723
5d	Deep violet	55	180	$C_{62}H_{52}N_6O_3I_2$ (1182)	62.94	3.39	7.10	62.94	3.39	7.10	545, 565, 610, 650	2277, 2046, 949, 577
5e	Deep violet	55	190	$C_{60}H_{46}N_8O_5I_2$ (1212)	59.40	3.79	9.24	59.38	3.78	9.23	540, 580, 600, 610	2211, 1317, 896, 892

Table 3. IR and ^{1}H NMR (mass) spectral data of the prepared compounds (2), (3b), (4a) and (5a).

Comp. No	IR Spectrum (KBr, Cm^{-1})	^{1}H NMR Spectrum (DMSO, δ); & (Mass data)
2	687, 754 (monosubstituted phenyl). 1133 (C-O-C cyclic). 1326, 1367 (C-N). 1495, 1454 (C=N). 1621, 1593, 1547 (C=C). 1723 (CHO).	7.2-8.2 (m, 10H, aromatic). 9.2 (s, 1H, CHO). M^+: 356.16
3b	694, 755 (monosubstituted phenyl). 878, 905 (o.disubstituted phenyl). 1158, 1083, 1046 (C-O-C cyclic). 1380, 1314 (C-N) 1496, 1442 (C=N). 1627, 1597 (C=C). 2925, 2857 (quaternary salt).	0.9-2.3 (m, 6H, $2CH_3$ of quinolinium). 2.8-3.6 (m, 4H, $2CH_2$ of quinolinium). 7-8.8 (m, 26H, aromatic + heterocyclic + 4-CH=).
4a	690, 756 (monosubstituted phenyl). 1027, 1126 (C-O-C cyclic). 1366 (C-N) 1496, 1447 (C=N). 1625, 1598 (C=C). 1717 (CHO).	7.2-8.6 (m, 14H, aromatic + 4-CH=). 9.2 (s, 2H, 2CHO). M^++1: 408.65
5a	752 (monosubstituted phenyl). 876 (o.disubstituted phenyl). 1123, 1154 (C-O-C cyclic). 1382 (C-N). 1493, 1449 (C=N). 1627 (C=C). 2924, 2856 (quaternary salt).	0.9-1.7 (t, 6H, $2CH_3$ of quinolinium). 3-3.4 (m, 4H, $2CH_2$ of quinolinium). 6.6-8.2 (m, 30H, aromatic + heterocyclic + 8-CH=).

Table 4. Solvatochromism of the dyes (3b) and (5a) in pure solvents having different polarities

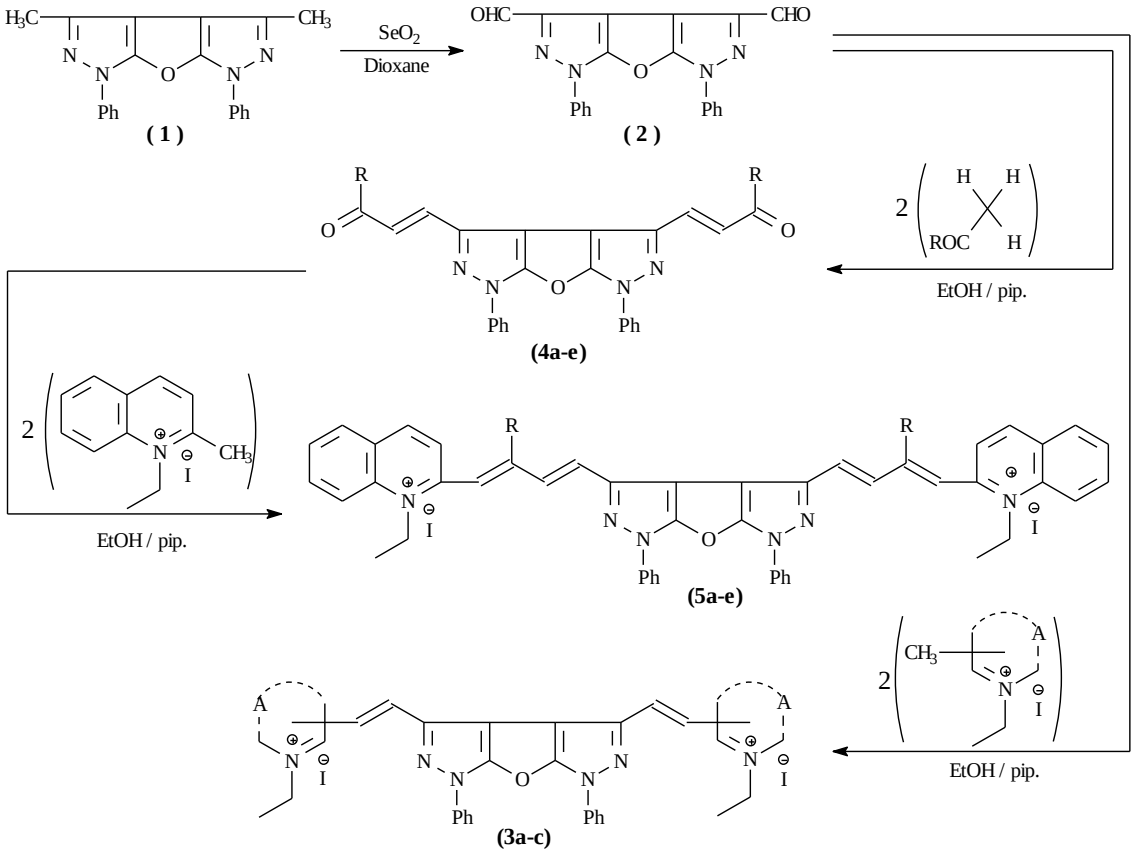
Solvent / Dye No.	H_2O		EtOH		DMF		$CHCl_3$		CCl_4		Dioxane	
	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)
3b	450 480 530	1795 2160 1922	480 540	2262 2253	425 460 580	1686 1692 1824	450 470 490 550	1655 1672 1599 1303	450 550 565	1655 1208 1281	450 550 570	1655 1270 1223
5a	450 490 510 560	1698 2071 1833 1293	450 520 540 570	2276 2229 2127 1332	420 450 480 560 580 650	935 1121 1179 880 864 396	430 480 570 635	1063 1008 891 513	420 460 480 550 640	1595 1602 1806 1835 774	430 460 480 580 645	1063 1008 985 834 450

Table 5. Halochromism of the dyes (3b) and (5a) in aqueous universal buffer solutions having varied pH values

pH / Dye No.	Universal Buffers															
	1.75		2.45		4.65		5.8		7.88		8.75		10.58		12.6	
	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^{-1}$)
3b	430 470 585	2502 1631 957	440 474 587	1194 1114 734	440 474 589	1980 1489 754	450 478 590	2468 1965 832	450 480 592	1164 1197 757	455 480 593	1281 1183 717	455 480 595	1568 1526 957	460 480 598	1233 1183 856
5a	460 500 615	1958 1467 725	470 505 620	1428 1304 481	470 512 622	1168 1052 634	475 515 628	1067 1017 583	475 515 630	1443 1017 711	480 510 635	1119 1131 673	485 530 638	1036 891 465	485 545 640	1165 996 412

Table 6. The variation of absorbance with pH at fixed λ for the dyes (3b) and (5a) in aqueous universal buffer solutions

		pH								
	Dye	**1.75**	**2.45**	**4.65**	**5.8**	**7.88**	**8.75**	**10.58**	**12.6**	**PKa**
Absorbance at fixed wavelength	**3b** **λ=480(nm)**	1.574	1.012	1.234	1.804	1.099	1.086	1.401	1.081	4.7 9.7
	5a **λ=600(nm)**	0.489	0.388	0.512	0.405	0.572	0.577	0.376	0.319	4.5 10.4



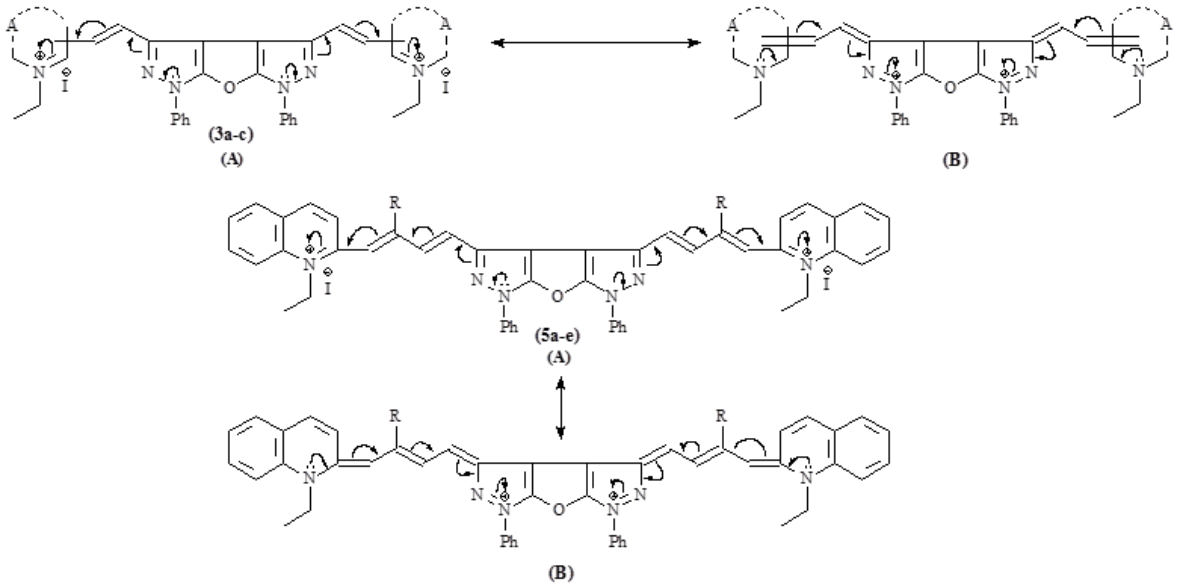
(1) (2) (4a-e) (5a-e) (3a-c)

Synthesis strategy of the prepared compounds (2), (3a-c), (4a-e) and (5a-e)
Scheme (1)

<u>Substituents in Scheme (1)</u>:

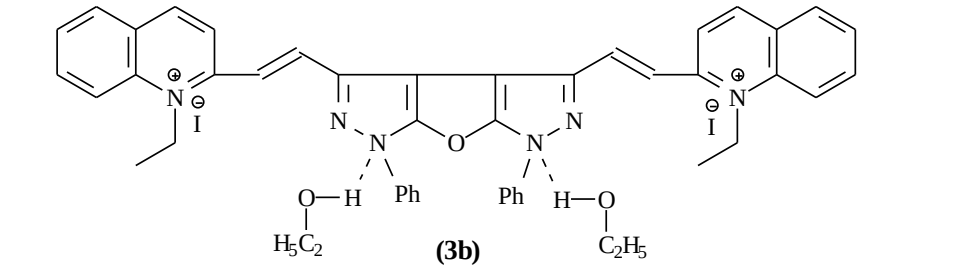
(3a-c): A=1-ethyl pyridinium-2-yl salt (a); 1-ethyl quinolinium-2-yl salt (b); 1-ethyl pyridinium -4-yl salt (c).

(4a-e), (5a-e): R = H (a); CH_3 (b); C_6H_5 (c); C_6H_4.p.OCH_3 (d); C_6H_4,p,NO_2 (e).

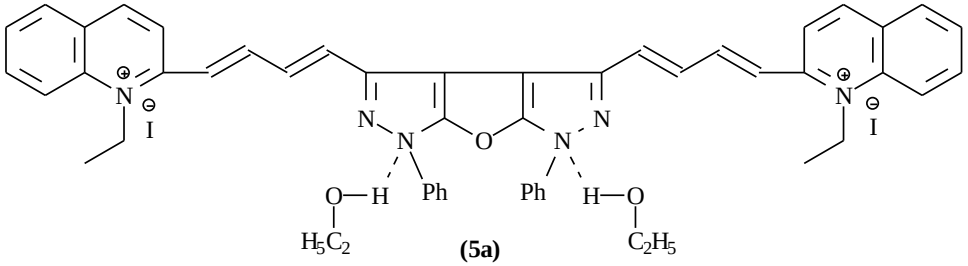


(3a-c) (A) (B) (5a-e) (A) (B)

Colour intensity illustration of the synthesized cyanine dyes (3a-c) and (5a-e)
Scheme (2)



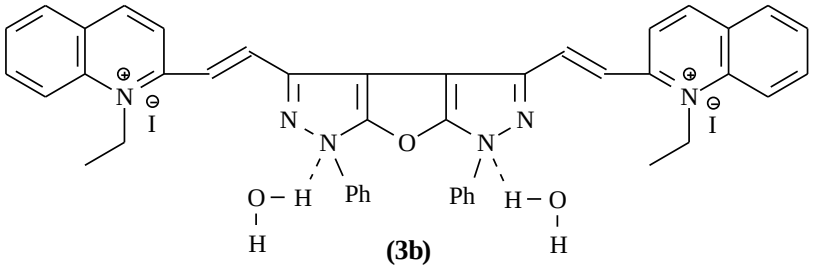
(3b)



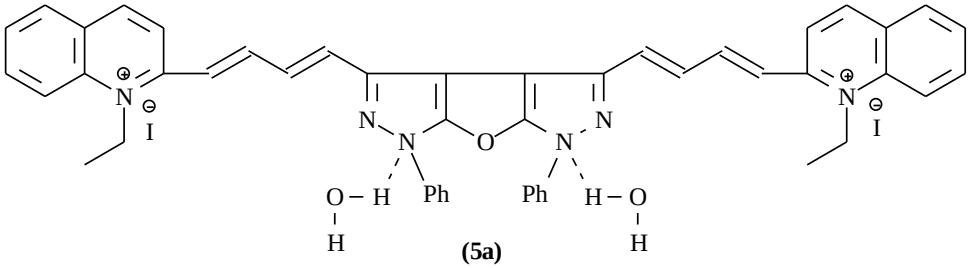
(5a)

Hdrogen bond formation between the cyanine dyes (3b), (5a) and ethanol molecules (specific solvent effect).

Scheme (3) (A)



(3b)

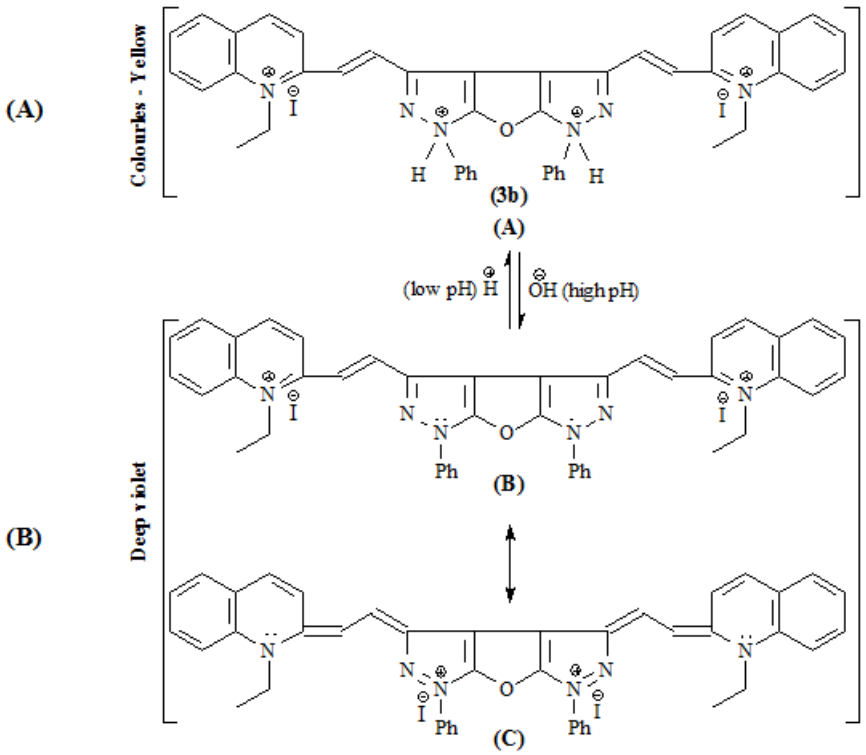


(5a)

Hdrogen bond formation between the cyanine dyes (3b), (5a) and water molecules (specific solvent effect)

Scheme (3) (B)

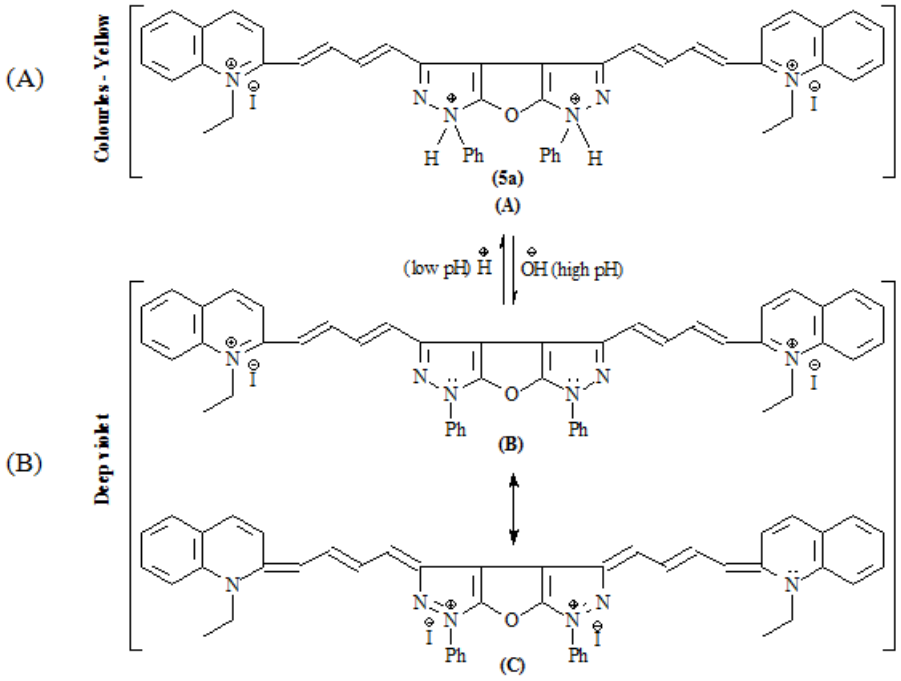
Effect of pH on discoloration efficiency of the cyanine dye (3b)



Decolourization (protonation) and colourization (deprotonation) of the cyanine dye (3b) in acid and base media, respectively (acido-basic equilibrium).

Scheme (4)

Effect of pH on discoloration efficiency of the cyanine dye (5a)



Decolourization (protonation) and colourization (deprotonation) of the cyanine dye (5a) in acid and base media, respectively (acido-basic equilibrium).

Scheme (4) Continue