Abstract
Preservation of Botanical Specimens
Retaining the Natural Colour Pigments

The history of preservation of botanical specimens goes back to the very ancient period. Moreover, the nature itself preserved a huge amount of plants particularly during Carboniferous period about 345 million years in the form of fossils. Ancient Egyptians preserved elaborate garlands and made detailed preparations using grains and herbs for their dead for metaphysical reasons for about 3000 years since 2600 BC. Medieval Monks harvested and dried flowers and herbs for medicinal purposes. Victorian ladies considered floral garlands to be an essential fashion accessory and they used to display dried flowers in glass domes. Flower craft enjoyed most popularity in Victorian England. The interest in dried flowers comes in waves bringing all the old applications and techniques along with fresh inspirations and ideas. In spite of using best chemicals for improvement of keeping quality and enhancement of vase life, the cut flowers lose charms due to microbial activities and ageing process. Dried flowers, herbs, grasses and to a lesser extent seed head are used by florists to design the semi-permanent maintenance-free, beautiful and decorative arrangements and the Pot-pourri mixes (A mixture of dried flowers and leaves used for making a room smell pleasant). Plant parts like Rose buds, Ferns, Pine cones, Lily pods, Marigold, etc. are the primary raw materials for the floral arrangements. These materials are used sometimes after dyeing and colouring. Botanical specimens which comprises, flowers, leaves, buds, stems, etc., preserved
in the fluid or pressed and dried as herbarium specimens, lose their original colours. Apart from the immediate changes, loss of water and desiccation of cell contents on drying, the most obvious short-term chemical changes for normally dried and stored specimens involve loss or change of colour. Little is known about long-term changes in the chemical composition of normally dried and stored plant materials. The main physical changes includes distortion of shape, loss of flexibility and brittlement, and these changes appear to be primarily caused by loss of water from the specimen rather than significant chemical changes (Bedford, David J., 1999). At the early stage flower and other parts of plants were dried simply by hanging them upside down. But for preserving the original colour and shape experimentation started in 1949 with all imaginable ways. Baker, G.E. (1949) preserved flowers in deep freeze. Collected flowers packaged in lined paper bags of the type sold for food processing and placed in a commercial deep freeze locker. Harris, R.H. (1964) described the drying technique of Vacuum dehydration and Freeze drying for Animals, Fungi, Algae and some flowering plants. In this method specimens first frozen at -15 to -20 °C for a few hours; then placed in a vacuum chamber at the same temperature together with a quantity of desiccant for absorption of the vapour. A paper by Bhartia et al. (1973) looked at combinational Microwave and hot air drying of various substances, including Silica gel. This work shows that microwaves enhance the rate of drying when compared with hot air drying. Davitt, Laurence M, Thomos Cochis (1978) of Anniston Museum of Natural History, in Anniston, Alabama, prepared Red-oat grass for the use in Savanna diorama. In 1979 NBRI Lucknow, India published a bulletin on 'Dehydration of Flowers & Foliage' by well known floriculturists and scientists M.A. Kher and J.C. Bhutani. Chase
and Hills, (1991) compared Silica gel with Drierite (CaSO\textsubscript{4}) as a suitable desiccant for drying leaf samples. Fuller and Barbe (1981), Hall (1981), Sauleda and Adams (1981) refer to drying specimens in a Microwave oven. Kathy Reid (2002) preserves flowers by freeze drying technique. Sharon Bale (2004) preserved the flowers by drying with the help of Silica gel. Drying can often be achieved in air if the relative humidity is sufficiently low or with the assistance of dry heat (Forman and Bridson, 1989). Pressing plants between moisture-absorbent sheets is a stock method that hasten drying (Thompson, 1926). Heat fixation in a Microwave oven is a potentially useful technique, for succulent plants (Fuller and Barde 1981, Stoddart, 1989). However, in view of problems with 'hot spots' of degraded tissue, this method should only be applied with caution. Dry preservation, while inexpensive, is inadequate to maintain the form, colour, texture and internal morphological integrity of many specimens, especially soft materials. (Reid, 1994).

In wet preservation different kinds of preservatives like Alcohol, Formalin, Acetic acid etc. are used. In the process of using these chemicals, plant tissues are preserved without much shrinkage there by keeping the forms of the specimen almost intact, but the colour of the tender and soft parts change within a very short duration because these preservatives destroy the natural pigments in the cells. The cause of changing of the colours of botanical specimens is due to the pigments which are made up of organic compounds and when they come in contact with acids or alkalis they get oxidised and start losing and changing their natural colours. Even slight change in a pigment structure changes the entire shade of the colour of the specimen. When the Magnesium-ion comes out from the Chlorophyll structure the green specimen turns brownish. Similarly other pigments
like Xanthophyll lose their original colour due to the effect of different chemicals on their structure. Preservation of such specimens without changing colour is a problem which encounter the museum personnel.

In 1838, Cleghorn suggested presenting the plant specimen with colour in a saturated solution of common salt in water. This method preserves the colour only for a few months; and traces of salt get deposited as dirt on the inner sides of the container. Mr. C.E. Jones (July, 1917) had been preserved green foliage and plants by boiling in a solution of Copper acetate and Acetic acid. Maltby, (1926) recommends a number of methods for preserving different types of plant parts with colour. For preserving green part of plant, he suggests, keeping the specimen in a solution of Copper sulphate and bubbling in Sulphur dioxide gas. Blaydes (1937) suggests storage of specimens in a 0.2% solution of Copper sulphate in the standard Formalin-acete-alcohol. Chandrashekar, M.S. (1958) has suggested preserving the green plant specimen with colour. The procedure suggested, requires the employment of two solutions - solution A, saturated solution of Copper sulphate in 10% aqueous Formalin; and solution B, 5% aqueous formalin. Knudsen, (1972) reveals that when preserving huge quantities of specimens in small containers, increase the percentage of Formalin. Use Formal-acetic alcohol (FAA) for preserving specimens intended for slide-making and histological studies, in the ratio of 93 parts of Formaldehyde to 3 parts of Glacial acetic acid.

In the light of above facts, some experiments were planned and conducted for retention of the colour of some selected plant specimens. The present research is a more detailed analysis of the effects of different grain size of Silica gel on shrinkage of petals and leaves, effects of temperature on natural colour pigments and physical appearance and so
on. For the preservation of pigments of plant specimens in liquid preservative, different experiments were also conducted.

Experiment for wet preservation were divided into two phases. In phase one experiments for fixing of colour pigments were conducted. In Phase two experiments for preservatives were conducted. The following plants were selected for the experiments which include: Dahlia, *Coccinia cordifolia*, *Iberis amara*, *Acalypha indica*, *Asparagus racemosus*, *Lantana indica*, *Bougainvillea spectabilis*, *Sonchus arvensis*, *Thevetia peruviana*, *Bauhinia sps*. All the plant specimens collected between 9:00 am to 11:00 am. For the preservation of plants in fluids, experiments were started with the evaluation of optimum quantity of Copper sulphate with 1 ml Formaldehyde for the fixing of Chlorophyll. The specimens *Coccinia cordifolia*, *Iberis amara*, *Acalypha indica*, *Asparagus racemosus*, *Lantana indica*, *Bougainvillea spectabilis*, *Sonchus arvensis*, *Thevetia peruviana*, and *Bauhinia sps.* and *Justicia* placed in different chemical concentrations for 24 hours for fixing of green colour of the specimens. *Coccinia cordifolia*, *Iberis amara*, *Acalypha indica*, *Asparagus racemosus*, *Lantana indica*, *Bougainvillea spectabilis*, *Sonchus arvensis*, *Thevetia peruviana*, *Bauhinia sps.* and *Justicia* were not fixed in the any above solutions and kept in the new set of solutions. Solution which contains Formaldehyde 2 ml, and Copper sulphate 1 gm shows retention of good green colour in *Iberis amara* plant, all other plants shown unsatisfactory results in all other solutions of this experiments. Solution which consist of Formaldehyde 2 ml, Copper Sulphahte 1 gm, and Propionic acid 1 ml given more satisfactory results for *Iberis amara*. But other specimens like *Coccinia cordifolia*, *Acalypha indica*, *Lantana indica*, *Bougainvillia spectabilis*, *Thevetia peruviana*, and *Bauhinia sps.* But green colour of *Sonchus*
**arvensis, and Justicia** was not fixed in the above solutions. Solution which comprises Formaldehyde 1 ml, and Copper sulphate 32 gm considered as the best fixative for *Asparagus racemosus* species. Specimens those shows satisfactory results in different fixative solutions, selected for testing of preservative solutions of different chemical concentrations. Samples of *Asparagus racemosus, Coccinia cordifolia, Acalypha indica, Bauhinia sps., Iberis amara, Lantana indica, Bougainvillia spectabilis,* and *Thevetia peruviana* revealed better results, hence selected for further experiments on preservatives. Samples which were best fixed taken out from the solution and washed three times with distilled water and placed in preservative solutions. Jars were sealed and stored at 18 °C ±2 in dark for further evaluation of colour retention. The specimens preserved in solutions consists of Formaldehyde 1 ml, and increasing quantity of Citric acid from 1 gm to 16 gm shows changes in colour of leaves after 3 days in increasing order in all samples. After one month leaves were started to fall from anterior side. Within two months green colour of leaves were faded completely in all the samples. Specimens preserved in solutions in which Citric acid were used as preservative, but none of the specimen found satisfactory. After two months of preservation, fungus growth developed in all five samples of this experiment and green colour of specimens destroyed. Specimens placed in the solution of distilled water, Formaldehyde and increasing quantity of Propionic acid from 1 ml to 16 ml, solution turns milky when quantity of Propionic acid exceeds from 4 ml. Specimens of solution (Formaldehyde 1 ml, Propionic acid 1 ml) reveals good green colour in all samples. No further colour changes found in these specimens for 15 months. All the samples that contains Formaldehyde 1 ml, Propionic acid 1 ml, and increasing quantity of Citric
acid from 1 gm to 16 shows no trace of green colour in any sample. Green colour faded in all samples and leaves were fallen in all the samples and none of the samples were found satisfactory. It is found that the quantity of Formaldehyde and Propionic acid are shown same result as preservative for *Coccinia cordifolia*, *Iberis amara*, *Acalypha indica*, *Lantana indica*, *Bougainvillia spectabilis*, *Thevetia peruviana*, *Bauhinia sps.* and *Asparagus racemosus* plants. Bluish white flowers of *Iberis amara* faded after six months, but green colour not faded even after 18 months. The experiments in which specimens were best preserved repeated for storage in dark and room light during fixing process and final storage. Specimens those stored in dark during fixing process and final storage process gives more better results than those specimens which were stored in dark only during final preservation process. The experiments shows that the quantity of Copper sulphate required for better fixation varies with specimen to specimen. Some specimens like Bougainvillea shows better fixing in 1 gm while other specimens like *Asparagus racemosus* shows good green colour in 32 gm. These results coincides with the findings of Mackenzie (1928), who used Copper sulphate with some other chemicals to preserve different plant parts, e.g., for mature dark-green foliage, a stronger Copper solution was used than for young tender light-green leaves. Chandrasekhar (1958) applied saturated solution of Copper sulphate to many other specimens found unsatisfactory. The results shows unnatural dark bluish green colour appeared on entire specimen.

The specimens showing good green colour after preservation and a fresh specimen of *Bougainvillia spectabilis* selected for the Spectrophotometric analysis of Chlorophyll contents. The method adopted for the estimation of Chlorophyll content was practiced by Mackinney in
1941. Spectrophotometric analysis for Chlorophyll contents reveals that the specimens firstly fixed in a solution of 1 gm Copper sulphate, 2 ml Formaldehyde, and 1 ml Propionic acid and secondly preserved in Formaldehyde 1 ml, Propionic acid 1 ml, in which the quantity of Chlorophyll $a$ 0.52 mg kg$^{-1}$ and quantity of Chlorophyll $b$ 1.03 mg kg$^{-1}$ measured in the sample whereas the quantity of Chlorophyll $a$ 0.97 mg kg$^{-1}$, and Chlorophyll $b$ 1.97 mg kg$^{-1}$ was measured in fresh samples of Bougainvillea spectabilis.

Specimens of Dahlia, Sonchus arvensis, and Thevetia peruviana containing yellow flowers were selected for experiments for the preservation of yellow colour pigment. Five solutions comprising Formaldehyde 0.5 - 8 ml and Propionic acid 1 ml prepared. Specimens placed in separate jars of fixing solutions for 24 hours in dark. *Thevetia peruviana* changed into brownish yellow after the treatment and the green colour of leaves faded. Although some amount of yellow pigments dissolved in the fixative solution. Yellow Dahlia flower and *Sonchus arvensis* fixed well in Propionic acid 1 ml and Formaldehyde 1 ml. Green colour of sepals faded in both specimens. Specimens stored in the different concentration of preservatives for the evaluation of their effectiveness. Solution which containing Formaldehyde 1 ml and Propionic acid 2 ml was satisfactory for *Sonchus arvensis*. Fifteen replicate specimens (Dahlia) of which were fixed in above solution prepared to find out appropriate concentration of different chemicals as a preservative. Solution consists of Distilled water 100 ml, Formaldehyde 1 ml, Propionic acid 1 ml, and Glycerine 2 ml were found better preservative for the yellow coloured Dahlia flower. Other samples were not found satisfactory. The above experiments shows that the same concentration of the Formaldehyde and
Propionic acid was works in both fixative and preservative solutions. It shows that the 1 ml Formaldehyde and 1 ml Propionic acid was sufficient for preservation of *Sonchus arvensis* and yellow Dahlia flower. In the final preservatives 2 ml Glycerine was also added to maintain the flexibility of specimens. Present experiments reveals that for the preservation of yellow coloured flowers of *Sonchus arvensis* and Dahlia, no preliminary fixation of colour is required. Proposed single solution method for the preservation of yellow coloured specimens concides with the work of Adriano and Youzen (1933). Further preserved specimens sealed carefully in glass jars. It has found that the preserved specimens hold good colour more than 24 months.

Bougainvillea, *Cosmos sulphureus*, Zinnia, *Helianthus debilis* (Beach Sun flower) and *Gaillardia pulchella* (Indian Blanket or Blanket Flower) were selected for the experiments on dry preservation of ornamental plants and flowers. Flowers were placed upside down for embedding in Silica gel. Pedicel of flowers reduced to two inches and buried in the desiccant material. Flowers of flat petals were selected for evaluation of shrinkage because they are easy to measure. Experiments were conducted to evaluate total shrinkage after drying and to find out optimum size of Silica gel to minimise shrinkage in petals particularly for the preservation of shape and texture. Six samples were prepared for the evaluation of shrinkage and they were dried in air by hanging upside down and treated with various grain size of Silica gel, such as 6-20 mesh, 15-20, 100-200, and 230-400 mesh. Average width sizes of petals of each flower were measured before and after drying. Experiments reveals that the natural drying and specimen hurried m.coarse .grains of Silica gel are not suitable to preserve shape and texture of flowers. The experiments
reveals that specimens which were dried in upside down hanging has maximum shrinkage, and when such shrinkage occurs, it is impossible to preserve texture of the specimen. In case of natural drying flowers lose their natural shape and maximum shrinkage occur and completely deform the texture. Course grained Silica gel also not suitable for the preservation of natural shape and texture due to their coarseness and heaviness which also damage petals. Heavy grains causes weight over the specimens which results in the deformation of flowers and leaves while stems and other hard parts like spines does not shows considerable shrinkage. Moreover large grains of Silica gel does not come in contact with the entire surface of petals and leaves which leave large intergranular spaces in comparison with fine grains. These intergranular spaces also provide enough space to shrink. Sharp edges of the grains touches the surface of specimens which damage petals by pinching and makes small holes. It is recommended for the spongy or hollow stems that a non-corrosive metal wire may be inserted to the spongy stems before drying to reduce the shrinkage. But woody stems shows minor shrinkage after drying process. It is observed that the intensity and duration of temperature is important for the colour preservation in flowers. The weight of flowers also taken before and after drying process in order to evaluate the dryness of the specimens. Blanket flower were dehydrated at different temperature. Then the specimens were weighed periodically until a constant weight achieved. The specimens were placed in a glass jar of appropriate size with 100 gm dehydrated self-indicating Silica gel for one hour to test the completion of dehydration. Weight of specimens taken five times after one hour interwell. The results shows that 76 percent water removed from specimens by this method. Samples which were dried at 140 °C, altered the colour of the
flower considerably and due to crispy nature of petals, it was difficult to remove the flower from the desiccant. Photographs were taken before and after drying process for showing colour changes in dried flowers. Specimens which were dried at 140 °C for 2 hours shows minimum colour retention though they were dried quickly. Specimens which were dried at 100 °C for 4 hours shows better results than earlier sample. The specimens dried at 37 °C for 72 hrs shows better colour retention than earlier sample. Results of the experiment matches with Pamela Westland, (1995) who reported that, In case of oven drying, over-processing makes the material unacceptably brittle. Flowers dried at temperature of 34 °C reveals brilliancy of colours while drying specimens at the temperature more than 37 °C fade the colours considerably. Although specimens dried at 34 °C takes more time to dry completely but they retain colour more than those specimens dried at 37°C which takes less time. Use of sieve plate of appropriate size is helpful for the safe removal of dried specimens from the desiccant. Earlier workers used to tilting of vessels to remove the specimens from the deciccant which damage specimens and is not convinient for safe removal of the specimens from the desiccant. Dried specimens must be stored quikly in air tight jars to prevent absorption of moisture from the air. It must be stored in sealed glass or plastic containers with some amount of coarse grained self-indicating Silica gel to maintain the moisture level. Preserved specimens should be stored at optimum temperature and relative humidity preferabely at the range of 18- 20 °C and RH 50- 55% for long lasting effect.

**Advantages of proposed technique of wet preservation:**

1. This process takes less time to complete the whole process of
preservation.
2. It ensure preservation of Chlorophyll contents in green specimens.
3. The proposed method is economical and technique is simple to adopt for small museum and labs.
4. The proposed methods will be helpful for museum curators to enhance the aesthetic look of their wet preserved collection.

Advantages of proposed technique of dry preservation:
1. It preserves natural appearance and texture of the specimens.
2. The dry preservation technique shows maximum colour retention.
3. Completely safe from burning of specimens, as mostly happen in the case of hot-sand method, Microwaves, and oven methods.
4. Dry preserved specimens can be used as display purpose, decorative pieces, commercial purpose and for reference material in botanical museums and herbarium.

Recommendations

In the light of above facts the following recommendations are made for the benefit of further workers:

1. Spectrophotometric analysis of Chlorophyll content must be performed at the time of collection of specimens and soon after the preservation process has completed for more deep analysis of the experiment.

2. It is necessary to use of suitable apparatus for dry air circulation during drying process. It will replace the humid in and around the flower with dry air. This process will enhance the drying rate and improve the quality of colours during preservation.
3. Drying of plants at a temperature less than 30 °C with circulated dry air in desiccant may be helpful for DNA preservation in the dried specimens.

4. Use of suitable equipment for colour measurement (Spectroscopy) before and after drying process is completed to show more accurate findings.

5. Injecting the synthetic colours prior to drying the specimens may give better colour retention and these type of specimens may be useful in floriculture industry.

6. Transgenic plants with highly sophisticated flowers having durable colours and strong petals which are supposed to resistant to various environmental conditions may be produced to achieve better results for the preservation of botanical specimens and would be beneficial for floriculture industry. Lyco Red company’s research team has developed new LRT (Lycopene rich tomato) varieties of tomato with twice as much lycopene as present conventionally (Ravishankar, G.A. and Vasudha Mudgil, 2004).