Chapter VI

Exploring bi-functional properties of certain Hibiscus *rosa-sinensis* (Chinese rose) flowers belonging to *Malvaceae* family

Flowers capture the attention of bystanders owing to their elegant fragrance, colourful patterns and beauty. Researchers are quite involved to reveal the mechanism and origin of colours exhibited by flower petals within a family. The colour of flowers is determined by the type and amount of pigments present, apart from their internal tissue structure. Although the pigments play a vital role, the importance of the morphology of the petals cannot simply be neglected towards their colouration. The biological importance of flower colour is not only to attract pollinators, but also to act as a protecting shield to the floral organs. Floral colouration is the result of scattered light from the structural inhomogeneities of the petal surface and the light absorption by the pigments. Basically, the pigments are distributed in the upper epidermis, but for the dark coloured petals, they can be found in palisade tissue and in the lower epidermis [1]. On the other hand, Whitney *et. al.* have described the epidermis of plants possessing the quasi regularly patterned structure of flowers, which is responsible for coherent scattering and may cause structural colouration [1]. Whitney *et. al.* have very well described that the epidermis of plants have cuticular folds that can lead to certain optical effects [2]. Vignolini *et. al.* have studied the epoxy replicas of Hibiscus trionum. The flower has been studied by Vignolini and has reported the iridescence effect of the flower [3]. They have very well explained the iridescence effect even for the epoxy replicas of *H. Trionum*. They did an optical analysis of the Hibiscus flower, indicating its diffractive effects, which gave us an idea regarding the cuticular folding in the flower colouration mechanism. They have studied the reflectance features at different angles of detection (θ_D = 15° to 75°) using ϕ=75° as the angle of incidence [3]. This study gave important insight to the iridescence of Hibiscus flower even under natural light conditions. But rarely have we found in the literature regarding the wettability properties of Hibiscus flowers. Study of wettability properties is important because of its several applications in different fields including industrial use. Nature has several examples of hydrophobic/superhydrophobic surfaces. *Lotus effect* described by Barthlott and

Neinhuis in 1997 paved a new way for understanding natural systems in terms of wettability properties [4]. Similarly, specimens of rose petals and gecko feet were carefully examined and adequate explanations have been put forward by different researchers for observable hydrophobic/superhydrophobic surfaces. While rose petal effect can be attributed to the pinning effect, the actual reason behind superhydrophobicity is the nanoscale surface architecture [5].

In this chapter, we discuss three varieties of Hibiscus flowers with apparent colours; red, pink and yellow. Infact, the structural colour can be manifested either by changing the refractive index (R.I.) or by changing the microstructural dimension [6]. Here, in order to exploit structural colour contribution, the reflectance properties of the petals were examined either: by dipping the petals in two independent solvents in order to change the refractive indices and also by allowing the petals for natural drying for four days (aging). Also, the wettability properties of the fresh and the petals have been studied, both in terms of static contact angle as well as dynamic contact angle measurement by using tilting plate methodology, where the tilting angle (β) is varied in the range, 0-90°.

6.1 Specimen collection and treatment

The samples chosen for our work are the three varieties of Hibiscus (*Hibiscus rosa sinensis*) belonging to the *Mallow* family (*Malvaceae*). The digital images of the samples are shown in Fig. 6.1 (a-c) below. The red appearing hibiscus has 5 petals with slight overlapping nature, the pink appearing hibiscus has 9 petals entrapped with one another in a bunch and the yellow appearing hibiscus has 15 non-overlapping petals. As shown in schematic, Fig. (6.1 (d)), pistil connects the root and style branch which holds stigmas. The stamen of the flower, essentially consists of stem-like filaments and witnessed only for red and yellow flowers. Here, each of the filament ends with an object, termed as 'anther' which is the chief source of pollen grains. The fresh flowers have been collected from our university garden. The petals are carefully sectioned and a small portion

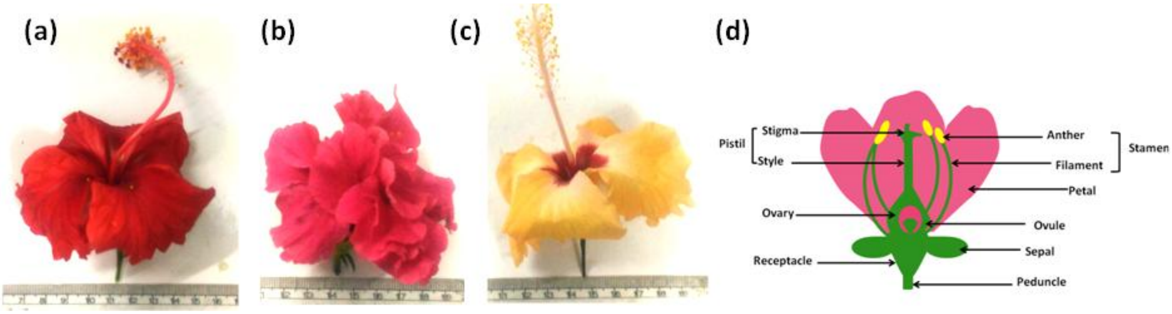

Figure 6.1: Digital photographs of (a) red (b) pink hibiscus and (c) yellow matured hibiscus flowers. A schematic highlighting various parts are depicted in (d).

(2 x 2cm) has been deliberately cut from the middle of the petal for both the optical and wettability studies.

6.2 Micro-morphological analysis of hibiscus petals

The outer surface of flower petals is governed by two main factors: surface structure of the epidermal cells and the cuticular waxy layer. The SEM images of the fresh and dried hibiscus petals are shown in Fig. 6.2. Like all flowers, the hibiscus flower petals consist of epidermal cells [3], which vary in fresh and dried ones, but all of them looks folded. The cuticular sculpture for the fresh red hibiscus petal exhibits the presence of folded papillae. The dimension of the papillae for the red hibiscus petal is 3.18± 0.45 µm. The micropapillae found are not densely packed, owing to a separation as large as 19.45 µm. Whereas, in dried petals, the micropapillae gets thinner and formation of wrinkles are quite visible in the SEM micrograph. The fresh petals of the pink appearing hibiscus have assembly of micropapillae, with an average spacing between the papillae as, 18.57 µm ,and dimension of 5.10±0.36 µm. Upon drying, the structure gets destroyed. The morphology of the papillae is lost, apparently due to the loss of moisture content. In case of yellow appearing hibiscus petals, we observe that the morphology is substantially different. The structure of the micropapillae in this case, is not very prominent and unlike the other two varieties. Also, it does

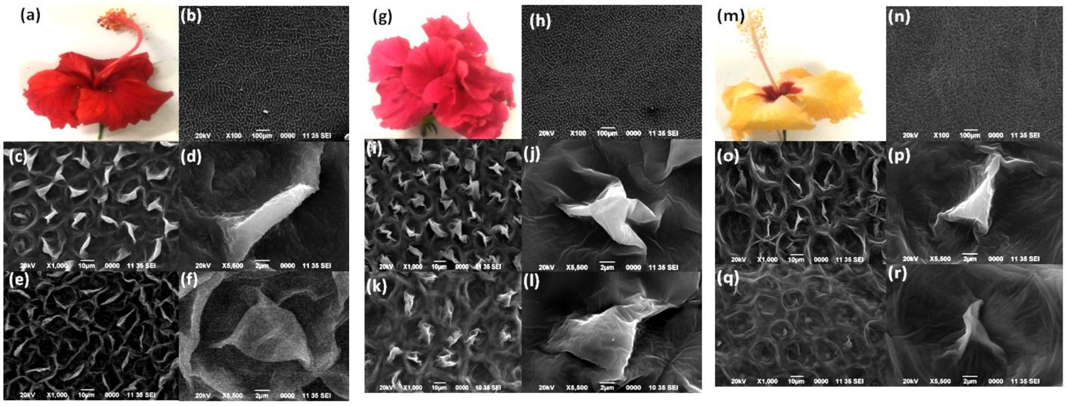

Figure 6.2: Digital photograph and SEM images of the (b-d) fresh and (e,f) dried (a) Red flower petal. Analogous images for (g-l) pink and (m-r) Yellow flower specimens are shown independently in col. (2) and (3); respectively.

not possess a well-built micropapillae structure, and comprises of thin papillae with an average width, 2.84±0.52 μm. Upon drying, the structure is further lost, and the presence of micropapillae becomes unclear.

The cuticle, infact, is visco-elastic in nature and is a polymer matrix which is affected by external factors, such as temperature, humidity etc [7, 8]. The visco-elastic nature of the cuticular layer (mainly cutin and wax) will be affected as the petals will be left for drying as this strongly depends upon the temperature and humidity. Upon drying the petals, we observe roughness to increase and to get induced. Orthogonal compressive and extensional stress leads to the formation of wrinkles on the petal surface [9]. As a result of drying, compressive stress will be imposed uniformly all along the petal, thus forming wrinkles and increasing the roughness as compared to the fresh ones. In case of flower petals, buckling mechanism has been studied and it is established that compressions laterally along the petal surface would lead to buckling instabilities [9]. Buckling basically describes the folds and bends that can be seen on the epidermal cells.

6.3 Assessment of surface wettability features of the fresh hibiscus petals

The upper panel of Fig. 6.3 (A) represents images of static water droplet on red, pink and yellow petals, whereas, the lower panel shows the water droplet shape on 0° and 90° degree of sliding. It is worth noting that the petal cell type plays a critical role in determining the wettability properties. As in case of rose petals, discussed in *Chapter V*, the conical shape and the hierarchical structure of the hibiscus petals impart them their hydrophobicity. In order to understand the wettability properties, we have assessed the water contact angle values for the fresh petals. Infact, wettability of rough surfaces is a complex phenomenon, which has been discussed by Cassie-Baxter (CB) and Wenzel earlier [10, 11]. But there is an intermediate state, where the water droplet on a rough surface can actually rest upon, referred to as the 'Cassie-impregnating regime'. The C-B model generally describes the suspended state of a water droplet on a pillar-like rough surface, where it does not fill up the grooves and air is likely to get entrapped beneath the water drop [10].

$\cos \theta_c = \varphi (1+ \cos\theta) - 1$, where, φ is the solid-water fraction. (6.1)

The Wenzel model is applicable to surfaces where the droplet makes its way in the grooves, and therefore signifies the collapsed state [11].

$\cos \theta_w = r_\varphi \cos \theta$, (6.2)

where r_φ is the roughness factor and θ is the contact angle for the smooth surface of a similar material system. Although there are several factors affecting the wetting-dewetting properties, but in plants, there are mainly two factors that can contribute to the hydrophobicity of the plants. Hydrophobicity is governed by the complex architecture and the waxy cuticular nature of the petal surface. Barthlott has categorised the wax structures into two broad categories, with several smaller sub-groupings [12]. The first category can be classified as the flat homogenous waxy layer, which can be further classified as films, smooth layers etc. with the tendency to form crusts or cracks on drying [13]. The second category, which is more attributed to the superhydrophobic phenomenon are termed as wax crystals. There exist various types of wax crystals, named as platelets, rodlets, tubules, and even thread like structures. The hibiscus petals, as observed for the three varieties are found to have irregular structures. In the yellow hibiscus petals, the waxy layer is observed to form coil like structure [14-

19]. The fresh petals exhibit hydrophobicity with water CA values of 110.7°, 102.9° and 101.1° as for the red, pink and yellow specimens; respectively. In plants, superhydrophobicity is mainly attributed to. the microstructure and nanostructure roughness characteristics, resulting from epidermal cell morphology and the wax crystals. The hierarchical nature of plants contributes to the superhydrophobicity nature. Moreover, there are certain plants which exhibit their superhydrophobicity nature even with flat smooth microstructure. The dense waxy nature of such plants contributes to their water-repelling properties. However, it has been reported that such plants do not retain their water repellency throughout the lifetime [12, 20].

Contact angle hysteresis (CAH) forms an important part of wettability studies [21]. The water droplet is first dropped carefully on the petal surface, and the water droplet is then allowed to tilt from 0° to 90°. As a result of sliding, the droplet tends to have several maxima and minima, known as advancing and receding angles. Fig. 6.3(B) and (C) depict the CA without and with tilting and consequently, maximum and minimum angles are determined over the tilting range. The difference between these angles gives the CAH value. Red hibiscus shows a low CAH value of 25.13°. Whereas, pink and yellow hibiscus petals offer relatively higher CAH values of 35.08° and 31.42° respectively. Pink and yellow hibiscus petals show hydrophobicity, but at the same time exhibit significant CAH value, which indicates higher adhesion to water. The '*Petal effect*' is already very well described, where the surface makeup is believed to be superhydrophobic and is highly adhesive to water [22]. Under such circumstance, the water droplet is pinned to the surface roughness and hence does not roll off but exhibits a higher static CA. Table 6.1 shows the dimension of contact line with tilting angle and Fig. 6.4 depicts it.

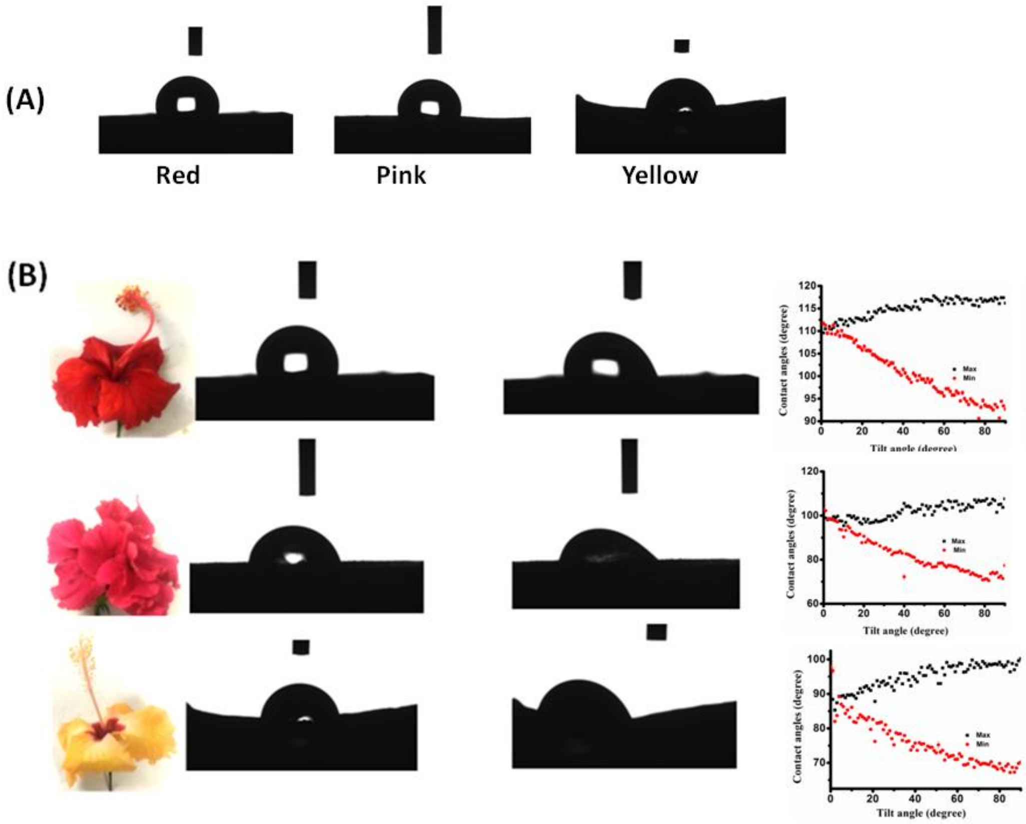

Figure 6.3 (A) Static water contact angle (CA) values of the (a) Red (b) Pink and (c) Yellow hibiscus (B) CA without (β=0°) and with base tilting (β=90°) (right panel) Advancing (maximum) and receding (minimum) contact angles measured in tilting base methodology as for (a) red, (b) pink and (c) yellow hibiscus petals.

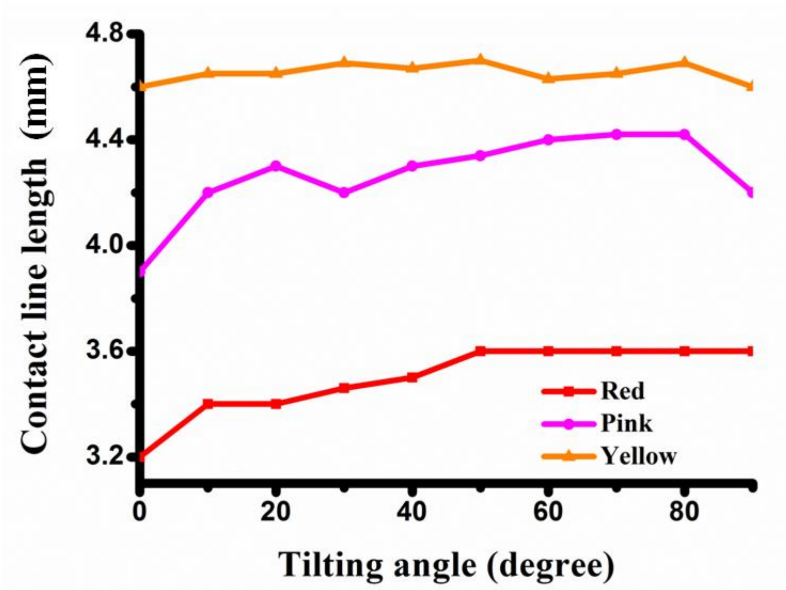

Figure 6.4: Contact line of the water droplet on hibiscus petals.

Table 6.1: Tilting Vs Contact line

Tilting angle (degree)	Measured dimension of contact line (mm)		
	Red	Pink	Yellow
0	3.2	3.9	4.6
10	3.4	4.2	4.65
20	3.4	4.3	4.65
30	3.46	4.2	4.69
40	3.5	4.3	4.67
50	3.6	4.34	4.7
60	3.6	4.4	4.63
70	3.6	4.42	4.65
80	3.6	4.42	4.69
90	3.6	4.2	4.6

6.4 Normal and variable incident angle reflectance features

6.4.1 Structural colouration character in different Hibiscus petals

The reflectance spectra for the three varieties of the hibiscus, namely red, pink and yellow are shown in Fig.6.5 (A). We discuss below the reflectance features of the untreated and treated petals of each of the flowers separately. Fig.6.5 (B) gives a comparative account of the reflectance spectra of the fresh and dried petals. The reflectance curve (Fig. 6.5 (A) (a)) for the fresh red hibiscus flower exhibits a peak at 367.3 nm and at ~550 nm, and then reflectance increases sharply towards the higher wavelength regime. As a result of dipping the petals in the ethanol (*R.I.* 1.36), it is highly expected that ethanol can fill in the microstructure, resulting in a change in the overall refractive index. The ethanol treated petals show an enhancement in the reflectance with the peak shifting by ~42 nm, but towards the higher wavelength, we observe the suppression of reflectance. In the wavelength regime 350-600 nm, there is an usual manifestation of reflectance as a result of ethanol dipping. Treating it with methanol results a small peak at ~316.7 nm. Small peaks/humps are observed at ~ 370 nm and 507 nm which appear as a result of methanol dipping for 24 h. Though at the higher wavelength, we observe a noticeable fall in reflectance. On the other hand, glycerine treated red hibiscus petals show similar response as the untreated one. Propanol treated petals, however, record a suppression in reflectance and offer almost a linear response. This indicates, therefore, removal of colour agent as a result of treating in glycerine. Whereas, the dried petals are found to exhibit a lowered reflectance feature, as drying results in the fading away of the colour as well as the loss of substantial moisture content.

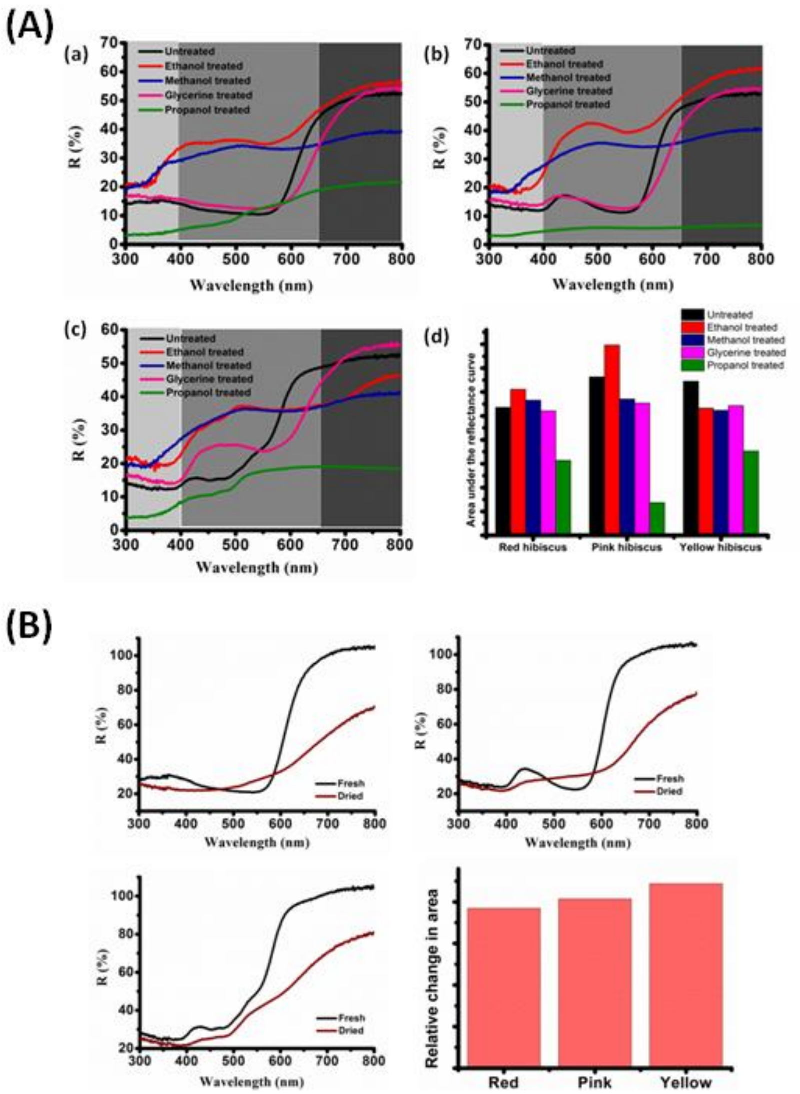

Figure 6.5: (A) UV reflectance spectra of untreated and liquid treated (a) Red (b) Pink and (c) Yellow hibiscus (d) histogram representation of the area under the curves of each spectrum (B) UV reflectance spectra of dried (a) Red (b) Pink and (c) Yellow hibiscus

The reflectance curves of the pink hibiscus are shown in Fig. 6.5 (A) (b). The spectra for the fresh pink hibiscus exhibit a peak at ~440.9 nm. At 550 nm, we observe an abrupt increase in reflectance towards the higher wavelength. The peaks get broadened for the ethanol treated petals and get shifted to 485 nm, with an enhanced reflectance of ~ 40%. A small peak also appears at ~308 nm as a result of methanol dipping. The glycerine treated petal suggests a similar response as that of the fresh ones, but also a suppression of reflectance in the higher wavelength regime. The propanol treated petals however, ensure a lowering of reflectance, and indicates the removal of colour present. As apparent from the figure, while exhibiting a decrease in overall intensity there exist no prominent peaks for the dried petals.

We have presented the reflectance curves for the yellow hibiscus in Fig. 6.5 (A) (c). Here, we observe a peak at ~ 424 nm for the fresh petal, with an abrupt increase of reflectance at ~ 500 nm, towards the higher wavelength side. As a result of immersion in ethanol for 24 h, we find that the reflectance is suppressed in the lower wavelength region. But an altered reflectance is observed beyond 434 nm, while the peak appears at 506 nm. Methanol treated petal showed a peak at 306 nm, following similar trend as the ethanol treated one, except for an increased reflectance. A broad peak at ~465.62 nm is exhibited for the glycerine treated yellow petal, unlike the other two hibiscus petals. Whatever, be the specimen colour, the dried petals showed a suppression of reflectance, in each case.

The reflectance features are strongly governed by the shape of the epidermal cells. The colour appears mainly due to the two reasons: pigments and the internal or surface tissue structure [1]. The pigments absorb the light that is incident on the petal surface and the remaining part of light is reflected from the surface structure, because of which we see the colour contrast. For all the varieties, we find that at a higher wavelength, the reflectance would increase sharply, which indicates that the NIR photons scatter more efficiently than other photons from the tissue/microstructures. The fresh tissues have airgaps and are filled with water, so the liquid will penetrate into the microgaps and cause change in the overall optical properties.

Using PerkinElmer Variable Angle accessory (VAR), the angle dependent reflectance response of the hibiscus petals have been investigated using incident angle variation from 15° to 75°. The schematic representation and the angle dependent reflectance spectra for the three specimens, *viz.* red, pink and yellow appearing hibiscus petals are shown in Fig. 6.6 and Fig. 6.7 respectively. From the spectra, it is observed that each of them would represent a wave like pattern with several dips/fluctuations. At a 15° angle of incidence, we observe a minimal reflectance in the lower wavelength regime, with a sharp rise in reflectance at ~ 577 nm, towards higher wavelength side. However, with changing angle of incidence, it showed a peak at ~487.6 nm, which is almost consistent in all the cases.

The reflectance response is maximum towards the higher wavelength region for all the incident angles under study. However, at ϕ=30°, the reflectance is maximum as compared to other angles of incidence. In case of pink hibiscus petal, the peak also appears at a 30° angle of incidence. As for the yellow hibiscus petals, we observe a linear rise in reflectance in the wavelength regime 400-600 nm, and beyond it, the reflectance is almost constant. Unlike the other cases, significant changes are observed for every varying angle of incidence. As observed from the morphological features, it is quite clear that the yellow petals would exhibit more exposed area as compared to the red and pink petals. More importantly, the cuticular folding is not very prominent. In *Hibiscus trionum*, the iridescence pattern is observed, which is attributed to the diffraction grating produced by the cuticular folding, as reported by Vignolini *et. al.* [3].

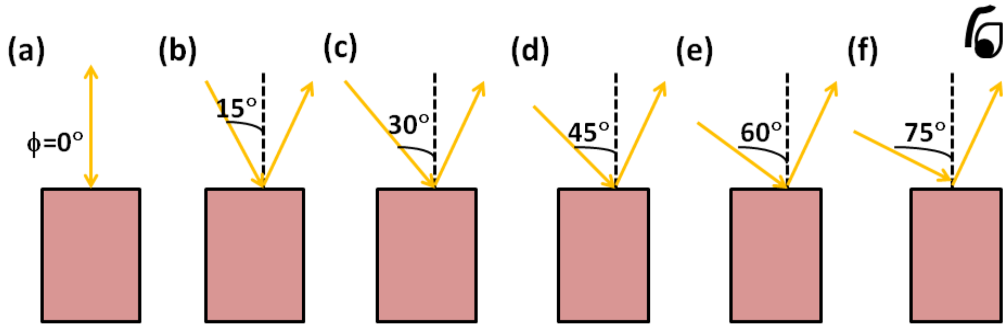

Figure 6.6: Schematic representation of the incident angle and the angle of detection.

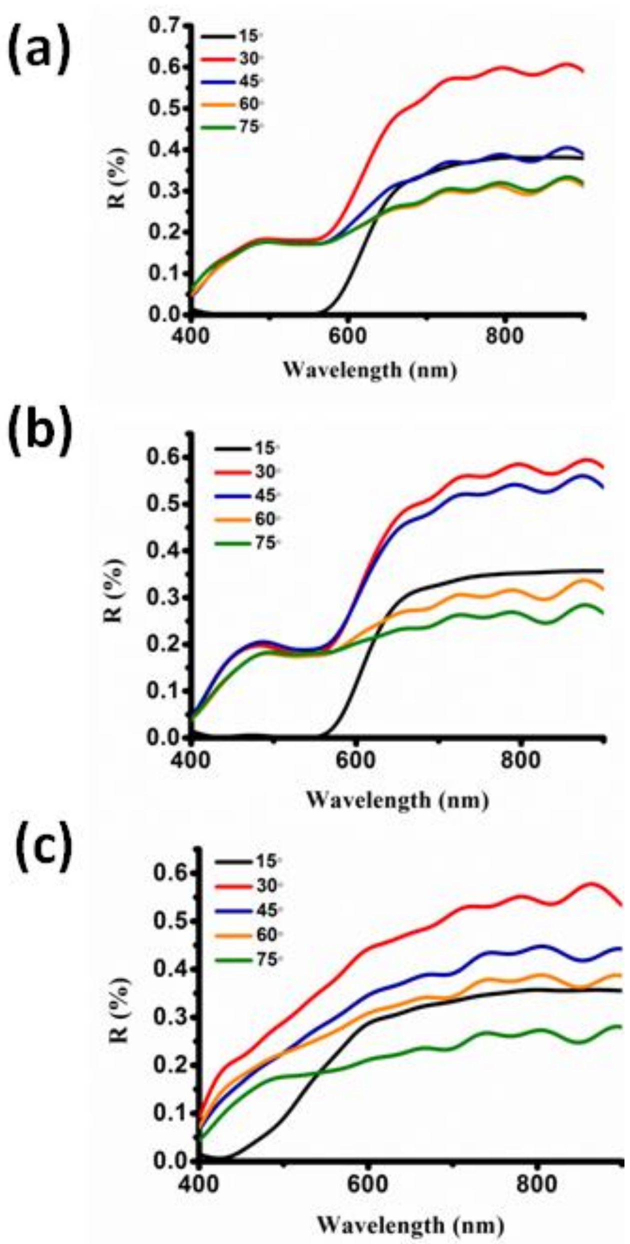

Figure 6.7: Angle dependent reflectance spectra of (a) red (b) pink and (c) yellow hibiscus petals.

Polarisation sensitive reflectance features of the three petals were also investigated using s and p polarisers. Fig. 6.8 depicts the polarisation dependent reflectance spectra of the red, pink and yellow hibiscus petals; respectively. For all the three specimens, the effective reflectance due to s-polarised light gives a higher reflectance response. The red hibiscus petals, infact, exhibit almost negligible reflectance in the regime 400-550 nm. As for the three cases, the reflectance response is higher towards the higher wavelength regime. Here, we can determine the degree of polarisation using the expression [23]:

$$p_z = \frac{R_s - R_p}{R_s + R_p} \qquad (6.3)$$

All the three specimens exhibit constant degree of polarisation (p_z). The yellow hibiscus, while exhibiting a lower degree of polarisation, is believed to be least sensitive to polarisation.

Floral colouration is a result of pigmentary and structural effects. Indeed, micro-structure plays prominent roles in selective reflectance and scattering events. The role of floral colouration in attracting pollinators has received much attention and

is a subject of typical interest in recent years. The complex plant-pollinator interaction has been described in several experiments [24]. The reflectance, to a great extent, depends on the inhomogeinity of the petal structure along with the thickness. *Hibiscus trionum* flower through the angle dependent and polarisation sensitive reflectance features.

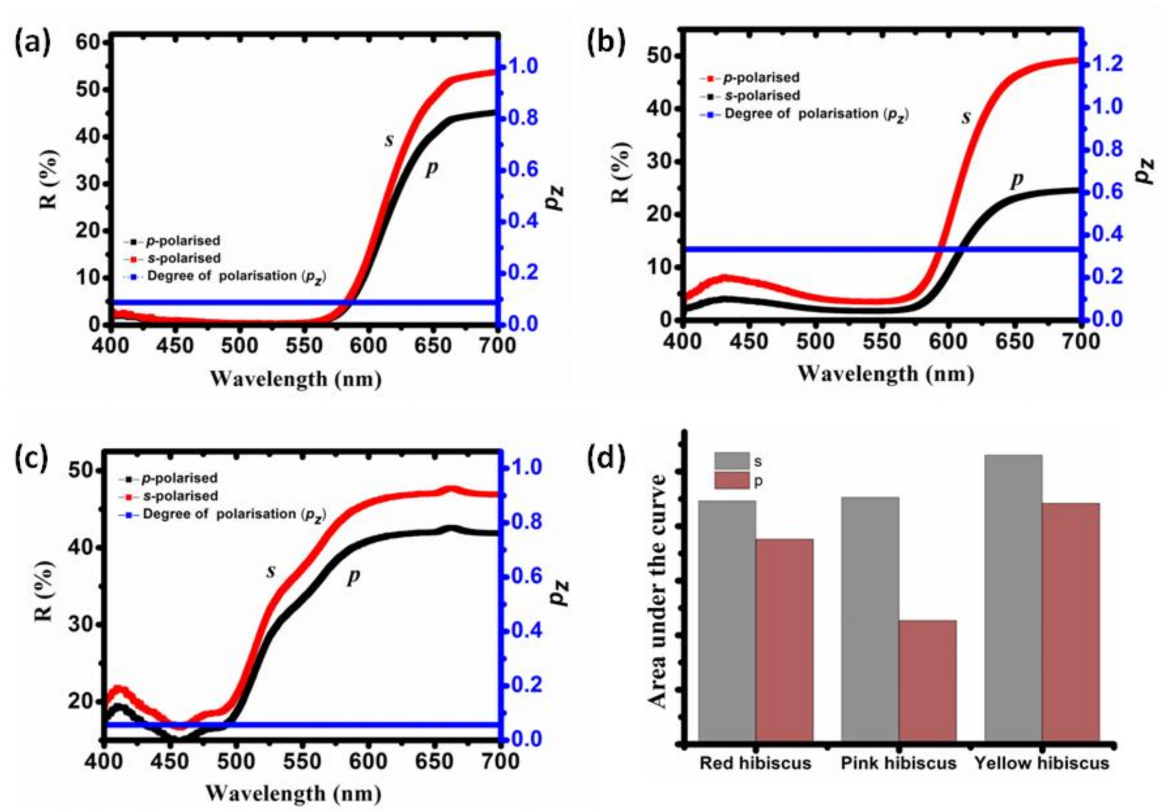

Figure 6.8: Polarisation sensitive reflectance features of (a) red (b) pink and (c) yellow hibiscus.

6.4.2 Chromaticity diagram

Fig. 6.9 depicts the chromaticity diagram of the fresh red, pink and yellow appearing hibiscus using 1931 CIE colour space [25]. Chromaticity diagram gives us a clear picture of the colour of any object. Here, the chromaticity diagram basically represents the loci of the intensity of the reflected wavelength. The co-ordinate (x, y) is defined as the chromaticity co ordinates in the colour space, where (0.33, 0.33) represents the white point of the loci, while each of the wavelength is converted to (x, y) co ordinates. For the three specimens, the chromaticity co ordinates (x,y) are inclined towards higher wavelength range.

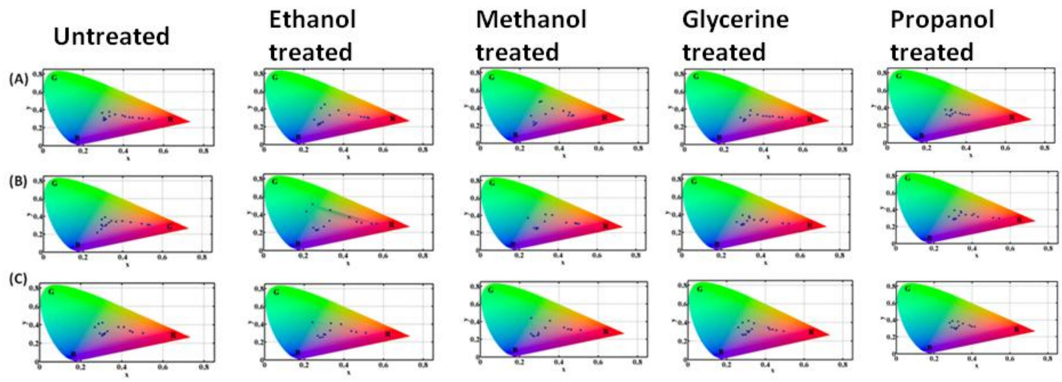

Figure 6.9: Chromaticity diagrams of the untreated and treated (A) red (B) pink (C) yellow hibiscus petals.

The treated petals, ethanol, methanol and glycerine ones have points accumulated over the complete colour space. The ethanol and methanol-treated petals show the scattered distribution of points over the wavelength range. The glycerine-treated petals, however show similar kind of trend as the untreated with increase in intensity. And the propanol treated ones have constant distribution of points, accumulated more at the centre, showing removal of colour.

6.5 Conclusions

We have studied the optical features of the three varieties of the hibiscus flower. The treatment of the petals with different media has caused a change in the reflectance features. In case of dried petals, the structure gets destroyed, and thus the reflectance decreases, because the microstructural roughness gets enhanced. Also the wetting-dewetting features for the fresh petals have been studied with high water CA value and high CAH value. Structure plays a vital role in the wettability properties. This study is important because it can pave a new way for exploiting newer micro structures at large.

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