Chapter II

Physical characterisation: tools and techniques employed

Several analytical techniques have been employed for evaluating specimens under study. To be specific, spectroscopic, imaging and wettability studies have been considered as per requirement and as discussed below. The specimens examined are naturally occurring soft matter systems. For instance, insect components, such as, dragonfly and butterfly wing parts have been considered as our first choice. Secondly, we opted for flower petals, namely, Indian rosacea and hibiscus for experimentation. Prior to the characterisation processes, we have carefully sectioned the specimens using sharp knives/scissors. With the help of forceps, specimens are deliberately seperated for the necessary studies. The samples were kept in the dessicator in order to avoid moisture uptake and contamination. Blowers have been used prior to each experiment so as to ensure the specimens dust-free. After taking care of precautions, samples were kept ready for the subsequent experimentation. As for the first-hand visual information, we have used digital scientific camera and an inverted optical microscope. In order to assess the optical responses over a broad range of wavelength, we have acquired and analysed the UV-Vis-NIR reflectance spectra, with special emphasis on variable incident angle accessory for viewing angle dependent studies. Also, polarisation sensitive reflectance features have been assessed using set of polaroids with necessary adjustment. Furthermore, for analysing the wetting-dewetting characteristics, water contact angles have been measured and in this regard, both static as well as dynamic modes have been examined for each sample.

2.1. Digital scientific camera and optical microscope

A Canon make digital scientific camera IXUS 951S is employed to view certain specimens and obtain first-hand information. The camera has a CCD sensor, of typical surface area ~28.6 mm^2, and capable of handling as high as 10^7 pixels. The pixel pitch is the separation between the two pixels, with a pitch value of 1.69, while the pixel area is 2.86 μm^2. A higher pixel value would indicate more light capturing capability.

Microscopic information could be revealed by using an inverted optical microscope (Leica DM IL LED), employing a 20X objective for imaging purposes. In this microscope, transmitted light has been used for optical imaging.

2.2 Scanning electron microscopy (SEM)

Scanning electron microscope is a type of electron microscope which uses back-scattered electrons for imaging purposes. Undoubtedly, it has added advantage over light microscope as regards both imaging and resolution. Electron microscopes have higher resolution and images can be captured distinctly without loss of information in a given region. In scanning electron microscopy, image of a sample is produced by scanning the surface with a focussed beam of electrons, thus providing information as regards the structural topography of the sample under study. In order to reveal morphological details of specimens under study (dragonfly, butterflies, rose petals etc.) scanning electron microscopy (SEM) technique was employed (SEM, JEOL, JSM 6390 LV) while the machine was operating at an accelerating voltage of 20kV. Prior to loading the surface of the carefully sectioned specimens, was subjected to a few layers (5-7 nm) of platinum coating so as to avoid charging effect during SEM imaging. The images of different specimens available are captured at different magnifications, depending on interesting features of a particular region. In case of dragonfly and damselfly wings, the microstructural details with packing of oblate shaped chitinous elements have been observed from the acquired images. Similarly the micrographs of the butterfly wings clearly revealed the presence of the wing scales, with the scale components comprising of longitudinal ridges, cross ribs, scutes, flutes and trabeculae [1]. The details are discussed in the individual chapters. As for the rose petals (*Rosacea* specimens), the micrographs revealed the presence of numerous micropapillae and further magnified images exhibited the presence of circular wrinkles, and nanofolders that make up the micropapillae [2]. In case of imaging rose petals, the sample holder was tilted upto~ 2°, in order to gain better insights to the micropapillae arrangement. On the other hand, the SEM images of the fresh as well as dried Hibiscus flower petals, exhibited presence of micropapillae on the petal surfaces, which tend to contract and result in microscopic wrinkles upon drying.

2.3 UV-Vis-NIR reflectance spectroscopy

Reflectance spectroscopy is an important tool for exploring structural colouration of given species. Here, the light reflected from the inhomogeneities of the samples is taken into account. In case of reflectance spectrophotometer, the light reflected from the sample (I) is compared to the light reflected from the white standard (I_0). The ratio is termed as reflectance (R%). Diffuse reflectance is important for the analysis of light propagation through inhomogeneous material, with light getting scattered off different points from the sample. Theoretical description of diffuse reflectance can be understood from the Kubelka-Munk model [3]. In this model, the geometric irregularities on an object are condensed to a single parameter, known as, scattering coefficient 'S' [4]. In our work, a series of reflectance dataset is acquired for each specimen through the UV-Vis-NIR reflectance spectroscopy at normal incidence (Shimadzu Co.). The UV-2450 system consists of two light sources (deuterium lamp D2, halogen lamp WI), the light emitted from these sources reflected by the mirrors get projected on the monochromator. For normal reflectance measurements, the slit width is 2 mm. The cross section of the light beam on the sample holder is about 1 mm wide. Through the monochromator, the data acquired is in the wavelength region 200-800 nm. For certain cases, we have also employed a

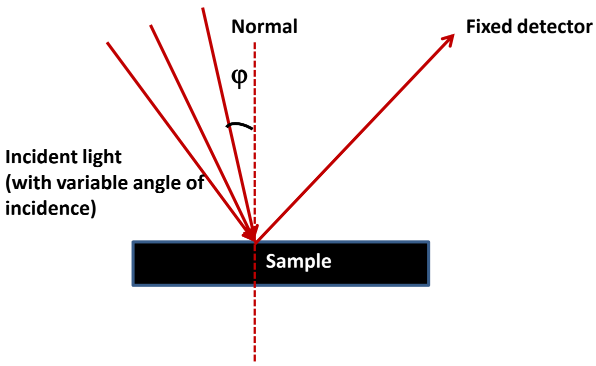

Figure 2.1: Schematic representation of varying incident light on the sample.

PerkinElmer 365 Lambda spectrophotometer with integrating sphere attachment to accomodate diffuse reflectance from all possible directions. The integrating sphere has a diameter of ~50 mm and is made of a versatile substance with barium sulphate coating. The schematic representations of the instruments are provided in the appendix III. As for the angle dependent studies, we have employed PerkinElmer 365 Lambda spectrophotometer with Variable angle accessory (VAR) attached to the main equipment while capable of obtaining reflectance measurements in the wavelength range 400-1100 nm. The incident angle could be varied between 15° and 75° in steps of 15°. The schematic representation is shown in Fig. 2.1. The reflectance measurements, with special considerations, are vital as it could pave the way for exploiting the colouration mechanism.

Apart from the above studies, we also acquired polarisation dependent reflectance features. In this regard, we have employed *s* and *p* polarisations using a set of polaroids (Holmarc optics), which transmit light in the wavelength range of 400-700 nm. The schematic representation of the polarised light is shown in Fig. 2.2.

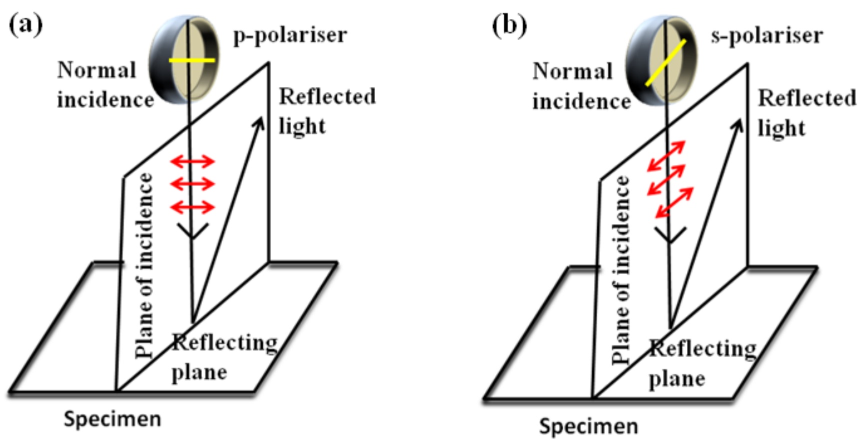

Figure 2.2: Schematic representation of the polarised incident light on the specimen surface.

2.4 Contact angle studies using contact angle meter

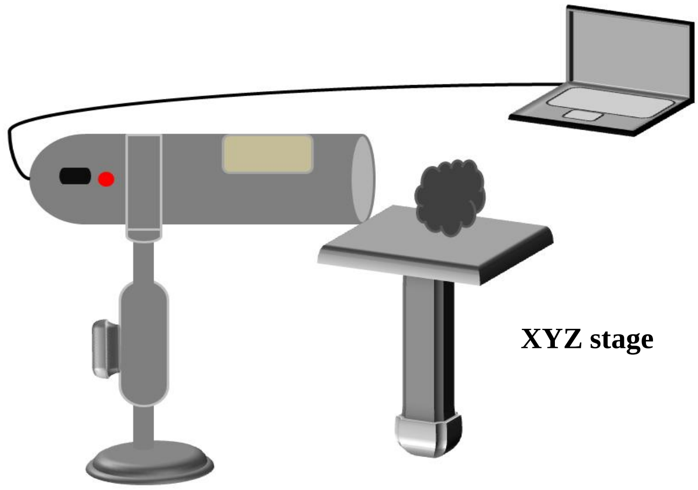

Figure 2.3: Schematic representation of the custom-made contact angle meter.

Both custom-made and sophisticated CA meters have been employed as per requirement. The schematic representation of the set-up is shown in Fig. 2.3. First, the desired specimen is kept horizontally on the platform of an XYZ stage that has 3D movement. One droplet of water of volume ~80 μl is then gently placed over the region under study. An independent PC—interfaced microscopic camera (Digimicro, 10×), fixed on a vertical stand, was screw-adjusted to image the solid-water interface along with the whole water droplet itself. The top view and the side view of the samples have been further captured for analysis. The image analysis was carried out by using *ImageJ* software® [5].

Apart from the custom made setup, we have employed a sophisticated contact angle meter (Kyowa Science Co. Ltd.), shown in appendix for measuring both the static as well as dynamic contact angles. As for static CAs, a liquid droplet of 2μl is placed over a specimen surface and CAs is measured at various locations. However, for the dynamic CA measurement, we had to employ tilting plate methodology, where a droplet on the sample surface is allowed to tilt. Tilting plate methodology is one of the most reliable methods, which must be taken into account for experimental precision. This method is also important in terms of its applicability to critical physical problems [6]. In real systems, we do not observe a single equilibrium CA, instead a number of contact angle values.

The contact angle of the sessile drop lies in between advancing (θ_a) and receding (θ_r) angles. The difference between these values is known as contact angle hysteresis (CAH). The contact angle hysteresis value is an important parameter while discussing/analyzing wettability features of a surface under study. This is worth mentioning here that, this has been a topic of debate for several years. The design of surfaces which allows green applications still remains a challenge to the researchers, because technically, the wetting transition also plays a crucial role in the fabrication of superhydrophobic surfaces [7]. On the other hand, dynamic CA study is beneficial as it could help us to deal with these issues. The schematic representation of the contact angle meter is shown in appendix and the representation of the advancing, receding angles are shown in Fig. 2.4.

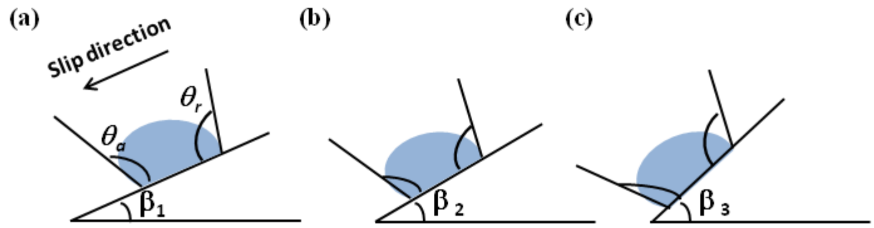

Figure 2.4: Schematic representation of the advancing and receding angles at different tilting angle.

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