

The chemical composition of glandular hairs have been studied following the techniques of Jensen² and Johansen³. Chemically the glandular hairs contain polyphenolic compounds, anthocyanins, glucose, sucrose and fructose. The foliar nectaries of *Cassia biflora* contain 15 amino acids⁴. Alston and Irwin⁵ similarly reported amino acids, phenols and anthocyanins in the flowers of 5 species of *Cassia* but without mention of toral glandular hairs.

In the closely related *Krameria* spp. Simpson *et al*^{6,7} reported two types of glands that produce saturated fatty acids and methyl esters, through a row of elongate epidermal cells that collect under the cuticle akin to those observed in the present study.

Based on the histochemical tests, it may be said that the glandular hairs are nutritive to various pollinators since the flowers are *enantiostylous*. It is thus clear that both the foliar glands as well as the toral glandular hairs presently described play useful role in maintaining plant/animal relationship.

We thank Prof. P. S. Rao, Department of Chemistry, Kakatiya University, for his help in the chemical analysis of glandular hairs. One of us (AC) is also thankful to Council of Scientific and Industrial Research, New Delhi, for financial assistance.

17 April 1985; Revised 23 July 1985

1. Bahadur, B., Kumar, P. V. and Reddy, N. P., *Proc. Natl. Symp. Poll. Ecol. Appl. Palyn.*, 1984 (in press).
2. Jensen, W. A., *Botanical Histochemistry*, Freeman & Co., San Francisco, U.S.A., 1962.
3. Johansen, D. A., *Plant Microtechnique*, Tata McGraw-Hill Publishing Company, New Delhi, 1940.
4. Baker, H. G., Opler, P. A. and Baker, I., *Bot. Gaz.*, 1978, 139, 322.
5. Alston, R. E. and Irwin, H. S., *Amer. J. Bot.*, 1961, 48, 35.
6. Simpson, B., Neff, J. L. and Seigler, D., *Nature (London)*, 1977, 267, 150.
7. Simpson, B., Seigler, D. S. and Neff, J. L., *Botanical Systematics and Ecology*, 1979, 7, 193.

IMMOBILIZED TRYPSIN ON GELATINATED PVC: PLATELET ADHESION

C. P. SHARMA, GEORGE JOSEPH and N. V. NIRMALA

*Division of Biosurface Technology,
Sree Chitra Tirunal Institute for Medical
Sciences & Technology,
Trivandrum 695012, India.*

A LARGE variety of synthetic polymers are increasingly used in biomedical applications. Polyvinyl chloride (PVC) with a wide choice of additives and ingredients such as plasticizers, fillers and stabilizers finds broad application in blood contacting devices. One of the disadvantages with this polymer is that it contains a large number of leachables which can adversely affect the blood components. In an attempt to reduce the leaching, the PVC sheet is plasma glow treated and a network of gelatine is developed on the surface which can effectively retard the migration of leachables to the surface. Trypsin is immobilized on the gelatine substrate using glutaraldehyde coupling to improve the blood compatibility of the surface.

Preparation of surfaces:

The PVC sheet was cut into pieces of size 2.5 cm × 3 cm, cleaned with 0.1% soap solution (Teepol) and washed thoroughly with distilled water and dried. The samples were plasma glow treated for 3 min with nitrogen gas at a pressure of 10⁻¹ m bar using Edwards Vacuum Coating Unit E306A. Then they were exposed to gelatine (500 mg %) dissolved in phosphate buffer pH 7.4 for 3 hr at reduced air/water interface¹. The samples were taken out rinsed in buffer and vacuum-dried. γ -irradiation was done (⁶⁰Co source) under N₂ atmosphere with a total dose of 0.275 M Rad. The irradiated samples were exposed to gelatine again and then cross-linked with glutaraldehyde (2.5%, v/v) by exposing for 2 hr. They were taken out and rinsed with the buffer thoroughly. Any unlinked aldehyde group was neutralized by a third exposure to gelatine (surface A). Alternatively instead of gelatine, trypsin was immobilized² by overnight exposure at 4 C to 50 mg % trypsin dissolved in phosphate buffer (surface B)

Contact angle studies:

The contact angle measurements on the surfaces were made with a goniometer (Kernco Instruments

Inc. Texas) using triple distilled water³. The angle made by a drop was observed for 25 min to note the changes. The contact angle was measured on the gelatine modified surface for a period extending to 9 weeks, to study the leaching phenomenon.

Platelet adhesion:

Washed platelets were prepared from citrated calf blood as described elsewhere¹ and suspended in tyrode solution⁴. The surfaces were exposed to the platelet suspension for 15 min and then rinsed with buffer under controlled flow rate for 2 min. The platelets were fixed with 2.5% glutaraldehyde and stained with Coomassie Blue. The platelet density was estimated using an optical microscope.

The migration of leachables to the surface of the PVC makes the initial contact angle high (i.e. hydrophobicity). The angle made by the water drop changes as the water penetrates the leachables.

As seen from table 1, the contact angle on the bare PVC changed from the initial value of 90° to 68° in 25 minutes. On the gelatinated surface also the angle changes due to the hydrophilic nature of gelatine. But the initial contact angle even after 9 weeks time is low compared to that of the bare PVC indicating that the gelatine network prevents the leachables from coming to the surface. The slight increase in the initial contact angle, with time may be due to some conformational changes of the gelatine molecules. The platelet adhesion results in table 2 demonstrate that the gelatine

Table 1 Sessile drop water contact angle

| Time (min) | Contact angle in degrees \pm S.D. ^a | | | |
|------------|--|----------------------------------|----------------|----------------|
| | Bare PVC | Surface A at different intervals | | |
| | | 0 | 6 weeks | 9 weeks |
| 0 | 90.0 \pm 1.0 | 50.5 \pm 4.0 | 57.0 \pm 2.0 | 63.0 \pm 2.0 |
| 10 | 76.0 \pm 0.5 | 26.0 \pm 2.0 | 52.0 \pm 3.0 | 57.0 \pm 2.0 |
| 20 | 71.5 \pm 0.5 | 21.0 \pm 1.0 | 49.0 \pm 3.0 | — |
| 25 | 68.0 \pm 0.5 | 20.0 \pm 1.0 | 47.0 \pm 3.0 | 47.0 \pm 1.0 |

^a Mean \pm Standard Deviation

Table 2 Platelet adhesion

| Surface | No. of platelets/mm ² \pm S.D. ^a |
|----------|--|
| Bare PVC | 16.0 \pm 2.0 |
| A | 10.0 \pm 1.7 |
| B | 7.5 \pm 1.7 |

^a Mean \pm Standard Deviation

modification has considerably reduced the number of adhering platelets compared to that on the bare PVC. The immobilized trypsin further reduced the platelet adherence (surface B in table 2). Hence such immobilization of enzymes on proteinated substrates may find application in developing blood compatible surfaces.

We appreciate the helpful discussions we had with Mr. Thomas Chandy and the help received from the Department of Atomic Energy, India.

29 November 1984; Revised 5 July 1985

1. Sharma, C. P. and Chandy, T., *J. Coll. Int. Sci.*, 1982, **89**, 479.
2. Ramesh, V. and Singh, C., *Biochem. Biophys. Res. Commun.*, 1980, **97**, 479.
3. Kaelble, D. H. and Moacanin, J., *Polymer*, 1977, **18**, 475.
4. Lee, E. S. and Kim, S. W., *Trans. Am. Soc. Artif. Int. Org.*, 1979, **XXV**, 124.

COMPARATIVE PHOSPHATE SOLUBILIZING CAPACITY OF SOME SOIL FUNGI

S. SURANGE

Maharashtra Association for The Cultivation of Science, Research Institute, Pune 411 004, India.

THE phosphate-solubilizing ability of different microorganisms has already been studied¹⁻⁵. This character, in respect of fifteen fungi, isolated from the lateritic forest soils of Maharashtra, was studied using Pikovskaya medium with tricalcium phosphate as the substrate. The fungi were inoculated to 50 ml aliquots of this medium, dispensed in 250 ml Erlenmeyer flasks in triplicate and medium without fungus served as control in each case. The flasks were agitated continuously on a rotary shaker for seven days at room temperature (27 \pm 2°C). Soluble phosphate in the medium was estimated by the 'molybdenum blue' method⁶. Pigments, whenever produced, were eliminated by adding activated charcoal followed by filtering. Colorimetric measurements were made on Beckman spectrophotometer (model 24) at 680 μ for standard as well as experimental phosphate concentrations (table 1).

Phosphate solubilized by the fungi was in the range of 1.6–20.9%. *Cylindrocarpon obtusisporum* and