CHAPTER II

MATERIALS AND GENERAL METHODS
MATERIALS

D-Glucose, D-fructose, D-galactose and D-mannose were the products of Aldrich Chemical Company, Milwaukee, Wis, USA. D-Xylose, D-arabinose and D-ribose were obtained from Sigma Chemical Company, St. Louis, Mo, USA. The only two furaldehyde derivatives used were 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde, which were obtained from Aldrich Chemical Company, Milwaukee, Wis, USA. 1-Deoxy-1-moropholino-D-fructose (DMF), N-acetyl neuraminic acid (Type IV, 98% purity), 4,4'-dicorboxy-2,2'-biquinoline (bicinchoninic acid) and nitroblue tetrazolium chloride (NBT) were obtained from Sigma Chemical Company, St Louis Mo, USA. L-Cysteine hydrochloride, o-cresol and L-fucose were the products of Aldrich Chemical Company, Milwaukee, Wis, USA.

Analar grade sulfuric acid (98%, specific gravity 1.84) used throughout the study, was from Ranbaxy Laboratories, S. A. S. Nagar, Punjab, India. Ninhydrin was obtained from Kemphosol, Bombay, India. Hydrochloric acid and glacial acetic acid were from BDH laboratories, Chemical division of Glaxo Laboratories India Ltd., Bombay, India. All other chemicals used in this study were analytical grade reagents.

Blood, saliva, thumb nail, toe nail and hair samples used in this study were collected from persons admitted to or attending the out patient clinics of the department of Oral Medicine and General Medicine, S. D. M. College of Dental Sciences, Sattur, Dharwad, Healthy volunteers comprised the control group.

METHODS

One-step method for estimation of sugars

In this method, both the compound of interest and the developer in an aqueous medium were exposed to heat of dilution, generated by the addition of
concentrated sulfuric acid. The maximum temperature attained in the reaction mixture was in the range, 115°C-118°C.

Unless stated otherwise, the following routine procedures were employed. To one ml aqueous solution of sugar 0.1 ml of ethanolic o-cresol (20 mg) was added and mixed, followed by the rapid addition of 3.0 ml concentrated sulfuric acid using a dispensette, freely and continuously, directed to the surface of the solution. The solution was mixed thoroughly using a vortex mixer. Both o-cresol and sulfuric acid were at room temperature (28-30°C) before use. The reaction system was allowed to cool at room temperature for 30 minutes and the absorbance values were measured in an Elico UV-visible spectrophotometer (model CL-27) at 500 nm for hexoses and 490 nm for pentoses, respectively. The final sulfuric acid concentration in the assay system was close to 75%.

**Two-step method for estimation of sugars**

In this method only the compound in aqueous medium without the developer, was exposed to heat of dilution, generated by the addition of concentrated sulfuric acid. After allowing the system to cool at room temperature for 30 minutes, the developer was added to the system, for color development.

Unless stated otherwise, to one ml aqueous solution of sugar, 3.0 ml concentrated sulfuric acid was added. The solution was mixed thoroughly. The reaction system was allowed to cool at room temperature (28-30°C) for 30 minutes before the addition of 0.1 ml of ethanolic o-cresol (20 mg). The chromogen formed was measured at 500 nm for hexoses and 490 nm for pentoses, 30 minutes after the addition of the developer.

**Pre-chilled acid method**

In this method the color reaction takes place at room temperature without exposing HMF/furaldehyde to heat of dilution. This method was employed
with HMF/furaldehyde solutions to determine whether destruction of HMF/furaldehyde takes place, when exposed to heat of dilution.

To 0.9 ml of distilled water, 3.0 ml of concentrated sulfuric acid was added, mixed and kept overnight at 4°C. A solution of furaldehydes in water (5-50 μg HMF/furaldehyde in 0.1 ml) was added to cold 75% sulfuric acid. No measurable increase in temperature of the system was observed at that stage. The contents in the system were mixed thoroughly and the reaction system was maintained at room temperature (28-30°C) for 30 minutes. Ethanolic o-cresol (20 mg) was added to the system for color development as before. After 30 minutes standing at room temperature, the chromogens formed were analyzed at 500 nm and 490 nm respectively for HMF and furaldehyde.

All the spectral analysis were made at 25°C. For all the above methods, blank solution consisted of distilled water, developer and sulfuric acid. The values reported are concordant, obtained after a minimum of five separate determinations.

Other methods are described under the relevant chapters.